Vascular function in the diagnostic categories of polycystic ovary syndrome

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BACKGROUND: Polycystic ovary syndrome (PCOS) is associated with increased risk of cardiovascular disease (CVD). However, it is unknown whether CVD risk is equivalent across the different diagnostic categories or phenotypes of PCOS, particularly in the new phenotypes that affect up to 50% of women with PCOS. Surrogate markers of CVD include endothelial function and arterial stiffness that are independent risk factors for CVD. The primary aim of this study was to assess whether milder phenotypes of PCOS have elevated CVD risk compared with controls.

METHODS: This was a cross-sectional study, conducted in an academic medical centre, of overweight premenopausal women with either National Institute of Health (NIH) PCOS (n = 29) or milder non-NIH PCOS (n = 25) and controls without PCOS (n = 27). Primary outcomes were markers of vascular health, including endothelial function [peripheral arterial tonometry (PAT), asymmetric dimethylarginine (ADMA) and plasminogen activator inhibitor-1 (PAI-1)] and arterial stiffness [central pulse wave velocity (PWVc)]. Secondary outcomes were insulin resistance, glucose tolerance and inflammation (C-reactive protein).

RESULTS: ADMA was significantly different across the three groups (P = 0.003). ADMA was similar for NIH and non-NIH PCOS (0.56 ± 0.01 versus 0.53 ± 0.02 μmol/l, P = 1.0) and higher for both NIH PCOS compared with controls (0.56 ± 0.01 versus 0.46 ± 0.02 μmol/l, P = 0.003) and non-NIH PCOS compared with controls (0.53 ± 0.02 versus 0.46 ± 0.02 μmol/l, P = 0.046). PAI-1 (P = 0.20), PAT (P = 0.95) and PWVc (P = 0.67) were similar for the three groups. All results were maintained after adjustment for age and body mass index where significant differences in these potentially confounding factors occurred between groups.

CONCLUSIONS: Women with NIH and non-NIH PCOS have elevated ADMA compared with controls independent of age and adiposity. This suggests that CVD risk, reflected by endothelial dysfunction, is increased in both traditional NIH and new milder non-NIH PCOS phenotypes. However, no differences in other markers of endothelial function or arterial stiffness were apparent between phenotypes in this PCOS cohort.

Key words: polycystic ovary syndrome / insulin resistance / endothelial function / arterial stiffness

Introduction

Polycystic ovary syndrome (PCOS) is a common condition affecting women of reproductive age. It is associated with reproductive (hyperandrogenism, menstrual irregularity, anovulation and infertility), metabolic (impaired glucose tolerance, type 2 diabetes mellitus (DM2), increased cardiovascular risk factors) and psychological dysfunction (worsened quality of life and increased anxiety and depression) (Norman et al., 2007; Moran and Teede, 2009; Moran et al., 2010; Wild et al., 2010). Metabolic features are underpinned by insulin resistance, an aetiological factor in PCOS which is inherently present in lean women with PCOS (Dunaif et al., 1989). Obesity and abdominal or visceral obesity further worsen insulin resistance and exacerbate the clinical features of PCOS (Balen et al., 1995; Cascella et al., 2008). As in the general population, insulin resistance and obesity in PCOS are associated with increased DM2 and other cardiovascular risk factors (Moran and Teede, 2009).

While the literature on the prevalence of cardiovascular disease (CVD) is controversial, recent data indicate that post-menopausal...
women with hyperandrogenism and a history of irregular menses have elevated angiographic coronary artery disease and worsened cardiovascular event-free survival (Shaw et al., 2008). PCOS is also associated with a range of traditional and novel risk factors for CVD including dyslipidaemia, hypertension, oxidative stress and inflammation (Wild et al., 2010). Non-invasive measures of vascular health as surrogate markers for CVD are being increasingly studied. These include endothelial function. This is measured through techniques such as peripheral arterial tonometry (PAT), which has potential benefits over techniques such as flow-mediated dilation (FMD) due to reduced variability and operator bias, and central pulse wave velocity (PWVc), which is accepted as the current gold standard measure of arterial stiffness (Laurent et al., 2006). Circulating markers of vascular function include plasminogen activator inhibitor-1 (PAI-1), an inhibitor of plasma fibrinolysis, and asymmetric dimethyl arginine (ADMA) that acts as an inhibitor of nitric oxide (NO) synthase. These have all been reported to independently predict cardiovascular events or mortality (Thogersen et al., 1998; Wilum-Hansen et al., 2006; Anderson et al., 2007; Matsuzawa et al., 2010) and to be abnormal in PCOS (Meyer et al., 2005b; Lowenstein et al., 2007; Moran et al., 2009, 2010).

PCOS has traditionally been diagnosed using the 1990 National Institute of Health (NIH) criteria of biochemical or clinical hyperandrogenism and anovulation (Zawadzki and Dunaf, 1992). With this definition, estimated prevalence of the condition was 8% (Diamanti-Kandarakis et al., 1999; March and Teede 2010). The European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) have now broadened the diagnosis of PCOS to require two of the following three features with exclusion of other aetiologies: polycystic ovaries on ultrasound (PCO), hyperandrogenism and anovulatory irregular periods (ESHRE/ASRM, 2004). These so-called Rotterdam criteria have increased clinical heterogeneity of PCOS, creating non-NIH phenotypes that are milder than those diagnosed using the NIH criteria. These milder reproductive phenotypes comprise either PCO and hyperandrogenism and regular menstrual cycles or PCO and irregular menstrual cycles but no hyperandrogenism. The application of the Rotterdam criteria has increased the prevalence of PCOS. The first community prevalence survey estimated a prevalence of Rotterdam-diagnosed PCOS of 12–18%, depending on whether imputed estimates of the presence of PCO were applied (March et al., 2010).

It is as yet unclear as to whether non-NIH PCOS involves the same adverse metabolic health risks as NIH PCOS. Limited and conflicting research suggests that non-NIH PCOS may present with a less adverse metabolic profile (Moran and Teede, 2009). However, it is not known whether this is an inherent feature of the different phenotypes or a consequence of differential expression of modulating factors such as insulin resistance, hyperandrogenism or adiposity. Furthermore, there is currently no research assessing non-invasive or circulating markers of vascular health as predictors of CVD in the different phenotypes of PCOS. Given the controversy surrounding the clinical relevance of milder non-NIH PCOS, the novel aim of this study was to assess the key clinical question in PCOS as to whether the recently defined non-NIH PCOS phenotypes have increased cardiometabolic risks compared with women without PCOS. We assessed a comprehensive range of cardiovascular risk factors and surrogate markers of cardiovascular health across the different PCOS phenotypes in comparison with non-PCOS control women using non-invasive assessments with the primary outcome measures of endothelial function and arterial stiffness.

**Materials and Methods**

**Subjects**

Overweight (body mass index (BMI) > 25 kg/m²) premenopausal (verified by serum follicle-stimulating hormone) women (aged 18–45) with PCOS (n = 54, n = 29 NIH and n = 25 non-NIH) and without PCOS (n = 27 controls) were recruited through community advertisement as previously reported (Moran et al., 2011). NIH PCOS was classified as two features of clinical or biochemical hyperandrogenism (Ferriman–Gallwey score > 8, total testosterone > 2.7 nmol/l or free androgen index (FAI) > 4.5) together with irregular periods (cycle length outside 21–35 days or < 8 cycles per year) and exclusion of other aetiologies (congenital adrenal hyperplasia, androgen-secreting tumours, Cushing’s syndrome, hyperprolactinaemia, thyroid dysfunction and adrenal disorders) (Zawadzki and Dunaf, 1992). Non-NIH PCOS was diagnosed by ESHRE/ASRM or Rotterdam criteria (ESHRE/ASRM, 2004), consisting of two of the three features of clinical or biochemical hyperandrogenism, PCO on ultrasound and irregular periods with exclusion of other aetiologies. The non-NIH subgroup consisted of the two additional new Rotterdam phenotypes of: (i) hyperandrogenism and PCO with regular menses and (ii) non-hyperandrogenism and PCO with irregular menses. Controls involved in this study had no history of diagnosed PCOS, were assessed for menstrual regularity, clinical and biochemical hyperandrogenism and PCO on ultrasound and presented with one or none of these features. Exclusion criteria included pregnancy, smoking, DM2, uncontrolled hypertension and non-regularity, clinical and biochemical hyperandrogenism and PCO on ultrasound and presented with one or none of these features. Exclusion criteria included pregnancy, smoking, DM2, uncontrolled hypertension and non-stable use of relevant cardiovascular risk factor-modifying medications (anti-hypertensives, lipid lowering, fish oil) during the study. Participants currently using hormonal (e.g. oral contraceptive pill) or insulin-sensitizing medication were excluded from the study unless willing to cease medication use for 3 months prior to study measurements. The institutional Human Research and Ethics Committee approved the study and all participants gave written informed consent.

**Clinical, biochemical and anthropometric measurements**

Participants were weighed lightly clothed without shoes following an overnight fast and BMI was calculated as (weight (kg)/height squared (m²)). Waist circumference was measured to the nearest 0.5 cm directly on the skin with a soft tape at the level midway between the lateral lower rib margin and the iliac crest. Dual-energy x-ray absorptiometry (DEXA; GE Lunar Prodigy, GE Lunar Corp., Madison WI using operating system version 9) was performed for the assessment of total, gynoid and android fat mass and fat free mass as previously described (Hutchison et al., 2011). Menstrual calendars were collected by participants for a minimum of 3 months to assess menstrual cyclicity. Wherever possible, all measurements were performed in the follicular phase of the menstrual cycle. Eleven women were assessed outside of the follicular phase, determined from serum progesterone. All analyses were performed with and without these women included with no statistical differences present. Fasting venous blood samples were taken for the analysis of glucose, insulin, testosterone, sex-hormone-binding globulin (SHBG), total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), PAI-1, ADMA and C-reactive protein (CRP) (by a high-sensitivity assay) as previously described (Moran et al., 2009). A 120-min 75-g oral glucose tolerance
test (OGTT) was performed with blood samples drawn for insulin and glucose at 0, 30, 60 and 120 min intervals. Homeostasis assessment of insulin resistance (HOMA) was calculated as \([\text{fasting glucose} \times \text{fasting insulin}] / 22.5\) and FAI was calculated as \([\text{testosterone} \times \text{SHBG}] / 100\).

**Non-invasive measures of arterial stiffness and endothelial function**

Arterial parameters were measured after a 4-h caffeine-free fast in a dark, quiet, temperature-controlled laboratory following 10 min supine rest. Supine-resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a Dinamap device (CRITIKON 1846 SX). Mean arterial pressure (MAP) was calculated as \([[(1/3 \times \text{SBP}) + (2/3 \times \text{DBP})]\]). For PWVc, continuous pulse pressure wave signals were recorded with hand-held tonometers (Millar Mikro-tip, SPT-301; Millar Instruments, Houston, TX, USA) as previously described and with accuracy and repeatability previously published (Liang et al., 1998; Meyer et al., 2005b). Transit distance was defined as the distance from sternal notch to femoral artery minus sternal notch to carotid. The start of systole was defined by the local maximum of the first derivative of the pressure signal. Mean transit time (\(\Delta t\)) between the feet of simultaneously recorded waves was determined from 10 consecutive cardiac cycles. PWVc was calculated from the transit distance and measured time delay (\(\Delta t\)) as \([\text{PWV} = D/\Delta t \text{ (m/s)}]\), where \(D\) is transit distance in metres. Endothelial function was measured by PAT (Itamar Medical Limited, Caesarea, Israel). Arterial pulsatile changes were measured using continuous recording of finger plethysmographic signals to assess vascular endothelial response to reactive ischaemia induced via a pneumatic tourniquet inflated around the upper arm to 200 mmHg for 5 min (Kuvin et al., 2003). Changes to PAT signal amplitude at consecutive 30-s intervals following the end of pressure cuff occlusion were analysed by EndoPAT 2000 Signal Analysis software (version 2.3.2, Itamar Medical Limited, Caesarea, Israel) with exclusion of signals affected by movement artefact or electrical interference and calculation of the mean signal amplitude of the baseline prior to the occlusion and the signal-to-baseline ratios at consecutive 30-s interval post-occlusion. The signal-to-baseline ratios from the occluded arm are normalized to signal from the non-occluded arm to compensate for potential systemic changes.

**Statistics**

Data were assessed for normality using Shapiro–Wilk tests and presented as mean \(\pm\) SEM except for non-normally distributed and ordinal data (median \(\pm\) interquartile range) and categorical data (proportions). Three women were previous smokers who had ceased prior to study commencement (\(n = 1\) NIH PCOS, \(n = 1\) non-NIH PCOS, \(n = 1\) control) and all analyses were performed both with and without these women included with no statistical differences noted. All data are presented for \(n = 81\) (\(n = 29\) NIH PCOS, \(n = 25\) non-NIH PCOS and \(n = 27\) control) except for \(n = 1\) for waist circumference, \(n = 3\) for ADMA and PAI-1, \(n = 6\) for OGTT glucose or insulin, \(n = 8\) for PAT, \(n = 10\) for CRP and \(n = 12\) for PWVc and blood pressure. Two-tailed statistical

**Figure 1** Participant recruitment.
analysis was performed using SPSS for Windows 14.0 software (SPSS Inc., Chicago, USA) with statistical significance set at a level of \( P \leq 0.05 \). Parametric or categorical data were analysed using one-way analysis of variance (ANOVA) or chi-square tests, respectively. Analyses were performed for: (i) all women with PCOS compared with controls with PCOS status as the between subject factor and (ii) different PCOS phenotypes (NIH and non-NIH) compared with each other and to controls with phenotype or control status as the between subject factor. Where differences existed between PCOS phenotypes or controls, post-hoc multiple comparisons were performed using Bonferroni adjustment. Univariate and multivariate analyses for ADMA, PAI-1, PWV and PAT were determined using linear and multiple linear regressions. BMI and/or age were used as covariates for all analyses, except for anthropometric variables where only age was used due to imbalances at baseline in these variables and their potential confounding effect on all analyses.

As no previous literature has assessed the primary outcomes of endothelial function and arterial stiffness in women with different PCOS phenotypes, we were not able to calculate prospective sample size. Previous work has reported differences of 0.77 \( \pm \) 1.0 m/s in PWVc, 0.52 \( \pm \) 0.51 in PAT, 0.74 \( \pm \) 0.1 \( \mu \)mol/l in ADMA and 1.0 \( \pm \) 1.1 U/ml in PAI-1 comparing women with NIH PCOS and controls (Lowenstein et al., 2007; Moran et al., 2009). With \( n = 25 \) in each group we would be powered to observe these differences to 78% for PWVc, 95% for PAT, 90% for PAI-1 and >99% for ADMA (\( P < 0.05 \)). On post-hoc power analysis, we were powered (\( P < 0.05 \)) to detect observed differences between only age was used due to imbalances at baseline in these variables and their potential confounding effect on all analyses.

Age, anthropometric and reproductive variables

For comparisons between women with NIH PCOS, non-NIH PCOS and controls, there were significant differences in anthropometric values and reproductive hormones as previously reported (Moran et al., 2011) (Table I). On post-hoc comparisons, NIH PCOS had greater weight, BMI, waist circumference (\( P \leq 0.001 \)), total fat mass (\( P = 0.02 \)), android fat mass (\( P = 0.006 \)), testosterone, FAI and decreased SHBG (\( P < 0.001 \)) compared with controls and non-NIH PCOS had greater testosterone, FAI and decreased SHBG (\( P < 0.001 \)) compared with controls (Table I).

Surrogate markers for CVD

There were no differences in lipid profile, PAT, PWVc, PAI-1 or MAP for women with PCOS across the three groups (Table II). With regard to arterial stiffness, on adjustment for MAP there remained no difference across the PCOS phenotypes and controls. A 120-min insulin and CRP were elevated across the PCOS phenotypes and controls such that NIH PCOS had elevated CRP (\( P = 0.01 \)) and 120-min insulin (\( P = 0.005 \)) compared with controls and non-NIH PCOS had elevated 120-min insulin (\( P = 0.02 \)) but no differences in CRP compared with controls. These differences were not maintained on adjustment for age and BMI (Table II). Women with PCOS had elevated ADMA compared with controls (0.54 \( \pm \) 0.01 versus 0.46 \( \pm \) 0.02 \( \mu \)mol/l, \( P = 0.001 \)). On comparison of women with

### Results

#### Subjects characteristics

The study compared \( n = 29 \) NIH, \( n = 25 \) non-NIH and \( n = 27 \) control participants. The \( n = 25 \) non-NIH PCOS comprised \( n = 22 \) hyperandrogenic regular menses PCOS and \( n = 3 \) non-hyperandrogenic irregular menses PCOS (Fig. I). Due to the small

### Table I Anthropometric and reproductive variables in the different PCOS phenotypes and controls.

<table>
<thead>
<tr>
<th></th>
<th>NIH PCOS (N = 29)</th>
<th>Non-NIH PCOS (N = 25)</th>
<th>Control (N = 27)</th>
<th>( P )-value overall PCOS versus controls</th>
<th>( P )-value age/BMI adjusted</th>
<th>( P )-value across NIH PCOS versus non-NIH PCOS</th>
<th>( P )-value age/BMI adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.0 ( \pm ) 1.1</td>
<td>33.4 ( \pm ) 1.2</td>
<td>36.4 ( \pm ) 1.7</td>
<td>0.03</td>
<td>N/A</td>
<td>0.07</td>
<td>N/A</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>98.6 ( \pm ) 4.4*</td>
<td>86.3 ( \pm ) 3.7b</td>
<td>78.3 ( \pm ) 2.4b</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>36.1 ( \pm ) 1.6a</td>
<td>32.5 ( \pm ) 1.1ab</td>
<td>28.7 ( \pm ) 0.8b</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>104.1 ( \pm ) 3.1a</td>
<td>99.7 ( \pm ) 3.2b</td>
<td>89.9 ( \pm ) 2.0b</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>45.1 ( \pm ) 2.5a</td>
<td>40.2 ( \pm ) 2.2ab</td>
<td>35.8 ( \pm ) 2.1b</td>
<td>0.022</td>
<td>0.14</td>
<td>0.023</td>
<td>0.13</td>
</tr>
<tr>
<td>Gynoid fat (kg)</td>
<td>8.2 ( \pm ) 0.5</td>
<td>7.2 ( \pm ) 0.4</td>
<td>6.9 ( \pm ) 0.3</td>
<td>0.128</td>
<td>0.39</td>
<td>0.067</td>
<td>0.18</td>
</tr>
<tr>
<td>Android fat (kg)</td>
<td>4.3 ( \pm ) 0.3a</td>
<td>3.7 ( \pm ) 0.3b</td>
<td>3.2 ( \pm ) 0.2b</td>
<td>0.010</td>
<td>0.06</td>
<td>0.008</td>
<td>0.04</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>49.7 ( \pm ) 1.5a</td>
<td>46.7 ( \pm ) 1.9</td>
<td>45.1 ( \pm ) 0.9</td>
<td>0.108</td>
<td>0.35</td>
<td>0.102</td>
<td>0.29</td>
</tr>
<tr>
<td>Testosterone (nmol/l)*</td>
<td>2.9 ( \pm ) 1.5a</td>
<td>2.0 ( \pm ) 1.6a</td>
<td>1.4 ( \pm ) 0.7b</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SHBG (nmol/l)*</td>
<td>30.8 ( \pm ) 22.0*</td>
<td>35.0 ( \pm ) 23.9*</td>
<td>55.1 ( \pm ) 24.8*</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>FAI*</td>
<td>7.7 ( \pm ) 6.3a</td>
<td>7.3 ( \pm ) 8.4a</td>
<td>2.6 ( \pm ) 1.7b</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean \( \pm \) SEM or median \( \pm \) interquartile where not normally distributed (indicated by *). Data were assessed by one-way ANOVA with PCOS status or PCOS phenotype as between subject factor with adjustment for age/BMI for all variables except for anthropometric variables where adjustments were performed for age only and age where adjustments were performed for BMI only.

*Study groups with different superscripts had significant differences on their post-hoc comparisons for comparisons not adjusted for age and BMI.

BMI: body mass index; FAI: free androgen index; NIH: National Institute of Health; PCOS: polycystic ovary syndrome; SHBG: sex-hormone-binding globulin.
NIH PCOS, non-NIH PCOS and controls, there were significant differences in ADMA (P = 0.003) which were maintained after adjustment of age and BMI. On post-hoc comparisons there were no differences between NIH and non-NIH PCOS for ADMA (P = 1.00) but higher ADMA in both NIH (P = 0.003) and non-NIH PCOS (P = 0.046) compared with controls (Table II).

### Univariate and multivariate analysis

Univariate analysis of surrogate markers is presented in Table III for all women combined. ADMA correlated with adiposity, insulin resistance and CRP. PAI-1 correlated with adiposity, insulin resistance and glucose tolerance and SHBG. PWVc correlated with age, glucose tolerance, CRP, lipid profile and MAP. PAT did not correlate with any study variables. Multivariate models were then constructed with the primary outcome measures of ADMA, PAI-1, PWVc and PAT as dependent variables. Total fat mass (parameter estimate (PE) 0.004 ± standard error (SE) 0.001, P < 0.0001) and HDL-C (0.072 ± 0.03, P = 0.023) predicted ADMA (r² = 0.297, P < 0.001). SHBG (−0.02 ± 0.007, P = 0.009) and HOMA-IR (0.113 ± 0.05, P = 0.03) predicted PAI-1 (r² = 0.265, P = 0.001). Age (0.04 ± 0.18, P = 0.028), glucose AUC (0.002 ± 0.001, P = 0.013), central MAP (0.04 ± 0.015, P = 0.007) and hsCRP (0.10 ± 0.05, P = 0.04) predicted PWVc (r² = 0.324, P < 0.001). No variables predicted the change in PAT. Androgens did not correlate with any variable and did not predict any variables in multiple regression models.

### Discussion

We report here a comprehensive assessment of novel and traditional cardiovascular risk factors across the phenotypes of NIH PCOS, non-NIH PCOS and controls. We report for the first time differences in markers of endothelial function, as reflected by elevated ADMA, in both NIH and non-NIH PCOS compared with controls. Arterial stiffness, non-invasive measures of endothelial function and PAI-1 did not differ between women with PCOS and controls or across the PCOS phenotypes. We also confirm previous reports of elevated CRP in NIH PCOS compared with controls and elevated insulin resistance (measured by OGTT insulin) in both NIH and non-NIH PCOS compared with controls (Carmina et al., 2005) although both of these differences were not maintained on adjustment of age and BMI. We confirm findings of similar insulin resistance between NIH and non-NIH PCOS (Diamanti-Kandarakis and Panidis, 2007). ADMA is an independent risk factor of DM2 and CVD (Anderson et al., 2007) and is a marker of endothelial function as a competitive inhibitor of NO synthesis. Elevated ADMA levels are also associated with reduced vascular tone and increased systemic vascular resistance (Boger, 2006). We have confirmed previous findings of elevated ADMA in NIH PCOS compared with controls (Heutling et al., 2008; Moran et al., 2009) although we note that this is not consistently reported (Pamuk et al., 2010; Soyman et al., 2011). We also report, for the first time, similar ADMA levels for NIH and non-NIH PCOS and higher ADMA in non-NIH PCOS compared with controls. Previous work has reported similar ADMA levels in lean and overweight women with PCOS, despite differences in insulin resistance or...
hyperandrogenism (Kilic et al., 2010; Turkcuoglu et al., 2010). This may indicate the contribution of factors, in addition to obesity, to elevated cardiovascular risk in PCOS. Here we also confirm significant associations in univariate or multivariate regression of ADMA with adiposity, insulin resistance and CRP (Heuting et al., 2008; Nakhjavani et al., 2010) possibly through mechanisms that have been previously proposed including hyperglycaemia, oxidative stress or dyslipidaemia (Eid et al., 2007). Although we report no association between androgens and ADMA in this study, ADMA may be modified by reproductive hormones with testosterone increasing ADMA in female-to-male transsexuals (Bunck et al., 2009). The regulation of ADMA in PCOS thus warrants further exploration. Importantly, we have previously reported that the markedly elevated ADMA in NIH PCOS is decreased by common pharmacotherapies used in PCOS including the oral contraceptive pill and insulin-sensitizing agents (Teede et al., 2010). These findings suggest that elevated cardiovascular risk factors associated with PCOS may be treated by therapy targeted to PCOS as opposed to traditional therapies targeting cardiovascular risk factors.

Existing literature examining endothelial dysfunction in PCOS is conflicting with either worse (Meyer et al., 2005b; Diamanti-Kandarakis et al., 2006; Cussons et al., 2009; Moran et al., 2009) or similar (Mather et al., 2000; Brinkworth et al., 2006; Baillargeon and Carpenter, 2007) endothelial function in women with PCOS compared with controls. Given the role of insulin resistance in up-regulating PAI-I gene transcription (Banfi et al., 2001) and stimulating NO synthase gene expression (Kuboki et al., 2000), the lack of a difference in PAI-I or PAT in this current study for women with PCOS and controls is unclear. The similar PAT findings across the PCOS phenotypes, despite differences in ADMA, are also surprising given that ADMA is a NO synthase inhibitor and that endothelial function is commonly measured by post-ischaemic-reactive hyperaemia which is mediated by shear-induced endothelial NO release (Nohria et al., 2006). This apparently anomalous result may be because NO-mediating accounts for only ~60% of PAT-measured digital artery dilation (Nohria et al., 2006) while FMD is almost entirely NO-mediated (Meredith et al., 1996). The observed lack of a difference in PAT despite differences in ADMA may therefore indicate similarities in other contributors to endothelial function such as lipids, glucose, androgens, CRP, oxidative stress, glucocorticoids, catecholamines and adipokines (Mertens and Van Gaal, 2002; Diamanti-Kandarakis et al., 2006). The independent role of PCOS status on worsening cardiovascular risk is still unclear.

We also report for the first time similar PWVc in women with NIH PCOS, non-NIH PCOS and women without PCOS. This is consistent with most (Cussons et al., 2009), but not all (Meyer et al., 2005b), previous studies comparing women with PCOS and controls. We were, however, able to confirm previous significant associations of PWVc with age, blood pressure, glucose tolerance and CRP in this study (Meyer et al., 2005a; Schumacher et al., 2009). The similarities in PWVc across the PCOS phenotypes in our study may be due to similarities in these known determinants of PWVc. Overall, we report here on a relatively more metabolically healthy PCOS cohort, potentially contributing to the lack of observed difference in PWVc, PAT and PAI-I. We note here similar lipids between women with and without PCOS, reduced PAI-I and ADMA for women with PCOS compared with our previous PCOS cohorts (Moran et al., 2009), and similar PAT

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Table III Univariate analysis of markers of vascular function in the combined group of women with and without PCOS.

<table>
<thead>
<tr>
<th></th>
<th>ADMA</th>
<th>PAI-I</th>
<th>Central PWV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-</td>
<td>-</td>
<td>R = 0.293, P = 0.011</td>
</tr>
<tr>
<td>Weight</td>
<td>R = 0.395, P &lt; 0.001</td>
<td>R = 0.327, P &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>BMI</td>
<td>R = 0.397, P &lt; 0.001</td>
<td>R = 0.328, P = 0.003</td>
<td>-</td>
</tr>
<tr>
<td>Waist circum.</td>
<td>R = 0.426, P &lt; 0.001</td>
<td>R = 0.353, P = 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Fat mass</td>
<td>R = 0.474, P &lt; 0.001</td>
<td>R = 0.327, P = 0.007</td>
<td>-</td>
</tr>
<tr>
<td>Android fat</td>
<td>R = 0.440, P &lt; 0.001</td>
<td>R = 0.375, P = 0.002</td>
<td>-</td>
</tr>
<tr>
<td>Gynoid fat</td>
<td>R = 0.432, P &lt; 0.001</td>
<td>R = 0.258, P = 0.024</td>
<td>-</td>
</tr>
<tr>
<td>HOMA</td>
<td>R = 0.234, P = 0.036</td>
<td>R = 0.515, P &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Glucose 0</td>
<td>-</td>
<td>R = 0.457, P &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Insulin 0</td>
<td>R = 0.315, P = 0.004</td>
<td>R = 0.455, P &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Glucose 120</td>
<td>-</td>
<td>R = 0.531, P &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Insulin 120</td>
<td>R = 0.324, P = 0.005</td>
<td>R = 0.294, P = 0.011</td>
<td>-</td>
</tr>
<tr>
<td>CRP</td>
<td>R = 0.366, P = 0.002</td>
<td>R = 0.261, P = 0.029</td>
<td>R = 0.287, P = 0.019</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-</td>
<td>R = 0.454, P &lt; 0.001</td>
<td>R = 0.286, P = 0.013</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-</td>
<td>-</td>
<td>R = 0.261, P = 0.024</td>
</tr>
<tr>
<td>LDL</td>
<td>-</td>
<td>-</td>
<td>R = 0.242, P = 0.037</td>
</tr>
<tr>
<td>HDL</td>
<td>-</td>
<td>R = -0.345, P = 0.002</td>
<td>-</td>
</tr>
<tr>
<td>MAP</td>
<td>-</td>
<td>-</td>
<td>R = 0.379, P = 0.001</td>
</tr>
<tr>
<td>SHBG</td>
<td>-</td>
<td>R = -0.457, P &lt; 0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

Data were analysed using linear regression.

ADMA: asymmetric dimethylarginine; BMI: body mass index; HOMA: homeostasis assessment of insulin resistance; CRP: C-reactive protein; MAP: mean arterial pressure; PAI-I: plasminogen activator inhibitor-I; PAT: peripheral arterial tonometry; PWV: pulse wave velocity; SHBG: sex-hormone-binding globulin.
and PWVc for women with PCOS compared with controls from previous cohorts (Meyer et al., 2005b; Lowenstein et al., 2007). Furthermore, the PWVc levels for all groups in this study are comparable with previously defined normal ranges (6.5 m/s) for a healthy population aged 30–39 years with no risk factors (2010). Potentially, the current findings include early and subtle abnormalities in vascular function in PCOS, indicated by elevated ADMA, which have not yet translated into clinical endothelial dysfunction or increased arterial stiffness (The Reference Values for Arterial Stiffness’ Collaboration).

The strengths of this study include the comprehensive assessment of CVD and DM2 risk factors, body composition, characterization of PCOS phenotypes and addressing of potential confounding factors including smoking status and menstrual cycle stage. Although powered on a priori calculation, we acknowledge limitations including relatively small sample sizes. However, we were powered to detect differences in our primary outcome of ADMA. Due to sample size restrictions, it was not possible to subdivide the non-NIH PCOS women into non-hyperandrogenic PCOS and ovulatory PCOS and analysis of these subgroups is warranted in future research. We also note the lack of matching for potential confounders such as age and BMI, although all statistical analyses were adjusted for age and BMI. The lack of a lean cohort also limits our ability to both mechanistically assess the effect of PCOS phenotype on endothelial function independent of obesity and to examine the full range of heterogeneity within PCOS phenotypes.

We report that overweight women with both NIH and non-NIH PCOS have elevated risk profiles for CVD as indicated by ADMA, as a marker of endothelial function, compared with women without PCOS. Of interest, this elevation in ADMA occurred despite no differences in a range of traditional and novel cardiovascular risk factors. This indicates differences in early and reversible markers of vascular dysfunction in young overweight women across the diagnostic spectrum of PCOS. This additionally highlights the potential utility of specific biomarkers to detect subtle degrees of metabolic risk in PCOS. Future research should focus on optimally sensitive predictive tools for the assessment of metabolic risk, the natural history of cardiometabolic complications in PCOS and the optimal treatment of metabolic risk in all women with PCOS. Clinically, this study highlights the importance of screening and monitoring both NIH and non-NIH PCOS for cardiovascular risk factors.

Authors’ roles

L.J.M., J.D.C., B.J.S. and H.J.T. had substantial contributions to the conception and design of the study; L.J.M. and H.J.T. had substantial contributions to acquisition of data, or analysis and interpretation of data; and all authors had substantial contributions to drafting the article or revising it critically for important intellectual content and final approval of the version to be published.

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PCOS phenotype and vascular function


