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Microvascular dysfunction in women with polycystic ovary syndrome

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BACKGROUND: Polycystic ovary syndrome (PCOS) is associated with multiple cardiovascular risk factors and an increased prevalence of arterial dysfunction. However, microvascular dysfunction in PCOS has not been assessed. METHODS: Subjects comprised 12 women with PCOS and 12 age-matched controls with normal ovaries. Microvascular function was assessed by observing forearm skin microvascular erythrocyte flux responses, to cumulative iontophoretic doses of 1% (w/v) acetylcholine (ACh) and 1% (w/v) sodium nitroprusside (SNP), using laser Doppler imaging. RESULTS: Basal microvascular perfusion was comparable in PCOS and controls. The increase in skin microvascular perfusion in response to ACh was however generally blunted in PCOS women ($P = 0.018$). Peak ACh-induced erythrocyte flux was also less ($p < 0.04$) in PCOS women (125.1 ± 21.7, i.e. 5.3-fold basal flux) than in controls (200.8 ± 28.5, i.e. 8.3-fold basal flux). Analysis of covariance indicated this effect was unrelated to differences in body mass index or serum testosterone but serum insulin may be a weak confounder. No differences were noted between the PCOS and control groups in their response to SNP. CONCLUSION: Despite its limited sample size studied, this is the first demonstration that women with PCOS exhibit microvascular endothelial dysfunction, indicated by an inhibited vasodilatory response to ACh.

Key words: acetylcholine/endothelium/erythrocyte flux/microcirculation/polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is associated with a range of cardiovascular risk factors including central obesity, dyslipidaemia and insulin resistance (Wild *et al*., 1985; Conway *et al*., 1992; Talbott *et al*., 1995). Consequently it has been estimated that women with the syndrome have a 7.4-fold increased risk of developing myocardial infarction (Dahlgren *et al*., 1992). This apparently straightforward relationship was challenged by the first study of mortality in women with PCOS (Pierpoint *et al*., 1998) which showed no increase in deaths from heart disease. This result was surprising in view not only of the clinical and metabolic profile of these women, but also that the results of the same study demonstrated higher than expected mortality from diabetes, a condition strongly associated with cardiovascular disease.

An association between coronary artery disease and PCOS was supported by the observation that the prevalence of coronary artery disease is 21% higher in women with the syndrome than in controls (Wild *et al*., 1990). Guzick (1996) and Talbott *et al*. (2000) reported increased intima-media thickness (IMT) in women with PCOS aged ≥45 years, compared to controls. Using an automatic arterial wall tracking system, we have reported increased IMT and decreased vascular compliance, in the carotid and femoral arteries in PCOS women aged <35 years (Lakhani *et al*., 2002, 2004). Furthermore, the response of femoral artery blood flow to intra-arterial methacholine chloride administration is inhibited in PCOS sufferers, suggesting compromise of endothelial nitric oxide (NO) production/release (Paradisi *et al*., 2001). However, it is not known whether the effects of PCOS are limited to arteries, or whether PCOS has a general vascular effect.

There is an urgent need to resolve the nature and degree of cardiovascular dysfunction in women with PCOS, given that coronary heart disease is the leading cause of death in women aged >50 years in developed counties, and that the syndrome is one of the commonest endocrinopathies in humans, affecting up to 12% of women (Dunaif and Thomas, 2001). This pilot cross-sectional study was designed to investigate endothelial and vascular smooth muscle function in the microcirculation of women with PCOS and control women with normal ovaries.

Materials and methods

A cross-sectional study was designed using two groups of women aged 18–35 years, recruited from the North Middlesex Hospital, London, UK. The PCOS group comprised 12 women attending the Reproductive

Endocrinology and Gynaecology clinics, who exhibited bilateral polycystic ovaries on transvaginal scan (Fox, 1999) and clinical features of menstrual irregularity, infertility, oligomenorrhoea (intermenstrual interval >35 days) or biochemical features of the syndrome with increased serum testosterone with/without hirsutism (score >7) (Ferriman and Gallwey, 1961) and/or increased serum concentrations of LH (Conway *et al*., 1989; Franks, 1989). The control group comprised 12 healthy women with normal ovaries on ultrasound, regular menstrual cycles (intermenstrual intervals of 21–35 days), no evidence of hyperandrogenaemia (hirsutism or acne), and who had not sought treatment for menstrual disturbance, infertility or hirsutism at any time. Women who smoked, had respiratory or cardiovascular disease, or who were on medication which could influence vascular resistance, such as oral contraceptives and aspirin, were excluded from both groups. The study was approved by the Local Research Ethics Committee, and written consent was obtained from each woman investigated.

Oligomenorrhoeic and control cases were studied at a time between days 4 and 7 of the menstrual cycle; there was no special timing for amenorrhoeic women. An overnight-fasted peripheral blood sample was obtained and serum prepared by centrifugation, then stored at −20°C until assayed for serum analytes. Patient height and weight were measured and used to calculate the body mass index (BMI). The minimum waist measurement between the pelvic brim and the costal margin and the maximum hip measurement at the greater trochanters were also taken and used to calculate the waist:hip ratio.

Iontophoresis and forearm skin microvascular flow measurements were performed (at ~17:00, i.e. 4 h post-prandially), with the women sitting comfortably in an armchair in a quiet room with an ambient temperature of 22±1°C. During a 20 min acclimatization period before iontophoresis, the volar aspect of the right forearm was gently cleaned with an alcohol wipe and swabbed with deionized water. Blood pressure and pulse were measured on the left arm at 2 min intervals throughout the assessment period using an automatic Dinamap device (Critikon Inc., Tampa, FL, USA).

Drug iontophoresis utilizes a potential difference between two chambers, adhered to the skin and filled with ionized drug solution, to induce drug ion migration into the skin. Drug dose is proportional to the duration and magnitude of the current (Morris *et al*., 1995). After acclimatization, therefore, two perspex iontophoresis chambers (ION1; Moor Instrument Ltd, Axminster, UK) were attached on the right forearm, 5 cm below the medial condyle with ≥10 cm between them, by means of double-sided adhesive rings avoiding hair, broken skin and superficial veins. The anodal chamber was filled with 0.25 ml 1% (w/v) acetylcholine chloride (ACh; Sigma–Aldrich Chemicals, UK) and the cathodal chamber with 0.25 ml 1% (w/v) sodium nitroprusside (SNP; Sigma–Aldrich Chemicals, UK). Simultaneous drug delivery from each chamber was controlled by a constant current iontophoresis controller (MIC-1e; Moor Instruments Ltd). Forearm cutaneous microvascular erythrocyte flux, as a measure of perfusion, was measured using a double chamber laser Doppler perfusion monitor (Laser Doppler DRT4 system; Moor Instruments Ltd), each chamber having a central laser Doppler probe.

Drug iontophoresis and forearm cutaneous microvascular erythrocyte flux were assessed using a cumulative dose–response protocol (Morris *et al*., 1995) with relatively low currents to limit galvanic effects which may cause non-specific vasodilation (Berghoff *et al*., 2002). Baseline erythrocyte flux was measured for 100 s without current, i.e. no drug was iontophoresed. This was followed by drug delivery at 10, 15 and 20 mA, sequentially, each for 100 s, followed by 800 s at zero current. Microvascular flux was continually assessed by laser Doppler; mean erythrocyte flux estimates were collected at the end of the baseline period, at 50 s intervals during iontophoresis, and at 100 s intervals thereafter. Flux responses to ACh or SNP were expressed as the ratio of flux at time '*t*' versus baseline flux at 100 s. Flux ratios were $\log_{10}$-transformed for statistical analysis to ensure homogeneity of variance.

Serum analytes, detailed in Table I, were analysed using standard methods in the Biochemistry Department, North Middlesex Hospital, London, UK. Serum LH, FSH, testosterone, estradiol and prolactin were measured using an ACS 180 Automated Chemiluminescence System with immunoassay kits (Ciba-Coring Diagnostics Ltd, UK), serum sex hormone-binding globulin by Chemiluminescent Immunolite kit (Diagnostic Products Corp., Los Angeles, CA, USA), cholesterol, triglycerides and high density lipoprotein by enzymatic–colorimetric assay kits (Boehringer Mannheim Immunodiagnostics Systems, UK), and glucose using an automated enzyme-linked immunosorbent assay (ES700; Boehringer Mannheim Immunodiagnostics Systems, UK).

Statistical analysis was performed using the program SPSS version 11 for Windows. Analysis of variance was used to test differences in microvascular flux responses and physical, endocrine and biochemical parameters, between the PCOS and control groups. The effect of confounding variables on flux response was assessed by analysis of covariance (ANCOVA). $P < 0.05$ was considered significant. Data are expressed as mean ± SD unless otherwise noted.

Results

There was no significant difference in age between the PCOS and control groups, neither were differences apparent in systolic and diastolic blood pressures, and heart rate. These latter parameters were also comparable pre- and post-iontophoresis.

Table I. Physical, endocrine and biochemical parameters in polycystic ovary syndrome (PCOS) and control subjects, pre- and post-iontophoresis where appropriate

Variable	PCOS	Control	*P* between groups
No. of women	12	12	
Age (years)	30.3 ± 4.4	27.7 ± 4.8	0.18
Body mass index (kg/m^2)	31.1 ± 7.1	24.1 ± 3.5	0.006*
Waist:hip ratio	0.78 ± 0.06	0.76 ± 0.04	0.58
Pre-systolic blood pressure (mmHg)	115 ± 14	110 ± 7	0.33
Pre-diastolic blood pressure (mmHg)	67 ± 12	64 ± 8	0.48
Pre-heart rate (beats/min)	73 ± 8	66 ± 10	0.10
Post-systolic blood pressure (mmHg)	117 ± 17	111 ± 12	0.32
Post-diastolic blood pressure (mmHg)	68 ± 11	67 ± 7	0.93
Post-heart rate (beats/min)	71 ± 10	65 ± 9	0.16
LH (IU/l)	10.8 ± 6.6	4.5 ± 3.4	0.09
FSH (IU/l)	6.3 ± 2.30	5.8 ± 2.60	0.69
Testosterone (nmol/l)	2.35 ± 0.78	1.70 ± 0.78	0.05*
Sex-hormone binding globulin (nmol/l)	53.7 ± 47.5	57.5 ± 26.4	0.83
Prolactin (mIU/l)	250.5 ± 113.0	273.4 ± 103.0	0.65
Estradiol (pmol/l)	257.3 ± 152.0	198.4 ± 126.0	0.35
Glucose (mmol/l)	5.05 ± 0.45	4.98 ± 0.50	0.74
Insulin (mIU/l)	20.3 ± 11.3	11.4 ± 7.8	0.06
Total cholesterol (mmol/l)	4.70 ± 0.97	4.51 ± 0.90	0.62
High-density lipoprotein (mmol/l)	1.40 ± 0.41	1.54 ± 0.41	0.40
Low-density lipoprotein (mmol/l)	2.99 ± 1.10	2.57 ± 0.85	0.31
Triglycerides (mmol/l)	1.02 ± 0.39	0.88 ± 0.36	0.40

*Significant.

Mean serum testosterone and BMI were elevated in women with PCOS compared with controls (Table I). Serum insulin (overnight-fasted) and LH levels also tended to be elevated in PCOS women, albeit significance was marginal ($P < 0.06$ and $P < 0.09$ respectively).

The coefficients of variation of microvascular perfusion measurements after ACh iontophoresis at 10, 15 and 20 μA, were 9.5, 10.8 and 12.6% respectively. Corresponding values for SNP were 16.6, 9.1 and 5.6% respectively. This indicated a reasonable degree of reproducibility in the estimates of skin microvascular blood flow. Basal microvascular perfusion was not significantly different in PCOS and control women (23.6 ± 3.4 versus 24.2 ± 4.2, respectively, all erythrocyte fluxes are in arbitrary units). The erythrocyte flux response to ACh, however, was generally decreased in women with PCOS, relative to controls ($P = 0.018$; Figure 1), in the absence of an effect on SNP responsiveness (data not shown). The peak ACh-induced erythrocyte flux occurred between 400 and 600 s in both groups, i.e. at the end of the 20 μA iontophoresis period, and was significantly less ($P < 0.04$) in the PCOS group (125.1 ± 21.7 at 500 s, i.e. 5.3-fold increase versus basal flux) than in controls (200.8 ± 28.5 at 500 s, i.e. 8.3-fold increase versus basal flux).

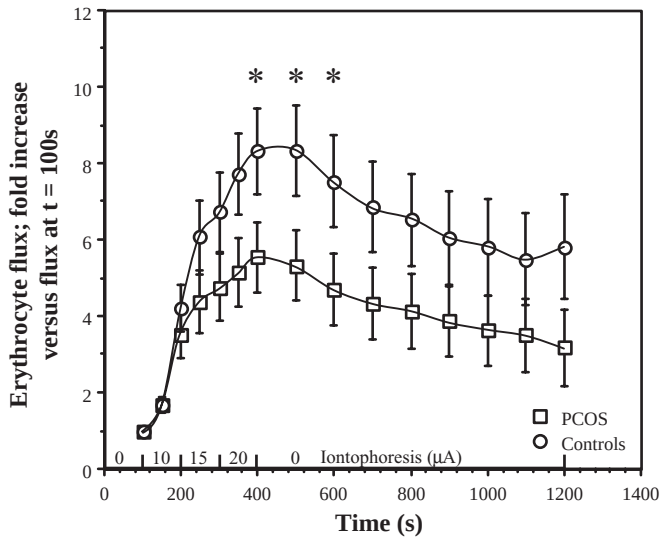

Figure 1. The fold increase (mean ± SEM) in forearm cutaneous microvascular erythrocyte flux in response to acetylcholine iontophoresis, relative to the baseline flux at 100 s, in polycystic ovary syndrome (PCOS, $n = 12$) and control ($n = 12$) women. The flux increase versus baseline was significantly less (*$P < 0.05$) in PCOS women than in controls.

BMI, serum testosterone and insulin levels varied, or tended to vary, between the control and PCOS groups. This variability might underlie the observed difference in erythrocyte flux response to ACh iontophoresis, as it is feasible that these parameters may influence microvasculature function.

To assess this possibility, ANCOVA was used to adjust for the effects of BMI and serum testosterone and insulin (individually and together), on the difference between control and PCOS subjects in the $\log_{10}$-transformed, flux response at 500 s versus baseline (Table II). Covariance analysis using BMI and/or serum testosterone had little effect on the adjusted PCOS–control difference and its significance. This suggests that the difference in ACh-induced vasodilation between PCOS and control subjects was unrelated to variation in BMI and serum testosterone. Adjustment with serum insulin, with or without the other potential confounders, decreased the adjusted difference to a small extent (~20%) and, more so, its significance. This latter effect was perhaps partly because of the decline in sample size, as insulin levels were only available for 18 of the 24 subjects. Variability in serum insulin was therefore probably not associated, to any great degree, with the blunted microvascular ACh response in women with PCOS, albeit statistical analysis supports the possibility that it may have a weak confounding effect.

Discussion

Women with PCOS were diagnosed according to criteria prevalent at the time of the study; however, inspection of their medical notes has shown that all would be diagnosed as such under current guidelines (Rotterdam ESHRE/ARSM-sponsored PCOS Consensus Workshop Group, 2004). The results of this pilot study demonstrate that these women with PCOS exhibit a blunted vasodilatory response to ACh in the forearm skin microvasculature, though their microvascular vasodilatory response to SNP was unaffected. These results are the first demonstration of endothelial dysfunction in the skin microcirculation, a surrogate marker for cardiovascular disease, in women with PCOS.

These results were obtained using the reproducible and validated methods of iontophoresis (Morris *et al.*, 1995; Caballero *et al.*, 1999; Berghoff *et al.*, 2002) to deliver ACh and SNP

Table II. The effect of potential confounding variables—body mass index (BMI) and serum testosterone (Test.) and insulin (Ins.)—on the peak/basal erythrocyte flux in polycystic ovary syndrome (PCOS) and control women during acetylcholine iontophoresis

	Unadjusted	BMI	Test	Ins	BMI/Test	BMI/Ins	Test/Ins	BMI/Test/Ins
n	24	24	23	18	23	18	18	18
Adjusted difference	0.20	0.27	0.20	0.16	0.26	0.21	0.17	0.21
95% CI lower	0.02	0.04	–0.02	–0.12	0.01	–0.10	–0.13	–0.11
95% CI upper	0.38	0.49	0.43	0.45	0.50	0.53	0.48	0.54
P	0.040	0.024	0.075	0.247	0.038	0.167	0.246	0.183

Analysis of covariance was used to adjust the mean difference between PCOS and controls in the log of this parameter, for confounders, singly and in groups. The adjusted difference and residual significance reflected the effect of PCOS/control status, while the degree of change from the unadjusted difference/significance indicated the effect of the confounder(s).
CI = confidence interval.

without trauma to the skin microvasculature in a controlled manner, and laser Doppler flowmetry to assess microvascular erythrocyte flux (Morris *et al*., 1995; Caballero *et al*., 1999; Berghoff *et al*., 2002). Although the galvanic effect of current and voltage application can cause non-specific vasodilation (Berghoff *et al*., 2002), the low current and charge densities used in the present study (<30 μA/cm^2, cumulative charge of 6.75 mC/cm^2) would limit such effects (Berghoff *et al*., 2002). Furthermore, any galvanic effect would also have occurred in the SNP chamber, where no PCOS–control differences were noted. Iontophoresis of ACh was thought to stimulate local C-nociceptive nerve fibres (Walmsley and Wiles, 1990), but local anaesthesia has no effect on ACh-induced vasodilation. Thus it is likely that ACh iontophoresis provides 'a reliable index of skin endothelium-dependent vasodilation' (Caselli *et al*., 2003).

The response to ACh was used to assess endothelial functionality *in vivo*, whereas the response to SNP tests the integrity of smooth muscle function (Morris and Shore, 1996). ACh interacts with the M_3 muscarinic receptor on the endothelial cell surface, which initiates a sequence of intracellular events—G protein activation, phospholipase A and/or C activation and stimulation of endothelial NO synthase activity—leading to NO synthesis, although prostacyclin and hyperpolarizing factor release may also be induced. This NO diffuses across the endothelial cell membrane and basement membrane, binds to guanylate cyclase within the vascular smooth muscle cell, leading to an increase in intracellular cyclic guanosine monophosphate and, ultimately, smooth muscle relaxation and vasodilation. By contrast, SNP decomposes to release NO *in vivo*, which interacts with the vascular smooth muscle guanyl cyclase to produce vasodilation in an endothelium-independent manner.

The reduced vascular response to ACh in women with PCOS, in the absence of an effect on the response to SNP, suggests that aspects of skin microvascular endothelial function are disturbed in women with PCOS, while smooth muscle function is unaffected. PCOS-related disturbances in the skin microvasculature are presumably at the arteriolar level, i.e. in vessels close to the skin surface containing a smooth muscle component, since NO presumably has little effect in small capillaries with no associated smooth muscle cells.

This abnormal response to ACh could be related to the metabolic or endocrine abnormalities present in women with PCOS. Insulin resistance is an obvious candidate since a similar impaired response to ACh has been found in non-insulin-dependent diabetus mellitus (Morris *et al*., 1995; Pitie *et al*., 1997; Caballero *et al*., 1999; Berghoff *et al*., 2002), and insulin resistance and glucose intolerance are present in 30–60% (Dunaif *et al*., 1992) and 8–40% (Dunaif *et al*., 1987; Robinson *et al*., 1993) of PCOS sufferers respectively. Indeed, the mean insulin level in overnight-fasted serum from PCOS subjects tended to be (P = 0.06) almost double that in controls in the present study. However, ANCOVA showed that serum insulin had only a minor confounding effect (~20%) on the difference in peak microvascular responsiveness to ACh between the normal and PCOS groups. Thus a large proportion of the effect of PCOS on this difference was unrelated to fasting serum insulin levels. The apparent limited effect of insulin is not unheard of, as the vasodilatory response to ACh is also impaired in obese patients (Steinberg *et al*., 1996), independent of insulin resistance. Alternatively, the limited confounding effect of serum insulin may occur because of the small number of subjects in this pilot cross-sectional study, or because fasting serum insulin levels are thought to be a poor index of insulin resistance and the severity of the metabolic syndrome. Ideally, more subjects should be investigated and insulin resistance should be estimated using a method which provides a better index, such as the euglycaemic clamp or the 2 h glucose tolerance test with insulin estimation. It should be noted that waist circumference, considered by some to be a better index of insulin resistance than fasting serum insulin or BMI, was different between women with PCOS and controls (88.7 ± 15.0 versus 75.9 ± 5.9 cm respectively, P = 0.024), but had no confounding effect on the PCOS-associated difference in $\log_{10}$ peak microvascular ACh responsiveness (mean adjusted difference = 0.25, 95% confidence interval 0.02–0.47, P = 0.033). The effect of insulin resistance on the changes in microvascular responsiveness noted in this study remains open to question.

In diabetic subjects the abnormal response to ACh is thought to result from decreased NO production or release (Morris *et al*., 1995; Pitie *et al*., 1997; Caballero *et al*., 1999; Berghoff *et al*., 2002). It has also been suggested that it may be due to accumulation of products formed by a non-enzymatic reaction between glucose and collagen in the diabetic microvascular basement membrane (Bucala *et al*., 1991), which impairs endothelium-dependent relaxation through the reduction of NO (Rodriguez-Manas *et al*., 1993). There is also evidence from animal studies of altered sensitivity of vascular smooth muscle to NO in diabetes (Okon *et al*., 2003). Of these potential mechanisms, the results of the present study support the possibility that endothelial NO production or release is compromised in the PCOS group, but the other mechanisms are unlikely to occur. Vascular smooth muscle sensitivity to NO was unaltered—there was no difference in the perfusion response to SNP (a direct NO donor). Furthermore, though serum insulin tended to be elevated in the PCOS group, no differences were seen in serum glucose (perhaps due to the age of the women), suggesting that basement membrane glycosylation would be similar in both groups.

A similar response to ACh is also seen in hypercholesterolaemic (Khan *et al*., 1999), and hypertensive (Panza *et al*., 1994) patients, independent of insulin resistance. BMI is increased in 35–60% of PCOS women and there is evidence of increased cholesterol and blood pressure (Wild *et al*., 1985; Conway *et al*., 1992; Talbott *et al*., 1995). These factors could therefore influence endothelial function in women with the syndrome. In the present study, BMI was elevated in the PCOS group; however, there was no evidence of altered serum cholesterol or blood pressure, perhaps due to the age of the women. Changes in microvascular perfusion in the PCOS group are therefore unlikely to be related to elevated cholesterol or blood pressure. ANCOVA was used to test for the effect of BMI on the difference in peak ACh responsiveness between the normal and PCOS subjects. The BMI-adjusted difference remained significant, suggesting that the effect of

PCOS on ACh-evoked microvascular erythrocyte flux was not related to BMI differences between the normal and PCOS groups.

Hyperandrogenaemia is the characteristic endocrine feature of PCOS and it is therefore of interest that testosterone influences vasocontractile responses, and impairs endothelium-dependent relaxation (Adams *et al*., 1995; Hutchinson *et al*., 1997) in hypercholesterolaemic rabbits and monkeys. Furthermore, androgen deprivation in adult men enhances endothelium-dependent relaxation (Herman *et al*., 1997). However, more recent studies suggest that the vascular effects of testosterone may be more complex. For example, acute exposure even to low nanomolar doses of testosterone significantly potentiates endothelin-1-induced vasoconstriction in porcine coronary artery rings (Teoh *et al*., 2000). This effect was insensitive to flutamide (an androgen receptor antagonist), was not blocked by *de novo* protein synthesis inhibitors and exhibited an acute time-course, all suggesting that it was not mediated *via* the classical androgen receptor influence on gene transcription. Nevertheless, this does not detract from the possibility that the diminished ACh-induced microvascular perfusion response seen in the present study resulted, at least in part, from the elevated levels of androgens in women with PCOS. ANCOVA was therefore used to test for the effect of testosterone on the difference in ACh-induced erythrocyte flux between the normal and PCOS groups. The testosterone-adjusted erythrocyte flux difference remained significant, suggesting that the effect of PCOS on ACh-induced microvascular blood flow was not related to differences in serum testosterone between the normal and PCOS groups.

It was notable that the microvascular endothelial deficit was found in women aged 18–35 years, with PCOS. 'Young' women were assessed because previous studies have shown vascular endothelial dysfunction—though not in the microvasculature—in obese women with PCOS, which was strongly associated with both elevated androgen levels and obesity/insulin resistance. The results of the present study raise the possibility that the endocrine and putative metabolic perturbations seen in women with PCOS, even those who are non-obese, have a detrimental influence on microvascular endothelial function from an early age; long-term exposure is not required. Indeed, it is possible that dysfunction occurs throughout the macro- and microvascular endothelium from an early age in women with PCOS, and that it predisposes to further damage to the endothelium. In essence, PCOS may lead to accelerated ageing of the vascular endothelium. This possibility cannot be assessed at present, as no studies have been performed to investigate the prevalence and age-related incidence of microvascular disease in women with PCOS. Defining the age-relationship of the onset of dysfunction is important, however. Although some findings indicate a greater incidence of vascular dysfunction in older women with PCOS, sometimes related to obesity, it is unclear whether the age of onset of dysfunction is earlier in such women. The observation of macrovascular dysfunction in PCOS women (aged 18–35 years) sufferers discussed above (Lakhani *et al*., 2002, 2004; Paradisi *et al*., 2001) suggest that this may be so, even in non-obese women (Lakhani *et al*., 2002, 2004). Confirmation of this finding leads to the possibility that preventative treatment could be administered to minimize vascular dysfunction which could lead to increased morbidity and mortality in later life.

In summary, this is the first report of microvascular endothelial dysfunction, a surrogate marker for cardiovascular disease, in women with PCOS. The ACh-induced increase in microvascular perfusion, which is dependent on endothelial NO release, was blunted in women with PCOS. This is perhaps a result of impaired endothelial NO release due to the endocrinological or metabolic changes in PCOS women. It was perhaps related to the differences in serum insulin between PCOS and control subjects to a minor extent, but not to changes in BMI or serum testosterone. Further studies are necessary to confirm the data and provide a mechanistic analysis of these findings.

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