Comprehensive Assessment of the Hemostatic System in Polycystic Ovarian Syndrome

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Abstract

Polycystic ovarian syndrome (PCOS) affects 12 to 19% of women and has reproductive and metabolic features (endothelial dysfunction, increased diabetes, and cardiovascular risk factors). It also appears to have altered coagulation and fibrinolysis with a prothrombotic state with epidemiological evidence of increased venous thromboembolism. We aimed to comprehensively assess hemostasis in women with PCOS versus control women. In an established case–control cohort of lean, overweight, and obese women with ($n = 107$) and without PCOS ($n = 67$), with existing measures of plasminogen activator inhibitor 1 (PAI-1), asymmetric dimethylarginine (ADMA), hormonal, and metabolic markers, we also assessed prothrombin fragments 1 and 2 (PF1 & 2), plasminogen, tissue plasminogen activator (tPA), and thrombin generation (TG). Higher levels of ADMA (0.70 vs. 0.39 μmol/L, $p < 0.01$), PAI-1 (4.80 vs. 3.66 U/mL, $p < 0.01$), and plasminogen (118.39 vs. 108.46%, $p < 0.01$) were seen in PCOS versus controls, and persisted after adjustment for age and body mass index (BMI). PF1 & 2 was marginally lower (180.0 vs. 236.0 pmol/L, $p = 0.05$), whereas tPA and TG were not different between groups, after adjustment for age and BMI. Significant relationships were observed between hormonal and metabolic factors with ADMA and PAI-1. We demonstrate impaired fibrinolysis in PCOS. In the context of abnormal endothelial function and known hormonal and metabolic abnormalities, this finding may underpin an increased risk of cardiovascular disease and venous thrombosis in PCOS.

Keywords

- polycystic ovarian syndrome
- hemostasis
- thrombosis
- plasminogen
- PAI-1

Polycystic ovarian syndrome (PCOS) is diagnosed based on ovulatory and menstrual disturbance, hyperandrogenism, and polycystic appearance of ovaries on ultrasound.[1] It is one of the most common endocrine disorders of females of reproductive age with 12 to 19% of women affected, depending on the population studied.[2,3] The etiology of PCOS remains to be elucidated, yet it is a complex disease similar to type 2 diabetes, rheumatoid arthritis, and cardiovascular disease (CVD), ~~underlined~~ by a combination of genetic predisposition and environmental factors with highly variable phenotypic expression.[4]

underpinned

PCOS metabolic abnormalities include insulin resistance (IR), hyperinsulinemia, dyslipidemia, and increased diabetes risk, all of which are cardiovascular risk factors.[1] Between 75 and 95% of women with PCOS have IR, which occurs independent of obesity and reinforces the pathophysiology of the disease.[5,6] Women with PCOS are also more likely to be overweight or obese than non-PCOS women, which appear to further increase IR and drive PCOS incidence and severity.[1,3,7,8] Women with PCOS also have comparatively higher body fat and central visceral adiposity inducing

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greater IR compared with body mass index (BMI)-matched control women; however, these findings do vary.[9] Hyperandrogenism also contributes to the pathophysiology of PCOS potentially driven by IR.[10] Overall, PCOS is a condition with inherent IR and hyperandrogenism, causing reproductive and metabolic features.

Recently, it has been reported that women with PCOS have significantly higher risk of cardiovascular[11] and venous thromboembolic diseases compared with their counterparts without PCOS.[12] These risks are exacerbated by high BMI and by some current PCOS management strategies. Concerns have been raised that important therapies, namely, the oral contraceptive pill (OCP) that targets reproductive features such as menstrual cycle regulation and hirsutism management, may also increase CVD and does increase venous thromboembolic risk.[1,12] PCOS combined with excess weight and the OCP renders this a high-risk group of young women.

Risks of increased arterial and venous thromboses from abnormalities in the hemostatic system are well accepted. We and others have previously noted, through a comprehensive review of the literature, that there may be additional cardiovascular risk factors in PCOS, potentially relating to disturbed hemostasis and endothelial dysfunction reflected by elevated asymmetric dimethylarginine (ADMA) and plasminogen activator inhibitor 1 (PAI-1).[13,14] Potential abnormal platelet function/activation and abnormal coagulation have also been documented in PCOS.[13,14] High fibrinogen levels are noted in PCOS women and enhanced inhibition of fibrinolysis reflected by high PAI-1 antigen and/or activity as well as increased tissue plasminogen activator (tPA) levels, most likely reflecting tPA–PAI-1 complexes.[13] However, studies performed on hemostatic factors in PCOS were small and had contradictory findings.[13,14] Correlations between hemostatic parameters and markers of metabolic and hormonal systems also revealed inconsistent findings.[13] Factors contributing to the heterogeneity include that many published studies did not consider BMI or did not include age-matched controls (as both BMI and age affect the hemostatic system).[13]

In this context, we aimed to comprehensively assess the hemostatic system in high-risk women with PCOS versus controls, adjusting for age and BMI. We aimed to evaluate components of the hemostatic system, including endothelial function, the coagulation cascade, the fibrinolytic system and relevant inhibitors, as well as assessing global markers of hemostatic function. We also investigated the relationships between hemostatic markers and hormonal and metabolic variables associated with PCOS, including IR/hyperinsulinemia and hyperandrogenism to identify potential underlying causes and future therapeutic targets.

Materials and Methods

Subjects

Citrate anticoagulated venous samples and data (endothelial and biochemical markers) from subjects collected and analyzed from the current observational study were taken from a biobank of clinical studies in women with and without PCOS.[5,15–17] The Southern Health Research Advisory and Ethics Committee approved all studies, and participants gave written informed consent. Recruitment of participants for this case–control study was undertaken from community advertisements. Recruitment, medical assessment, and sample collection were performed in a single center (Monash University) and under the supervision of a single, expert academic clinician (H.T.). All participants were premenopausal women aged 18 to 45 years with (i.e., cases) and without PCOS (i.e., controls).[5,15,16] All cases met the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine criteria (also known as the Rotterdam diagnostic criteria) with two of (1) irregular menstrual cycles (< 21 or > 35 days), (2) clinical (hirsutism, acne) or biochemical (elevation of at least one circulating ovarian androgen) hyperandrogenism, and (3) polycystic ovaries on ultrasound.[18] Exclusion criteria were secondary causes of amenorrhea and hyperandrogenism (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing syndrome, hyperprolactinemia, thyroid dysfunction, adrenal disorders, and pregnancy), smoking, uncontrolled hypertension, type 2 diabetes, and nonstable use of antihypertensive, lipid-lowering ,or fish oil medications. In all cases, participants were required to cease OCPs, endocrine hormonal treatment, or insulin-sensitizing agents (e.g., metformin) for 3 months before clinical measurements, and all participants received standard diet and lifestyle advice.[5,15,16] All studies were completed in the same center with the same staff and study protocol as described earlier. All controls had regular menstrual cycles (21–35 days), displayed no evidence of clinical or biochemical hyperandrogenism, and ceased medication as described earlier, 3 months before sample collection.

Clinical Measurements

All subjects were weighed in lightly weighing clothes with no shoes in the same center and the BMI was calculated by weight (kg) divided by squared height (m^2). Waist circumferences were measured at the umbilicus. Measurements were performed by an experienced operator.

Biochemical, Endothelial, and Hemostatic Measurements

IR was assessed by homeostasis model assessment (HOMA score) and a 75-g oral glucose tolerance test (glucose and insulin at 0, 30, 60, and 120 minutes) as previously described.[15] The HOMA score was calculated as fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5.[5,15–17] Insulin was assayed using the analyzer AxSYM[Q2] that is based on the microparticle enzyme immunoassay technology.[5,15–17] Total testosterone was measured using a chemiluminescent immunoassay (Beckman Coulter, Fullerton, CA).[5,15–17] Total cholesterol and triglycerides were measured using enzymatic reagents (DADE Diagnostics, Brisbane, Australia). High-density lipoprotein (HDL) cholesterol was measured by homogeneous assay techniques (HDLC-Plus; DADE Diagnostics) adapted to a DADE Dimension RXL chemistry analyzer. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation (LDL cholesterol = [total cholesterol − HDL cholesterol] − [triglycerides/

2.2]), adapted to SI units.[5,15–17] All tests were performed in the same commercial pathology laboratory at Monash Health.

Endothelial function markers were assessed on a subset of subjects. As previously published, PAI-1 activity was assayed using the commercial chromogenic assay Berichrom PAI (Marburg, Germany)[15,16,19,20] with an intra- and interassay coefficient of variation (CV) of 1.3 ± 0.6 and 4.3 ± 0.5%, respectively. ADMA was assessed using a chromogenic immunoenzymatic assay kit from DLD Diagnostika (Hamburg, Germany) with an intraassay CV between 5.7 and 6.4% and an interassay CV between 8.3 and 10.3%.[15,20]

Hemostatic markers were assessed on 174 citrate anticoagulated venous blood samples (107 PCOS and 67 controls). These had been centrifuged, separated, and plasma stored at −80°C until assayed. To prevent the deteriorative effects of freeze-thaw cycles on coagulation factors, simultaneous testing of coagulation markers was undertaken. We aimed to have a systematic approach when assessing the hemostatic system. Therefore, measurement of the system's components was undertaken as well as assessing hemostasis overall. Not all measures were completed on the full dataset because of some inadequate stored samples. Endothelial dysfunction was assessed by measuring the nitric oxide inhibitor ADMA and the endothelially synthesized product PAI-1 (as previously described).[15,16,19,20] In vivo coagulation cascade activation was assessed through prothrombin fragments 1 and 2 (PF1 & 2) levels using an enzyme-linked immunosorbent assay (ELISA) method (Siemens Enzygnost F1 + 2 monoclonal, Marburg, Germany) with an intraassay CV between 3.6 and 5.5% and an interassay CV between 4.4 and 11.2%. The fibrinolytic system was assessed through plasminogen and tPA. Plasminogen activity was assayed using the STA Stachrom Plasminogen colorimetric assay performed on the automated analyzer Stago STA-R (Asnieres sur Seine, France) with an intraassay CV between 1.8 and 1.9% and an interassay CV between 2.4 and 3.4%. tPA was assayed using an ELISA method (TriniLIZE tPA Antigen, Tcoag, Bray, Ireland) with an intraassay CV between 4.9 and 5.5% and an interassay CV between 3.5 and 5.4%. Inhibition of fibrinolysis was assessed through PAI-1 activity. Finally, assessment of thrombin generation (TG) looked at the hemostatic system overall, evaluating the net synthesis of thrombin through coagulation activation but also evaluated the simultaneous effects of inhibitors of the coagulation cascade. TG was assessed using the calibrated automated thrombogram (CT[Q3]) method (Stago) with an intraassay CV of 4.3% and an interassay CV of 6.1%.

CAT Q3

Statistics

At the inception of the study, we did a power calculation based on detecting a minimum hypothesized difference in PAI-1 of 1.32. The results of the power calculation indicated that we needed 40 participants in each group with a power of 90% and an α at 0.05. All data are presented as mean ± standard deviation with the exception of insulin, HOMA score, ADMA, PAI-1, PF1 & 2, and tPA data that are presented as median and interquartile range. Two-tailed statistical analysis was performed using SPSS for Windows 22.0 software (SPSS, Inc., Chicago, IL) with statistical significance accepted when $p < 0.05$. Data were assessed for normality and logarithmically transformed where appropriate. Demographic and hemostatic differences between PCOS women and controls were assessed using the Student *t*-test for normally distributed data and the Wilcoxon–Mann–Whitney test for nonnormally distributed data. Multiple linear regression analysis was used to examine the independent association between predetermined metabolic/hormonal parameters (i.e., HOMA score, fasting insulin, and total testosterone) and all hemostatic variables in PCOS women and controls (after adjusting for age and BMI).

Results

Baseline, Metabolic, and Hormonal Results

Baseline characteristics of subjects (PCOS and controls) including age, BMI, waist circumference, as well as biochemical (metabolic and hormonal) markers are displayed in ►**Table 1**. Significantly higher BMI, waist circumference, cholesterol, LDL, triglycerides, insulin, HOMA-IR, and testosterone levels were observed in PCOS compared with controls.

Endothelial and Hemostasis Function: Differences between PCOS and Controls and Relationships to Hormonal and Metabolic Markers

Although there were 174 participants in this study ($n = 107$ PCOS and $n = 67$ controls), not all parameters were measured for all subjects with variable availability of adequate samples for complete testing (►**Table 2**). With endothelial function, significantly higher levels of ADMA and increased PAI-1 activity were seen in PCOS versus controls ($p < 0.0001$ for both parameters) (►**Fig. 1**), which persisted after adjustment for age and BMI ($p < 0.0001$ and $p = 0.005$, respectively). These differences also persisted after adjustment for waist circumference ($p = 0.003$ and $p < 0.0001$, respectively; data not tabulated) and after adjustment for all lipid markers for which we noted differences in PCOS and controls: cholesterol ($p < 0.0001$ for both, respectively; data not tabulated), LDL ($p < 0.0001$ for both; data not tabulated), and triglycerides ($p = 0.009$ and $p < 0.0001$, respectively; data not tabulated). Linear regression models using fasting insulin, HOMA score, and total testosterone demonstrated significant relationships between these metabolic and hormonal factors with both ADMA ($p < 0.01$ for all three factors) and PAI-1 ($p < 0.01$ for all three factors) after accounting for age and BMI (►**Table 3**). A significant difference was noted between PCOS and controls in PF1 & 2 levels ($p = 0.028$); however, this was only borderline when adjusted for age and BMI ($p = 0.05$), with higher levels in controls versus the PCOS group. After adjusting for age and BMI, no significant relationship was noted between PF1 & 2 with fasting insulin and HOMA score ($p = 0.08$ and $p = 0.07$, respectively) and only a borderline relationship was noted with testosterone levels ($p = 0.05$) (►**Table 3**). A significant difference between PCOS and controls was noted in plasminogen activity ($p < 0.0001$) following adjustment for age and BMI ($p = 0.024$) (►**Fig. 2**) that also persisted following adjustment for waist circumference ($p < 0.0001$; data not tabulated), cholesterol ($p < 0.0001$; data not tabulated), LDL

Table 1 Demographics[Q4] (age, BMI, waist circumference, hormonal, and metabolic parameters) for PCOS and controls

Variable	PCOS ($n = 107$)	Controls ($n = 67$)	p-Value
Age (y)	32.8 ± 6.7	34.1 ± 7.9	0.224
BMI (kg/m^2)	35.6 ± 7.8	31.3 ± 7.0	0.0004
Waist circumference (cm)	104.7 ± 16.7	94.9 ± 16.6	< 0.0001
Fasting insulin (mU/L)	17.60 (11.0–23.09)	9.94 (5.20–16.45)	0.0001
HOMA score	3.58 (2.20–5.20)	2.05 (0.10–3.36)	< 0.0001
Glucose levels (fasting) (mmol/L)	4.72 ± 0.57	4.70 ± 0.38	0.619
Cholesterol (mmol/L)	5.29 ± 1.02	4.90 ± 0.91	0.007
HDL (mmol/L)	1.26 ± 0.36	1.30 ± 0.34	0.408
LDL (mmol/L)	3.43 ± 0.93	3.10 ± 0.77	0.038
Triglycerides (mmol/L)	1.35 ± 0.70	1.0 ± 0.46	< 0.0001
Total testosterone (nmol/L)	2.5 ± 1.0	1.4 ± 0.6	< 0.0001

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; PCOS, polycystic ovarian syndrome.
Note: Part data[5,15–17]; means ± SD reported and ANOVA for age, BMI, testosterone, waist circumference, glucose levels, cholesterol, HDL, LDL, and triglycerides. Median (interquartile range) and p-values from log transformed data for insulin and HOMA score.

($p < 0.0001$; data not tabulated), and triglycerides ($p < 0.0001$; data not tabulated), with higher plasminogen activity observed in the PCOS group. Linear regression models using insulin, HOMA score, and testosterone did not demonstrate significant relationships between these factors and plasminogen activity after accounting for age and BMI ($p = 0.16$, 0.11, and 0.13, respectively) (►**Table 3**). Although a significant difference was noted between PCOS and controls as regard to tPA antigen levels ($p = 0.025$), these relationships disappeared when BMI and age were taken as confounders ($p = 0.55$). This fibrinolytic marker did not show a significant relationship to insulin, HOMA score, or testosterone ($p = 0.85$, $p = 0.81$, and $p = 0.88$, respectively) (►**Table 3**). No significant difference in TG was noted between these two groups.

Discussion

In the setting of increased cardiovascular risk factors and higher risk of CVD and venous thromboembolism in PCOS,[11,12] limited studies have evaluated the hemostatic system and its relationship to IR/hyperinsulinemia and hyperandrogenism as the key hormonal abnormalities in PCOS. Here, we have assessed the hemostatic system, evaluating both pro- and antithrombotic elements and overall hemostatic activity and explored hemostatic relationships

Table 2 Comparison of hemostatic variables between PCOS and controls

Variable	PCOS ($n = 107$)	Non-PCOS ($n = 67$)	p-Value	p-Value adjusted (age and BMI)
ADMA (μmol/L)[a] ($n = 134$)	0.70 (0.55–1.13)[b]	0.39 (0.30–0.48)[c]	< 0.0001	< 0.0001
PAI-1 (U/mL)[a] ($n = 134$)	4.80 (3.28–6.64)[b]	3.66 (2.46–4.37)[c]	< 0.0001	0.005
PF1 & 2 (pmol/L) ($n = 75$)	180.0 (154.0–255.5)[c]	236.0 (180.3–358.5)[d]	0.028	0.05
tPA (ng/mL) ($n = 91$)	11.35 (8.35–14.18)[c]	9.20 (7.40–10.40)[d]	0.025	0.545
Plasminogen activity (%) ($n = 128$)	118.39 ± 13.93[b]	108.46 ± 15.86[c]	< 0.0001	0.024
TG (nM/min) ($n = 123$)	2,179.38 ± 533.51[c]	2,071.62 ± 546.19[c]	0.271	0.495

Abbreviations: ADMA, asymmetric dimethyl arginine; PAI-1, plasminogen activator 1; PCOS, polycystic ovarian syndrome; PF1 & 2, prothrombin fragments 1 and 2; tPA, tissue plasminogen activator; TG, thrombin generation.
Note: Means ± SD and ANOVA for plasminogen activity and TG. Median (interquartile range) and p-values from log transformed data for ADMA, PAI-1, PF1 & 2, and tPA.
[a]Part data[15–17].
[b]$n = 74–83$.
[c]$n = 51–63$.
[d]$n = 22–31$.

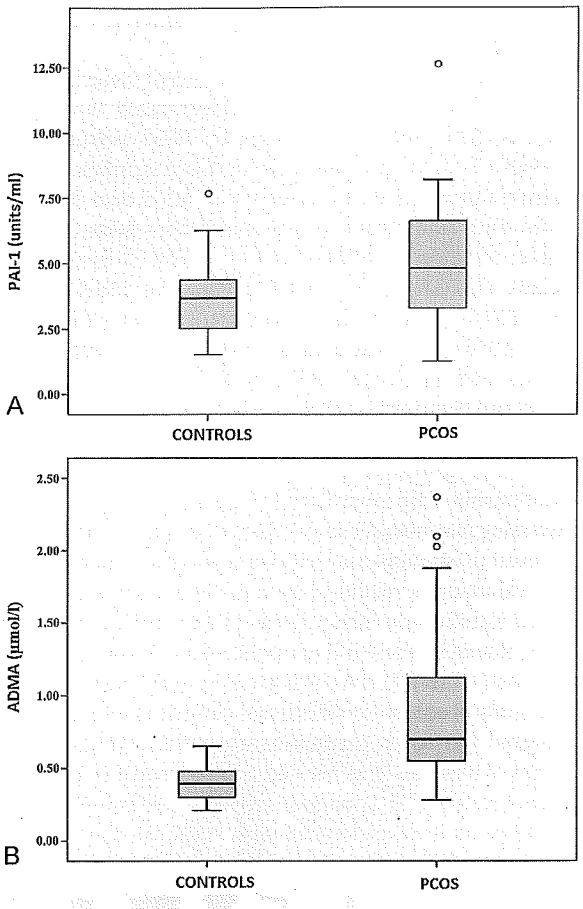

Fig. 1 Significant difference in endothelial function markers (A) PAI-1 (units/mL) and (B) ADMA (µmol/L) between PCOS and controls with higher levels for both markers noted in the PCOS group. (Part data[15–17]). ADMA, asymmetric dimethyl arginine; PAI-1, Plasminogen activator 1; PCOS, polycystic ovarian syndrome.[Q5]<>

Q5
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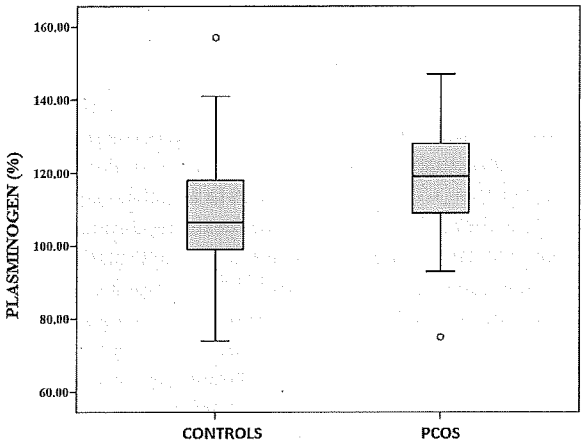

Fig. 2 Significant difference in fibrinolytic marker plasminogen (%) between PCOS and controls with higher levels noted in the PCOS group. PCOS, polycystic ovarian syndrome.

to metabolic and hormonal factors in women with PCOS compared with healthy controls. In this comprehensive study of the hemostatic system, our main findings include elevations in markers of endothelial dysfunction (ADMA and PAI-1) as well as reduced fibrinolysis (PAI-1 and plasminogen), with no significant difference in coagulation activity. Fasting insulin, HOMA score, and total testosterone levels showed a significant relationship with endothelial function markers ADMA and PAI-1.

Recent research has shown increased risk of venous thromboembolic diseases in PCOS with a 1.5-fold increased risk in comparison to women without PCOS.[12] Risks of CVD have also been reported as significantly higher in PCOS when compared with women in the general population.[11] It is well established that endothelial dysfunction can predispose to CVD.[21] In accordance with the literature,[20,22–35] our results support evidence for endothelial dysfunction in PCOS versus controls, reflected by

Table 3 After adjustment for age and BMI, evaluation of the effects/associations of metabolic (insulin and HOMA score) and hormonal (testosterone) parameters on the hemostatic parameters (differences) between PCOS and controls

Variable	Fasting Insulin		HOMA score		Testosterone	
	B coefficient and 95% CI	*p* (R^2) value	*B* coefficient and 95% CI	*p* (R^2) value	*B* coefficient and 95% CI	*p* (R^2) value
ADMA	0.70 (0.59 to 0.86)	< 0.01 (0.45)	0.70 (0.55–0.86)	< 0.01 (0.45)	0.75 (0.58–0.91)	< 0.01 (0.46)
PAI-1	0.22 (0.06–0.37)	< 0.01 (0.25)	0.22 (0.07–0.37)	< 0.01 (0.25)	0.24 (0.07–0.40)	< 0.01 (0.23)
Plasminogen	4.43 (−1.10 to 9.96)	0.16 (0.26)	4.43 (−1.10 to 9.92)	0.11 (0.26)	4.56 (−1.35 to 10.5)	0.13 (0.26)
PF1 & 2	−0.22 (−0.48 to 0.03)	0.08 (0.15)	−0.23 (−0.48 to 0.02)	0.07 (0.16)	−0.27 (−0.53 to 0.01)	0.05 (0.13)
tPA	0.02 (−0.15 to 0.18)	0.85 (0.28)	0.02 (−0.15 to 0.19)	0.81 (0.28)	− 0.02 (−0.21 to 0.18)	0.88 (0.26)

Abbreviations: ADMA, asymmetric dimethyl arginine; CI, confidence interval; HOMA, homeostasis model assessment; PAI-1, plasminogen activator 1; PCOS, polycystic ovarian syndrome; PF1 & 2, prothrombin fragments 1 and 2; tPA, tissue plasminogen activator.
Note: Data are assessed by multivariable linear regression models including age and BMI as covariates. A *p*-value of < 0.05 was considered significant.

elevated levels of ADMA and PAI-1 activity. These differences persist even after adjustment for age and BMI, which are among the strongest determinants of endothelial dysfunction (►**Table 2**). Linear regression analysis showed that the metabolic (fasting insulin level and HOMA score) and hormonal (testosterone) abnormalities in PCOS are related to the endothelial dysfunction (►**Table 3**). Other PCOS factors that may contribute to endothelial dysfunction include coexisting chronic inflammation, increased visceral adiposity, or elevated plasma levels of cholesterol and/or triglycerides.[9,36–40] Our results, however, do not indicate significant effects from increased waist circumference or lipid abnormalities on the aberrant endothelial function. The differences noted between PCOS and controls with regard to both PAI-1 activity and ADMA levels still persisted after adjustment for waist circumference ($p = 0.003$ and $p < 0.0001$, respectively; data not tabulated), cholesterol ($p < 0.0001$ for both; data not tabulated), LDL ($p < 0.0001$ for both; data not tabulated), and triglycerides ($p = 0.009$ and $p < 0.0001$, respectively; data not tabulated). Elevations in both PAI-1 and ADMA, and endothelial dysfunction pose significant CVD risks for women with PCOS, as endothelial dysfunction has been connected with an increased risk of future CVD events via contribution to atherosclerosis.[20] ADMA is also an independent marker for CVD morbidity and mortality.[34] Further research into the mechanisms and potential for amelioration of endothelial dysfunction in PCOS is needed.

In terms of coagulation activity, PF1 & 2 is a useful test for detecting a prothrombotic state and elevated levels of PF1 & 2 have been linked with a high risk of thrombosis.[41] To our knowledge, this current study is the first to assess PF1 & 2, to evaluate the presence of subclinical in vivo coagulation-mediated thrombosis, in women with PCOS. After adjustment for age and BMI, the differences in PF1 & 2 levels between women with and without PCOS were only of borderline significance (►**Table 2**). HOMA score and insulin were not associated with PF1 & 2 levels with a borderline link demonstrated between the hemostatic marker and testosterone (►**Table 3**). Further study with larger sample sizes may be warranted here. Our study also investigated TG in PCOS, a test that assesses the formation of thrombin following activation of the coagulation cascade by tissue factor. TG is a global hemostatic assay that looks at both the procoagulation effects of the coagulation cascade, but also at inhibitory substances that prevent the generation of thrombin, mainly tissue factor pathway inhibitor, antithrombin (AT) and protein C.[42] TG is very sensitive to variations of prothrombin and AT, as well as to the activity of activated protein C system.[42] Our study showed no significant difference between PCOS and controls with regard to TG (►**Table 2**). Although the PF1 & 2 and TG results do not suggest an overall prothrombotic state, the TG assay does not take into account the effects of fibrinolysis, the markers of which mainly act upon fibrinogen and fibrin, activated downstream from thrombin in the coagulation cascade. The TG assay also does not assess the inhibitory effects of PAI-1 that counteracts the effects of the fibrinolytic system. However, based on our current study, isolated coagulation activation does not appear to be increased in women with PCOS compared with healthy controls.

In terms of fibrinolysis, hypofibrinolytic capacity through net reduced fibrinolysis was demonstrated via both the plasminogen and PAI-1 results in PCOS versus controls (►**Table 2**). In the current study, we noted significantly higher plasminogen activity in women with PCOS than in controls, even after adjustment for age and BMI (►**Table 2**). Adjustments for waist circumference ($p \leq 0.0001$[Q6]; data not tabulated), cholesterol ($p < 0.0001$; data not tabulated), LDL ($p < 0.0001$; data not tabulated), and triglycerides ($p < 0.0001$; data not tabulated) also did not affect the outcomes and significant differences still persisted between PCOS and controls for plasminogen activity. Linear regression analysis revealed that the metabolic (insulin and IR) and hormonal (testosterone) factors do not have any significant relationship with plasminogen activity (►**Table 3**). Studies assessing plasminogen in PCOS have revealed either similar[43] or lower plasminogen activity compared with controls[44] with no evaluation of relationship to metabolic markers. We also noted significantly higher levels of tPA in PCOS versus controls. However, these did not persist after adjustment for age and BMI (►**Table 2**). tPA levels are elevated in PCOS,[26,27,45] but one study observed no differences compared with controls.[22] Elevated tPA levels predominantly reflect tPA–PAI-1 complexes, which can be generated as a consequence of elevated PAI-1 levels.[22,26] It is unclear whether tPA levels are linked with insulin levels in women with PCOS. Lin and Yongmei[26] showed that there is a relationship, but our study (►**Table 3**) and others[45] do not support this conclusion.

The elevated plasminogen activity in PCOS versus controls noted in our study most likely reflects a prevention of conversion to plasmin rather than enhanced fibrinolytic capacity (though further investigation would need to be undertaken to confirm this). Elevated PAI-1 levels may contribute in part in preventing the conversion of plasminogen to active plasmin, though other factors may also be responsible. Linear regression analyses supports this notion and shows a significant effect of PAI-1 on plasminogen after adjustment for age and BMI ($p = 0.01$; data not tabulated). PAI-1 acts to inactivate tPA thereby reducing conversion of the proenzyme plasminogen to its active and functional role (plasmin). The Stachrom colorimetric assay used to assess plasminogen in our study uses a plasmin substrate, and therefore will measure both plasminogen and plasmin present in plasma samples. Plasmin in fluid phase, however, is almost immediately converted to plasmin–α2 antiplasmin or neutralized by α2 macroglobulin.[46,47] α2 antiplasmin, a principal plasmin inhibitor, is present in the plasma at high enough concentrations to immediately neutralize up to 50% of the available plasmin.[46,47] The plasmin–α2 antiplasmin reaction is the most rapidly known protein–protein interaction.[46] The remainder of the 50% is neutralized by α2 macroglobulin. Our study has shown that the aberrant lipid profile (increased cholesterol, LDL, and triglycerides), frequently found in women with PCOS, is not the contributing factor (or sole contributing factor) to the elevated plasminogen activity or PAI-1 activity. Further studies would need to be undertaken to confirm this. Recently, plasminogen has been

shown to play several biological roles, apart from fibrinolytic capacity including a role in the inflammatory process and atherosclerosis.[46,47] The mechanism of action or role of plasminogen in inflammation is yet to be defined; however, elevated activity would then not be surprising in a syndrome where increased inflammatory markers, including C-reactive protein levels, have been well documented.[38,48,49] Future research is needed to explore the physiology of plasminogen in PCOS.

Limitations of our study include the analyses of hemostatic markers in women recruited for a study designed to address another research question. However, methods used in the original study were rigorous, women were well characterized, and samples were well prepared. We also acknowledge that not all end points were measured on all samples with inadequate sample available in some cases. Also, there was a lack of assessment of marker/s that evaluate primary hemostasis, including the potential for an increased or aberrant platelet function or number. Finally, the cross-sectional design of the study precludes the establishment of any firm conclusion about causality. Strengths include a systematic approach to hemostatic assessment, a well-characterized population on no medications, adjustment for age and BMI, and prior sample size calculation to attain adequate statistical power to detect statistical significance.

In conclusion, our case–control study shows no coagulation abnormalities in PCOS compared with controls but clear evidence of impaired capacity for fibrinolysis. With increased activity of the inhibitor of fibrinolysis, PAI-1, as well as increased plasminogen (perhaps arising due to reduced conversion to active plasmin), a mild-to-moderate hypofibrinolytic state occurs. The reasons for these changes are unclear. The mechanisms driving this hypofibrinolytic state may be linked to abnormal endothelial function with significant effects from the aberrant hormonal (hyperandrogenemia) and metabolic (IR/hyperinsulinemia) changes in PCOS. Further studies are needed to confirm these cross-sectional results, including studies that therapeutically modulate the hemostatic system in PCOS. A hypofibrinolytic state in this condition represents a further CVD risk factor and risk marker in PCOS, which may contribute to the development of arterial and venous thromboembolic diseases.

Conflict of Interest

All the authors have no conflict of interest to declare.

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