

ORIGINAL ARTICLE

Cardiovascular risk in women with polycystic ovarian syndrome (PCOS)

A S T Bickerton, N Clark, D Meeking, K M Shaw, M Crook, P Lumb, C Turner, M H Cummings

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Aims: Studies have suggested that polycystic ovary syndrome (PCOS) is associated with increased cardiovascular risk. The aim of this study was to examine cardiovascular risk profiles in women with PCOS compared with healthy age and weight matched control subjects using novel biochemical and biophysical markers.

Methods: After ethics committee approval, 11 women with PCOS and 12 controls were recruited (mean age, 32; SD, 6.5 years; mean body mass index (BMI), 33.1; SD, 5.9 kg/m²). Serum was analysed for lipid and lipoprotein profile (total and high density lipoprotein cholesterol, triglycerides, apolipoprotein B-100, apolipoprotein A1, lipoprotein (a)), and sialic acid, fibrinogen, homocysteine, and C reactive protein (CRP) concentrations. Endothelial function was also assessed by a standard venous occlusion plethysmography technique to measure reactive hyperaemic forearm blood flow (RH), and expressed as per cent increase from baseline.

Results: There were no significant differences in glucose, lipid, or lipoprotein concentrations between the two groups. Furthermore, sialic acid (PCOS: mean, 70.5; SD, 149 mg/litre; controls: mean, 71.3; SD, 112 mg/litre), fibrinogen (PCOS: mean, 3.1; SD, 1.0 g/litre; controls: mean, 3.3; SD, 0.7 g/litre), CRP (PCOS: mean, 4.6; SD, 4.2 mg/litre; controls: mean, 5.4 SD, 5.5 mg/litre), and RH (PCOS: mean, 158.7; SD, 135.5%; controls: mean, 200.1; SD, 114.2%) were similar.

Conclusions: There were no differences in surrogate markers of the processes linked to enhanced cardiovascular risk between patients with PCOS and weight matched controls.

See end of article for authors' affiliations

Correspondence to:
Dr A Bickerton, Academic
Department of Diabetes
and Endocrinology, Queen
Alexandra Hospital,
Cosham, Portsmouth PO6
3LY, UK; alex.bickerton@
oxlip.ox.ac.uk

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There is increasing evidence that patients with polycystic ovary syndrome (PCOS) have increased cardiovascular risk compared with age matched controls. It has been estimated that myocardial infarction is seven times more likely in patients with PCOS,¹ and cardiac catheterisation studies have shown more extensive coronary artery disease in these patients than in women with normal ovaries.² Furthermore, significant subclinical carotid atherosclerosis has been demonstrated on carotid artery ultrasound in women with PCOS.^{3 4}

This increased cardiovascular risk is probably the result, in part, of the metabolic disturbance associated with PCOS. Dyslipidaemia, diabetes, and obesity are all potent cardiovascular risk factors that tend to cluster in women with PCOS. However, it is not known whether the increased cardiovascular risk seen in PCOS is mediated through obesity per se or is independent of body mass index (BMI) and the result of other metabolic factors.

“Dyslipidaemia, diabetes, and obesity are all potent cardiovascular risk factors that tend to cluster in women with polycystic ovary syndrome”

In recent years, interest has grown in novel biochemical and biophysical markers of cardiovascular risk. C reactive protein (CRP) has been shown to be a good predictor of vascular events. In addition to being a marker of inflammation, there is evidence that CRP may have a direct role in atherogenesis via adhesion molecule expression, complement activation, and mediation of low density lipoprotein (LDL) uptake by macrophages.⁵ Sialic acid has been proposed as a predictor of cardiovascular mortality, although the reason for this association remains unclear.^{6 7} Similarly, raised fibrinogen⁸ and homocysteine^{9 10} concentrations have been

associated with an increased risk of ischaemic heart disease and atherosclerosis. Fibrinogen may promote cardiovascular disease by a variety of mechanisms including increased blood viscosity, thrombus formation, or platelet aggregation.⁸ Homocysteine is postulated to damage the vascular endothelium directly.¹¹ Finally, microalbuminuria is a well established predictor of cardiovascular morbidity in the diabetic, and possibly the non-diabetic, population.^{12 13} Endothelial dysfunction is thought to occur at a very early stage of atherosclerotic plaque development, and is an early marker for atherosclerosis.¹⁴ It is now possible to investigate endothelial dysfunction in vivo using several techniques, including venous plethysmography to examine reactive hyperaemia (RH).¹⁵

To date, few studies investigating processes linked to increased cardiovascular risk in PCOS have collectively used novel biophysical and biochemical techniques. Such results have been conflicting, in part because of the inadequately matched characteristics of the PCOS and control groups (particularly with reference to weight). There have been no studies assessing RH responses in this population. Consequently, we set out to compare these biochemical markers between women with PCOS and an age and weight matched control group.

AIM

To examine whether women with PCOS exhibit endothelial dysfunction or increased susceptibility to atherothrombosis or vascular inflammation independent of BMI.

Abbreviations: ACR, albumin-creatinine ratio; BMI, body mass index; CRP, C reactive protein; CV, coefficient of variation; HDL, high density lipoprotein; LDL, low density lipoprotein; PCOS, polycystic ovary syndrome; RH, reactive hyperaemia

SUBJECTS AND METHODS

Subjects

Our study comprised two groups of women (11 with PCOS and 12 controls) between the ages of 18 and 50 years old (premenopausal women). Patients with PCOS were recruited from the endocrine and gynaecological clinics of Portsmouth Hospitals, UK. PCOS was defined as an ovarian ultrasound diagnosis, with a history of menstrual disturbance (amenorrhoea/oligomenorrhoea), in association with raised serum testosterone and luteinising hormone, and a low or normal follicle stimulating hormone.¹⁶ Control subjects were healthy volunteers with a normal menstrual cycle and with no clinical or biochemical features of hyperandrogenism, thereby excluding the diagnosis of PCOS in this group. Controls were recruited from hospital staff and were matched with the patients for age and weight. Local ethical committee approval was given before the start of the study. Written consent was obtained from every subject. A full medical, family, and drug history was taken. The Rose questionnaire¹⁷ was used to screen for cardiovascular disease. A full clinical examination, including measurement of BMI, waist circumference, and waist to hip ratio, and an electrocardiogram were performed. Subjects were excluded if there was clinical (Rose questionnaire) or electrocardiographic evidence of coronary artery disease, a family history of coronary artery disease, a history of smoking, or concurrent oestrogen, antihypertensive, or lipid lowering medication. Therefore, we deliberately chose a group of patients with PCOS who had no cardiovascular risk factors that may have confounded the interpretation of the results.

Laboratory tests and endothelial function

A fasting blood sample was drawn on day five of each subject's menstrual cycle for plasma hormone profile (luteinising hormone, follicle stimulating hormone, oestradiol, testosterone, free androgen index, androstenedione), CRP, glucose, sialic acid, fibrinogen, homocysteine, and lipid profile (total cholesterol, triglyceride and lipoproteins; high density lipoprotein (HDL) cholesterol, apolipoprotein B-100, apolipoprotein AI, and lipoprotein (a)). An early morning urine sample was sent for albumin-creatinine ratio (ACR) measurement.¹⁸ Lipid and lipoprotein measurements were analysed by automated techniques on a Cobas Fara 2 analyser and fibrinogen and high sensitivity CRP on a Behring BN2 analyser (interassay coefficient of variation (CV), 2.7% and 4.4% for fibrinogen and CRP, respectively). Sialic acid was measured by a previously described colorimetric method on a Cobas Fara 2 analyser (interassay CV, 3.1%).¹⁹ Homocysteine was measured by stable isotope dilution and electro spray tandem mass spectrometry (interassay

CV, 7% at 12 μ mol/litre).²⁰ The intraassay CVs for all the biochemical analyses were less than 10%.

Endothelial function was assessed using a standard venous occlusion plethysmography technique to measure forearm blood flow.²¹ Forearm blood flow was measured at rest (baseline), and during RH. To induce RH, blood flow to the forearm was prevented by the inflation of a cuff on the upper arm to suprasystolic pressure (20 mm Hg above systolic blood pressure).¹⁵ The duration of arterial occlusion was five minutes. Ten seconds after the release of the cuff, the forearm blood flow was remeasured and recorded continuously for two minutes, or until the forearm blood flow returned to baseline values. RH was taken as the first three recordings of blood flow after cuff release.

Statistical analysis

Unpaired *t* tests were used to compare biochemical and biophysical variables in both groups. Pearson's correlation coefficients were calculated to assess correlations between subject characteristics and the novel markers of cardiovascular risk measured. Significance was taken as 5%.

RESULTS

Table 1 shows the clinical characteristics and hormone profiles of the two groups. There were no significant differences between the groups in age, BMI, waist circumference, or waist to hip ratio. All subjects were above ideal body weight (BMI > 25). Table 2 shows the results of the biochemical markers of cardiovascular risk and the RH forearm blood flow. The results were normally distributed and are expressed as mean (SD). There were no significant differences in lipid or lipoprotein concentrations between the two groups. Furthermore, sialic acid, fibrinogen, homocysteine, and CRP were similar. Although there was a significant difference in fasting blood glucose between the two groups neither group was diabetic. Basal forearm blood flow and RH, expressed as percentage change in blood flow, were not significantly different between the two groups. There were no significant correlations between RH and BMI, weight, waist to hip ratio, or the novel biochemical risk markers in either group.

DISCUSSION

The main findings of our study were that there were no differences in endothelial function, as measured by RH, or any of the biochemical parameters that assess atherothrombosis risk or vascular inflammation in women with PCOS compared with age and weight matched controls.

Although it has been suggested that the increased cardiovascular risk seen in PCOS may be the result, in part, of endothelial dysfunction,²² we found no differences in

Table 1 Baseline characteristics and hormone profiles of subjects

	PCOS (n = 11)	Controls (n = 12)	p Value
Age (years)	33.5 (6.1)	30.7 (6.7)	0.31
Weight (kg)	92.4 (21.1)	84.4 (12.4)	0.28
BMI (kg/m ²)	35.3 (6.8)	31.0 (4.1)	0.08
Waist circumference (cm)	102.4 (19.6)	93.5 (13.5)	0.21
Waist to hip ratio	0.9 (0.1)	0.8 (0.1)	0.48
Systolic BP (mm Hg)	125 (17)	120 (11)	0.28
Diastolic BP (mm Hg)	77 (12)	67 (10)	0.06
Testosterone (nmol/l)	2.8 (1.1)	1.8 (0.5)	0.02
Free androgen index	10.6 (5.5)	3.9 (1.5)	0.004
SHBG (nmol/l)	28.5 (6.5)	53.1 (15.5)	0.0003
LH (IU/l)	11.0 (3.5)	4.2 (2.4)	0.004
FSH (IU/l)	5.3 (1.2)	3.7 (1.8)	0.03

Values are mean (SD).

BMI, body mass index; BP, blood pressure; FSH, follicle stimulating hormone; LH, luteinising hormone; PCOS, polycystic ovary syndrome; SHBG, steroid hormone binding globulin.

Table 2 Biochemical markers of cardiovascular risk and RH forearm blood flow

	PCOS (n = 11)	Controls (n = 12)	p Value
Glucose (mmol/l)	4.7 (0.3)	5.1 (0.4)	0.02
ACR (mg/mmol)	2.0 (2.4)	2.1 (5.3)	0.93
Cholesterol (mmol/l)	5.6 (1.4)	4.7 (0.9)	0.11
Triglyceride (mmol/l)	1.8 (1.3)	1.0 (0.5)	0.09
HDL (mmol/l)	1.1 (0.4)	1.2 (0.2)	0.38
Apolipoprotein A1 (g/l)	1.4 (0.3)	1.5 (0.2)	0.24
Apolipoprotein B-100 (g/l)	1.1 (0.4)	0.9 (0.3)	0.21
Lipoprotein a (g/l)	0.05 (0.08)	0.1 (0.11)	0.22
Fibrinogen (g/l)	3.1 (1.0)	3.3 (0.7)	0.55
Sialic acid (mg/l)	705 (149)	713 (112)	0.88
CRP (mg/l)	4.6 (4.2)	5.4 (5.5)	0.73
Homocysteine (μ mol/l)	10.5 (3.3)	9.4 (2.0)	0.35
Basal forearm blood flow (ml/100 ml/min)	3.2 (6.6)	1.0 (0.6)	0.27
RH blood flow (ml/100 ml/min)	9.5 (21.7)	2.8 (1.8)	0.3
% Change in blood flow	158.7 (135.5)	200.1 (114.2)	0.44

ACR, albumin-creatinine ratio; CRP, C reactive protein; HDL, high density lipoprotein; PCOS, polycystic ovary syndrome; RH, reactive hyperaemia.

endothelial function between women with PCOS and age and weight matched controls. Two previous studies have examined endothelial function in PCOS. Mather *et al*, using brachial artery ultrasound, found no evidence of endothelial dysfunction in healthy women with PCOS compared with age but not weight matched controls.²³ Conversely, Paradisi *et al*,²² using leg blood flow responses to the vasodilator methacholine chloride, compared age and weight matched controls with obese patients with PCOS. They were able to demonstrate endothelial dysfunction in the patients with PCOS compared with the control group. The reasons for these discrepant findings remain unclear. It is possible that the slightly differing exclusion criteria and baseline characteristics of the subjects in these studies may have affected the results. Paradisi *et al* did not exclude smokers and neither study was performed at a predetermined point in the subjects' menstrual cycle. Another possible contributory factor to the discordant results is insulin resistance. Insulin resistance, hyperinsulinaemia, and obesity are all seen in patients with PCOS.²⁴ However, the association between these metabolic factors is not straightforward because insulin resistance is seen equally in both obese and non-obese women with PCOS.²⁴⁻²⁵ Peripheral insulin resistance is linked to both atherosclerotic processes²⁶ and, in the healthy obese, to endothelial dysfunction.²⁷ Therefore, it is conceivable that endothelial dysfunction is only present in insulin resistant women with PCOS. This question was addressed in the Paradisi and Mather studies²²⁻²³; however, the results were conflicting and the issue remains unresolved.

"We found no differences in endothelial function between women with polycystic ovary syndrome and age and weight matched controls"

An alternative explanation that has been postulated for the increased cardiovascular risk in PCOS is hyperandrogenism. Possible underlying pathophysiological mechanisms include a correlation between free testosterone and systolic blood pressure,²⁸ and a link between increased androgens and abnormal lipid metabolism.²⁹ However, the association between hyperandrogenism and cardiovascular risk is not universally accepted.²⁹

None of the novel surrogate biochemical markers of cardiovascular risk was raised in our women with PCOS. To our knowledge, there are no data on sialic acid concentrations or ACRs in patients with PCOS. CRP has previously been shown to be higher among patients with PCOS than in controls.³⁰ Unfortunately, these results are confounded by the

observation that both groups contained smokers, and smoking is associated with raised CRP concentrations.³¹⁻³² One study of homocysteine showed raised homocysteine concentrations in non-obese (BMI < 30 kg/m²) patients with PCOS compared with controls.³³ However, it is difficult to compare this with our present study because our subjects were obese. Fibrinogen concentrations in patients with PCOS have been assessed previously, with some discordance of results. Atiomo *et al* found higher concentrations of fibrinogen in patients with PCOS compared with controls.³⁴ However, the authors have questioned the interpretation of these data because the participants of the study were not weight matched. Two other studies have shown no increases in fibrinogen concentrations in non-obese women with PCOS compared with weight matched controls.³⁵⁻³⁶ Although our study participants were more obese than those of Atiomo *et al*, our findings were similar. Dahlgren *et al* found a positive correlation between BMI and fibrinogen concentrations,³⁵ and it may be that our study subjects were not sufficiently obese to demonstrate raised fibrinogen concentrations.

We found no differences in the lipoprotein parameters of our patients with PCOS compared with the control group. Several previous studies have shown high triglyceride and low HDL values in women with PCOS compared with controls, a pattern that is typical of insulin resistance.³⁶⁻³⁷ This combination of lipid parameters is known to be particularly atherogenic and, in women with PCOS, cannot be explained solely by obesity. One possible explanation is reduced insulin sensitivity. This is supported by the observation that the typical disturbance of lipid parameters seen in PCOS (high triglycerides, high LDL, and low HDL) is associated with the presence of insulin resistance.³⁷⁻³⁸

There were limitations to our study. First, we did not directly measure insulin sensitivity. However, because our patients were matched for weight and displayed no other characteristics typical of the insulin resistant state (dyslipidaemia, hypertension, and abnormal fasting glucose), the

Take home messages

- There have been reports of increased cardiovascular risk in subjects with polycystic ovary syndrome (PCOS)
- However, we found no differences in surrogate markers of the processes linked to enhanced cardiovascular risk between patients with PCOS and weight matched controls

PCOS and control groups were probably similarly insulin resistant. The second limitation of our study is that we did not examine lean controls. However, the homocysteine, fibrinogen, ACR, sialic acid, and CRP values in our study groups were similar to those seen in control groups matched for weight from other studies. If it is obesity rather than PCOS that determines the concentrations of cardiovascular risk markers then we would expect lower concentrations in lean patients.

In conclusion, none of the markers associated with increased risk of atherothrombosis, endothelial dysfunction, or vascular inflammation differed between women with PCOS and weight matched controls.

Authors' affiliations

A S T Bickerton, N Clark, D Meeking, K M Shaw, M H Cummings, Academic Department of Diabetes and Endocrinology, Queen Alexandra Hospital, Cosham, Portsmouth PO6 3LY, UK
M Crook, P Lumb, C Turner, Department of Chemical Pathology, Kings College London, London SE5 9RS, UK

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