



## Polycystic ovary syndrome is associated with severe platelet and endothelial dysfunction in both obese and lean subjects

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### ABSTRACT

Platelet hyporesponsiveness to the anti-aggregatory effects of nitric oxide (NO) occurs commonly in association with myocardial ischemia and coronary risk factors, often co-exists with endothelial dysfunction and represents an independent marker of long-term cardiovascular risk. We sought to determine whether polycystic ovary syndrome (PCOS), which has been postulated as a cardiovascular risk factor in women, is independently associated with this phenomenon. Twenty-four young women with PCOS (mean age  $32.1 \pm 1.3$ ) were evaluated in lean ( $n = 12$ ) and obese ( $n = 12$ ) subgroups, and compared with age-matched lean normals ( $n = 12$ ). Platelet aggregation and its inhibition by the nitric oxide donor sodium nitroprusside (SNP) were assessed and compared with vascular endothelial function. Plasma concentrations of malondialdehyde (MDA),  $N^G,N^G$ -dimethyl-L-arginine (ADMA) and hs-CRP were measured as markers of oxidative stress, endothelial dysfunction and inflammation, respectively. Circulating endothelial progenitor cell (EPC) counts were also documented. In both PCOS subgroups, which demonstrated hyperaggregability to ADP, responses to SNP inhibition of aggregation (the principal end-point of the study) were significantly impaired ( $P < 0.01$  for both), as were their endothelium-dependent vascular responses to salbutamol ( $P < 0.05$  for both). However, vasomotor responses to nitroglycerin and circulating EPC counts did not vary between groups. PCOS subjects also had significantly elevated ADMA, MDA and hs-CRP levels relative to normals (all  $P < 0.05$ ). Impairment of SNP response remained unaltered after mean  $30 \pm 2.4$  months follow-up in PCOS subjects. We conclude that in PCOS subjects, independent of obesity and associated insulin resistance, profound and reproducible impairment of platelet responsiveness to NO is an additional component of cardiovascular homeostatic disturbance.

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### 1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting premenopausal women and has major implications for reproductive health [1,2]. Although PCOS is diagnosed on the presence of variable combinations of hyperandrogenism, ovulatory disturbances and polycystic ovaries [3], it is also a multifaceted metabolic disease linked with insulin resistance, obesity and metabolic syndrome [4–8]. A number of investigations have addressed the possibility that PCOS may also be associated with incremental biochemical and physiological mark-

ers of cardiovascular risk, including endothelial [9–14] and platelet [15] dysfunction. However, these studies have been performed in insulin-resistant PCOS subjects. Furthermore, it remains uncertain whether PCOS is actually associated with increased cardiovascular morbidity and mortality, largely by virtue of lack of prospective evaluation studies. A critically important conceptual issue therefore is whether PCOS is independently associated with abnormal cardiovascular physiology, or whether such anomalies are engendered by associations with insulin resistance/metabolic syndrome.

It has recently been demonstrated [16] that patients with a variety of cardiac risk factors (e.g. hypertension, obesity, hyperglycemia and hyperlipidemia) frequently exhibit diminution of platelet (and vascular) responsiveness to nitric oxide (NO) relative to normal subjects and that this “NO resistance”, which is particularly prominent in the presence of overt myocardial ischemia, is an independent marker of increased mortality [16–18]. Although NO resistance may frequently co-exist with vascular endothelial dysfunction, there are

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fundamental differences in underlying mechanisms: for example, NO resistance is independent of the biochemical processes responsible for NO release [16]. The major objective of the current study was to determine whether PCOS is associated with platelet resistance to nitric oxide, and whether this disturbance is related to obesity, metabolic syndrome, or to other markers of disturbances of vascular homeostasis, such as endothelial dysfunction and/or inflammatory activation.

## 2. Methods

### 2.1. Study population

The planned study had the primary objective of comparing a PCOS population with age-matched lean normal subjects, and a principal secondary objective of comparing lean and obese PCOS women who were otherwise healthy. Women who had been previously diagnosed with PCOS (according to the definition below) responded to advertisements in the local media.

We sought to exclude women who had other cardiovascular risk factors, were on treatment for management of PCOS, or who were mildly overweight, in order to obtain well-defined cohorts of lean and obese PCOS subjects without other factors associated with abnormal platelet and vascular function. Thus, subjects were eligible for investigation if they were healthy, aged between 18 and 40 years and had not received hormonal contraception for a minimum of two months. Other exclusion criteria were smoking, current antiplatelet therapy, known presence of diabetes mellitus and the use of medication that might affect blood pressure, glucose or lipid metabolism. The two groups selected were: (1) lean women with a body mass index (BMI) of less than 25 kg/m<sup>2</sup> and (2) obese women with a BMI greater than 30 kg/m<sup>2</sup>. Aged-matched lean healthy women were enrolled voluntarily as the normal group. They had regular menses every 27–31 days, no hirsutism or abnormal androgen levels, were not smokers or on any hormonal replacement. The study was approved by the Ethics of Research Committee of The Queen Elizabeth Hospital and written informed consent was obtained before study entry.

### 2.2. Study protocol

After a 12 h overnight fast, subjects were evaluated between 8 and 9 a.m. Following 30 min of rest, venous blood was taken via an antecubital vein for the determination of platelet function (see below) and biochemical parameters including fasting lipid profile, high-sensitivity C-reactive protein (hs-CRP), glucose and insulin levels. Homeostasis model of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were determined as surrogate indices of insulin resistance. Levels of testosterone, estradiol, prolactin and thyroid function were also measured. Plasma concentrations of the endogenous nitric oxide (NO) synthase inhibitor N<sup>G</sup>,N<sup>G</sup>-dimethyl-L-arginine (ADMA), its inactive stereo-isomer N<sup>G</sup>,N<sup>G</sup>'-dimethyl-L-arginine (SDMA) and of arginine, the substrate of NO synthase, were measured [19]. Circulating endothelial progenitor cell (EPC) counts were performed utilizing flow cytometric analysis (FACScan, Becton Dickinson) of cells positive for both cell surface antigens, CD34 and CD133 as previously described [20]. Malondialdehyde (MDA), a marker of oxidative stress [21] was determined by a modification of the thio-barbituric acid (TBA) reaction as previously described [22].

### 2.3. Platelet aggregation studies

Blood was collected in plastic tubes containing 1:10 volume of acid citrate anticoagulant (2 parts of 0.1 mol/L citric oxide to 3

parts of 0.1 mol/L trisodium citrate). Aggregation in whole blood was studied using a dual-channel impedance aggregometer (Model 560, Chrono-Log) as previously described [23]. Aggregation was induced by adenosine diphosphate (ADP) using three concentrations: 1, 2.5 and 5 μmol/L. In order to evaluate the presence and extent of putative platelet NO resistance [16,18], the NO donor sodium nitroprusside (SNP; 10 μmol/L) was utilized to quantitate platelet responsiveness to NO (expressed as percent inhibition of aggregation) [23] and was added to samples one minute before the addition of ADP. Aggregation was monitored continuously for 7 min and responses were recorded for extent of aggregation (Ohms). Reproducibility of findings was assessed by repeat determination of responsiveness to SNP at a follow-up interval of 30 ± 2.4 months in 7 of the controls and 10 PCOS subjects.

### 2.4. Pulse wave analysis

Pulse wave analysis together with administration of the endothelium-independent vasodilator nitroglycerin (NTG) and the endothelium-dependent vasodilator salbutamol (β<sub>2</sub>-agonist) was used to assess arterial function as previously described [24,25]. NTG was administered as a single sublingual dose (25 μg), corresponding approximately to ED<sub>40</sub> values in normal individuals in preliminary studies. At least 20 min following NTG, salbutamol was given as a single 400 μg dose by inhalation with a spacer device [24]. Effects of NTG and salbutamol were quantitated by determination of drug-induced changes in rate-adjusted augmentation index (AI<sub>x</sub>) [24]. Response to NTG was expressed as area under the δAI<sub>x</sub>: time curve, while that to salbutamol was expressed as peak δAI<sub>x</sub>.

### 2.5. Definitions

PCOS was defined by the ESHRE/ASRM criteria which specify the presence of any two of the following three components: (i) oligo-/anovulation; (ii) clinical or biochemical evidence of hyperandrogenemia and/or (iii) polycystic ovaries [3]. Other causes of hyperandrogenism were excluded. The National Cholesterol Education Program's Adult Treatment Panel (ATP) III criteria was used to diagnose the metabolic syndrome [26]. HOMA-IR was measured by multiplying fasting plasma insulin (mU/L) and fasting plasma glucose (mmol/L) divided by 22.5 [27]. QUICKI was derived by the inverse of sum of logarithmically expressed values of fasting insulin and glucose [28].

### 2.6. Statistical analysis

The primary comparison was between the twenty-four PCOS women and the normal subjects, with secondary analyses to evaluate the potential impact of obesity and the extent of insulin resistance on major parameters measured. Based on previously published data from a normal subject cohort [29], this study had approximately 80% power to detect >25% difference in SNP response between groups. Thus, the primary hypothesis tested was that non-diabetic young women with PCOS exhibit NO resistance at the level of platelet aggregation. The principal secondary hypothesis was that these putative anomalies occur independent of variability in body mass index and/or insulin resistance.

Characteristics of participant groups (normal vs. PCOS) were compared utilizing non-paired *t*-test. Differential effects of obesity and comparisons of EPC counts were examined utilizing one-way analysis of variance (ANOVA). Potential differential relationships between aggregation and platelet responsiveness to NO donor in these groups were evaluated with analysis of covariance (ANCOVA). Concentration-response data were analyzed by two-way ANOVA. Data for hs-CRP were compared via the non-parametric Kruskal–Wallis test. The limit of statistical significance

was set at  $P < 0.05$ . All data were expressed as mean  $\pm$  standard error of mean (S.E.M.), unless otherwise stated.

### 3. Results

#### 3.1. Subject characteristics

The study population consisted of twelve normal subjects and twenty-four women with PCOS, of whom twelve were lean (L-PCOS) and twelve were obese (O-PCOS). As envisaged, the PCOS subjects represented highly selected subsets from a total of one hundred and sixty-eight screened individuals; the most frequent bases for exclusion were smoking, intermediate BMI and/or concurrent therapy.

The normal and PCOS subjects differed significantly in several respects (Table 1), although these differences largely reflect characteristics of the O-PCOS subgroup (Table 2). Plasma testosterone concentrations were equally elevated in L-PCOS and O-PCOS. However, systolic and diastolic blood pressures, fasting insulin concentration, HOMA-IR, QUICKI score, triglycerides, total and LDL cholesterol were abnormal only in the O-PCOS group. Neither HOMA nor QUICKI indices varied between normal and L-PCOS groups, indicating similar responsiveness to insulin in these two groups. Only one subject in the O-PCOS group met the criteria for the metabolic syndrome, whereas no subject in the L-PCOS group met the criteria for the metabolic syndrome.

#### 3.2. Platelet aggregation

Platelet responsiveness to ADP (1–5  $\mu\text{mol/L}$ ) in blood samples obtained from normal, L-PCOS and O-PCOS subjects are depicted in Fig. 1A. Aggregation in response to ADP was significantly greater in both L-PCOS and O-PCOS groups compared to the normal group (ANOVA,  $P < 0.001$  for both).

#### 3.3. Platelet sensitivity to nitric oxide

In accordance with the principal objective of the study, inhibition of platelet aggregation by the NO donor SNP was evaluated in

**Table 1**  
Baseline characteristics of all participants.

Parameter	Normal (n = 12)	PCOS (n = 24)
Age (year)	30.5 $\pm$ 1.7	32.1 $\pm$ 1.3
Body mass index (kg/m <sup>2</sup> )	21.2 $\pm$ 0.3	27.5 $\pm$ 1.3*
Waist circumference (cm)	68 $\pm$ 1	86 $\pm$ 4*
Systolic blood pressure (mmHg)	97 $\pm$ 3.1	105 $\pm$ 3
Diastolic blood pressure (mmHg)	66.8 $\pm$ 2.2	73 $\pm$ 2 <sup>†</sup>
Fasting glucose (mmol/L)	4.4 $\pm$ 0.1	4.5 $\pm$ 0.1
Fasting insulin (mU/L)	6.3 $\pm$ 0.8	11.1 $\pm$ 2.0
HOMA-IR <sup>‡</sup>	1.19 $\pm$ 0.16	2.28 $\pm$ 0.44
QUICKI <sup>§</sup>	0.378 $\pm$ 0.009	0.359 $\pm$ 0.009
Total cholesterol (mmol/L)	4.1 $\pm$ 0.2	4.9 $\pm$ 0.2 <sup>†</sup>
HDL cholesterol (mmol/L)	1.5 $\pm$ 0.1	1.6 $\pm$ 0.1
LDL cholesterol (mmol/L)	2.3 $\pm$ 0.1	3.0 $\pm$ 0.2 <sup>†</sup>
Triglyceride (mmol/L)	0.8 $\pm$ 0.1	0.9 $\pm$ 0.2
Testosterone (nmol/L)	1.23 $\pm$ 0.13	2.56 $\pm$ 0.25*
Estradiol (pmol/L)	360.0 $\pm$ 70.4	230.5 $\pm$ 37.4

\*  $P < 0.01$  for comparison with normal group.

<sup>†</sup>  $P < 0.05$  for comparison with normal group.

<sup>‡</sup> HOMA-IR (homeostatic model assessment: insulin resistance). Calculated as the product of the fasting plasma insulin level (in mU/L) and fasting plasma glucose level (in mmol/L), divided by 22.5. Higher scores indicate insulin resistance.

<sup>§</sup> QUICKI (quantitative insulin sensitivity check index). QUICKI was derived by calculating the inverse of the sum of logarithmically expressed values of fasting insulin and glucose. Lower scores indicate insulin resistance.

all 3 groups utilizing ADP concentrations of 2.5 and 5  $\mu\text{mol/L}$ . Mean inhibition of platelet aggregation by NO in blood samples from normal subjects were 73  $\pm$  1 and 43  $\pm$  8% for ADP 2.5 and 5  $\mu\text{mol/L}$ , respectively (Fig. 1B). In contrast, platelet responsiveness to NO was approximately halved in both the L-PCOS and O-PCOS groups ( $P < 0.01$ ).

We also assessed whether the observed low anti-aggregatory response to NO in the PCOS groups (Fig. 1B) might be a reflection of the increased platelet aggregation in these groups (Fig. 1A). As there was no difference in the extent of aggregation or percent inhibition of aggregation between the L-PCOS and O-PCOS groups, these data were pooled for further analysis. Analysis of covariance revealed that the aggregation:NO response relationship differed significantly ( $P < 0.001$ ) between PCOS and normal subjects (Fig. 2).

**Table 2**  
Baseline characteristics of subgroups.

	Normal (n = 12)	Subgroups	
		L-PCOS (n = 12)	O-PCOS (n = 12)
Age (year)	30.5 $\pm$ 1.7	31.0 $\pm$ 2.0	33.3 $\pm$ 1.6
Body mass index (kg/m <sup>2</sup> )	21.2 $\pm$ 0.3	22.2 $\pm$ 0.6	33.0 $\pm$ 1.1 <sup>*,†</sup>
Waist circumference (cm)	68 $\pm$ 1	71 $\pm$ 1	100 $\pm$ 4 <sup>*,†</sup>
Systolic blood pressure (mmHg)	97.0 $\pm$ 3.1	101.5 $\pm$ 3.3	110 $\pm$ 4.0 <sup>‡</sup>
Diastolic blood pressure (mmHg)	66.8 $\pm$ 2.2	70.5 $\pm$ 3.2	75.1 $\pm$ 2.0 <sup>‡</sup>
Fasting glucose (mmol/L)	4.4 $\pm$ 0.1	4.3 $\pm$ 0.1	4.6 $\pm$ 0.1
Fasting insulin (mU/L)	6.3 $\pm$ 0.8	7.4 $\pm$ 2.4	14.9 $\pm$ 2.8 <sup>‡</sup>
HOMA-IR	1.19 $\pm$ 0.16	1.49 $\pm$ 0.55	3.07 $\pm$ 0.63 <sup>‡</sup>
QUICKI	0.378 $\pm$ 0.009	0.385 $\pm$ 0.011	0.332 $\pm$ 0.007 <sup>§,  </sup>
Total cholesterol (mmol/L)	4.1 $\pm$ 0.2	4.4 $\pm$ 0.2	5.3 $\pm$ 0.3 <sup>§,¶</sup>
HDL cholesterol (mmol/L)	1.5 $\pm$ 0.1	1.7 $\pm$ 0.1	1.4 $\pm$ 0.1 <sup>¶</sup>
LDL cholesterol (mmol/L)	2.3 $\pm$ 0.1	2.6 $\pm$ 0.1	3.3 $\pm$ 0.3 <sup>§,¶</sup>
Triglyceride (mmol/L)	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	1.3 $\pm$ 0.3 <sup>¶</sup>
Testosterone (nmol/L)	1.23 $\pm$ 0.13	2.58 $\pm$ 0.26 <sup>#</sup>	2.54 $\pm$ 0.44 <sup>‡</sup>
Estradiol (pmol/L)	360.0 $\pm$ 70.4	305.3 $\pm$ 76.1	206.3 $\pm$ 31.4
CD34+/CD133+ cell count (per 100,000 events)	49.6 $\pm$ 11.1	47.6 $\pm$ 8.5	66.6 $\pm$ 15.7

\*  $P < 0.001$  for the comparison with normal group.

<sup>†</sup>  $P < 0.001$  for the comparison with L-PCOS group.

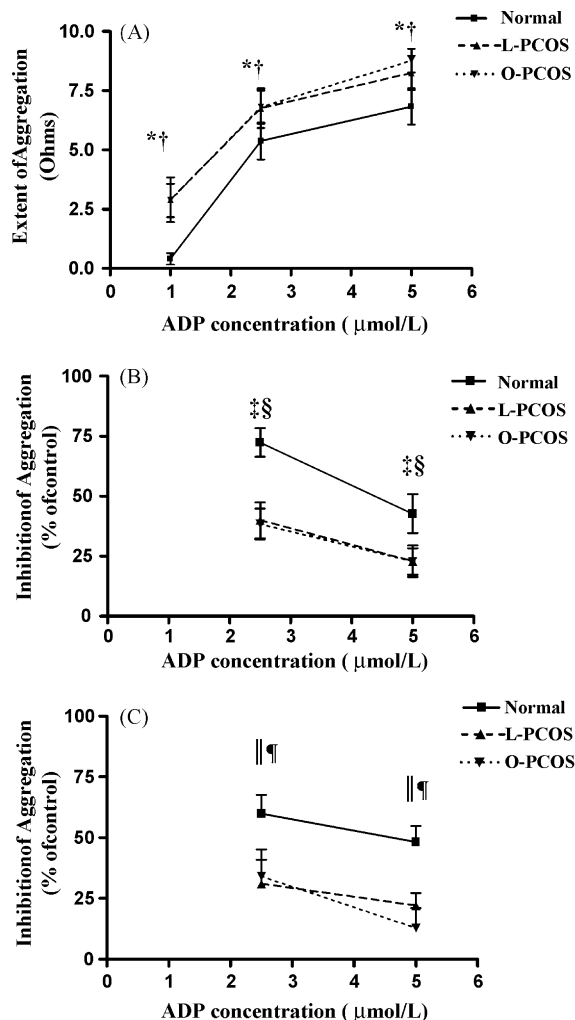
<sup>‡</sup>  $P < 0.05$  for the comparison with normal group.

<sup>§</sup>  $P < 0.01$  for the comparison with normal group.

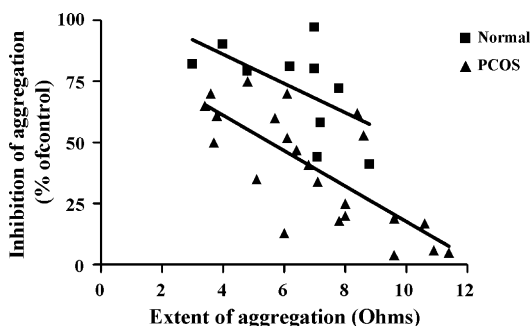
<sup>||</sup>  $P < 0.01$  for the comparison with L-PCOS group.

<sup>¶</sup>  $P < 0.05$  for the comparison with L-PCOS group.

<sup>#</sup>  $P < 0.01$  for the comparison with normal group.



**Fig. 1.** Effect of PCOS on platelet aggregation (Panel A), initial NO response (Panel B) and NO response at follow-up (Panel C). Panel A shows extent of ADP-induced whole blood platelet aggregation for the L-PCOS, O-PCOS and normal groups. L-PCOS and O-PCOS groups were hyperaggregable compared to normal subjects ( $P < 0.001$ , ANOVA, <sup>†</sup>L-PCOS and <sup>‡</sup>O-PCOS, respectively). Panel B shows inhibition of ADP-induced platelet aggregation by the NO donor SNP (10  $\mu\text{mol/L}$ ). In comparison with the normal group, L-PCOS and O-PCOS groups had significantly impaired platelet responses to SNP at both ADP concentrations ( $P < 0.01$ , ANOVA, <sup>‡</sup>L-PCOS and <sup>§</sup>O-PCOS, respectively). Panel C shows inhibition of ADP-induced platelet aggregation by the NO donor SNP (10  $\mu\text{mol/L}$ ) at mean follow-up of  $30 \pm 2.4$  months. Both PCOS subgroups show persistently impaired platelet responses at both ADP concentrations ( $P < 0.01$ , ANOVA, <sup>¶</sup>L-PCOS and <sup>#</sup>O-PCOS, respectively).



**Fig. 2.** ADP-induced (2.5  $\mu\text{mol/L}$ ) aggregation:NO response relationship. There is a significant difference between the aggregation:NO response relationships for PCOS and normal subjects ( $P < 0.001$ ,  $F$ -ratio = 106.7, ANCOVA).

**Table 3**  
Biochemical Markers.

	Normal	PCOS	Subgroups	
			L-PCOS	O-PCOS
MDA ( $\mu\text{mol/L}$ )	$0.17 \pm 0.02$	$0.25 \pm 0.02^*$	$0.25 \pm 0.02^\dagger$	$0.25 \pm 0.02^\ddagger$
ADMA ( $\mu\text{mol/L}$ )	$0.51 \pm 0.01$	$0.57 \pm 0.01^\S$	$0.56 \pm 0.02$	$0.59 \pm 0.02^\ddagger$
hs-CRP (mg/L) <sup>  </sup>	$0.39 \pm 0.06$	$1.77 \pm 0.29^*$	$0.81 \pm 0.20$	$2.73 \pm 0.38^{*,\#}$

\*  $P < 0.01$  for comparison with normal group.

†  $P < 0.05$  for comparison with normal group.

‡  $P < 0.05$  for comparison with normal group.

§  $P < 0.05$  for comparison with normal group.

<sup>||</sup> Data for hs-CRP are expressed as medians because 5 subjects in the normal group had levels below the quantitation level of 0.25 mg/L.

<sup>¶</sup>  $P < 0.001$  for comparison with normal group.

<sup>#</sup>  $P < 0.001$  for comparison with L-PCOS group.

The extent of platelet NO response within PCOS subjects was not correlated with HOMA-IR or QUICKI score, BMI or testosterone levels (data not shown).

After  $30 \pm 2.4$  months follow-up, differences in SNP response between normal subjects and both PCOS subgroups remained undiminished ( $P < 0.01$ ) (Fig. 1C).

#### 3.4. Endothelium-dependent and --independent vasodilation and endothelial progenitor cell counts

Baseline  $\text{AI}_x$  was  $5.5 \pm 2.9\%$  for normal subjects,  $6.3 \pm 3.4\%$  for L-PCOS and  $17.6 \pm 5.2\%$  for O-PCOS groups ( $P < 0.05$  vs. normal and L-PCOS subjects). Fig. 3A shows the change in  $\text{AI}_x$  over 20 min following salbutamol inhalation for all three groups. The L-PCOS and O-PCOS groups had significantly reduced responses (ANOVA,  $P < 0.05$  for both) when compared to the normal group (maximum responses  $1.2 \pm 2.1$  and  $1.9 \pm 1.6\%$  vs.  $-5.2 \pm 1.7\%$ , respectively), consistent with endothelial dysfunction. Endothelium-independent responses to NTG were normal and did not differ significantly between groups (Fig. 3B). Furthermore, EPC counts in PCOS subjects did not vary from those in normal subjects (Table 2).

#### 3.5. Biochemical markers

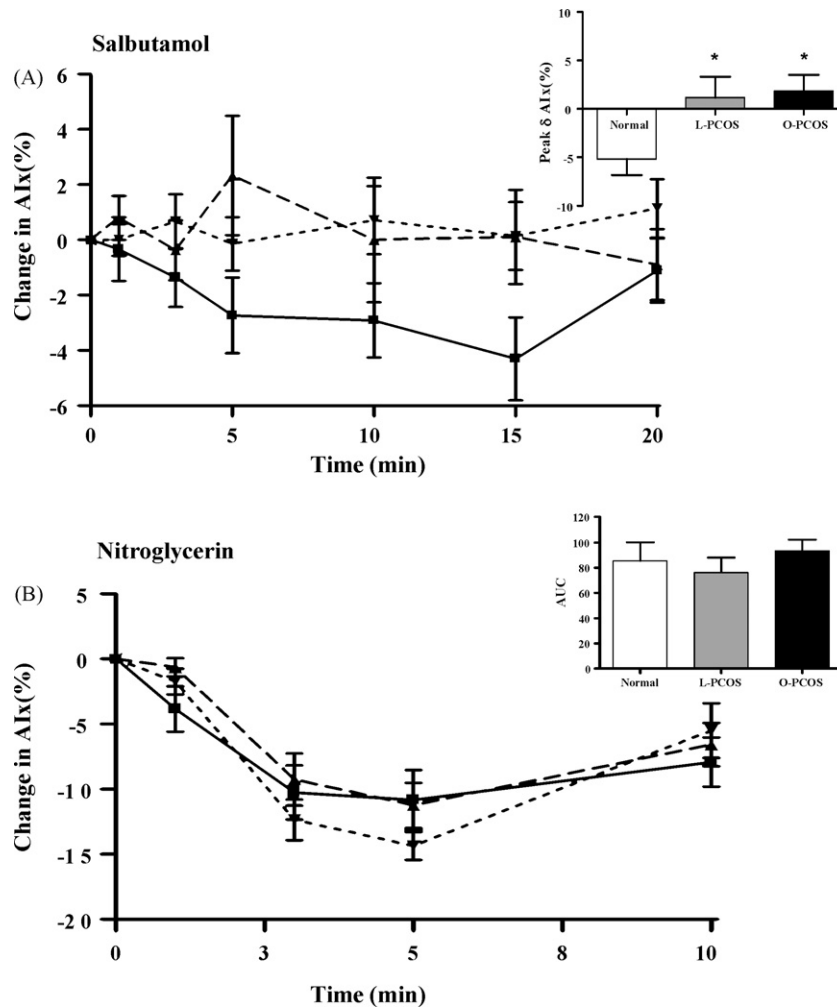
Plasma concentrations of MDA, ADMA and hs-CRP are summarized in Table 3.

MDA concentrations were elevated ( $P < 0.05$ ) in PCOS subjects independent of subgroup. ADMA concentrations were elevated in PCOS subjects relative to normals ( $0.57 \pm 0.02 \mu\text{mol/L}$  vs.  $0.51 \pm 0.01 \mu\text{mol/L}$ ;  $P < 0.01$ , ANOVA). However, this difference resulted largely from elevation in the O-PCOS group. Arginine:ADMA ratios were lower in PCOS than in normal subjects ( $P < 0.01$ ), whereas SDMA concentrations did not vary between groups (data not shown). Hs-CRP levels were also elevated in PCOS subjects ( $P < 0.02$ ), primarily due to marked elevation in the O-PCOS group.

## 4. Discussion

This highly selected cohort comparison study demonstrates that subjects with PCOS have markedly impaired platelet responsiveness to NO, irrespective of the presence/absence of obesity. This is a completely distinct additional abnormality of platelet function to previously described [15] hyperaggregability, and which is mechanistically linked [16] to the previously described endothelial dysfunction in such patients.

Given that platelet resistance to NO in patients with ischaemic heart disease is an adverse prognostic marker [18], this finding



**Fig. 3.** Endothelium-dependent and --independent responses.

Endothelial function was assessed by changes in augmentation index ( $AI_x$ ) in response to endothelium-dependent (salbutamol, Panel A) and --independent (NTG, Panel B) vasodilators. Peak change in  $AI_x$  in response to salbutamol (400  $\mu$ g) were significantly reduced in L-PCOS (–▲–), O-PCOS (–▼–) compared to the normal (–■–) group (insert Panel A, \* $P < 0.01$ ). No difference in area under the  $\delta AI_x$ :time curve of NTG was observed between the groups (insert, Panel B). AUC = area under the  $\delta AI_x$ :time curve.

provides an additional potential basis for regarding PCOS as a contributor to cardiovascular risk in women. The attenuation of NO responses in PCOS subjects is indeed similar in severity to that previously documented in patients with myocardial ischaemia [23,29,30].

The study evaluated a cross-section of clinical and biochemical parameters relevant to comparisons between control subjects, and L-PCOS vs. O-PCOS subsets: these constitute demographic information rather than study end-points. As regards the secondary end-points of the study, apart from the similarity in extent of disturbances of platelet and endothelial function in the O-PCOS vs. L-PCOS subgroups, the current findings are essentially confirmatory of the results of previous investigators.

Specifically, the study confirmed the presence of vascular endothelial dysfunction [9–14], together with platelet hyperaggregability [15] and activation of inflammatory markers [11,14,31]. Thus, the PCOS cohorts studied were similar to those characterized in a number of recent investigations of platelet and vascular function.

An area of some controversy in previous investigations has been the potential role of obesity-associated metabolic syndrome as a basis for physiological anomalies. In the current study, all but one woman did not meet the ATP III diagnostic criteria for metabolic syndrome. Thus, metabolic syndrome does not provide the sole

basis for either platelet or vascular functional anomalies in PCOS.

Given that platelet response to NO was diminished in PCOS subjects, it might have been expected that vascular responses to NO donors such as NTG would also be diminished, as found by Kravariti et al. [12], but not by other investigators [31,32]. It should also be acknowledged that not all investigators have demonstrated an association between PCOS and endothelial dysfunction [33–35]. It is likely that platelet responsiveness to NO is more markedly affected by increased superoxide anion concentrations than vascular responsiveness [36]. Similarly, endothelial progenitor cell counts, which partially reflect NO stimulation of bone marrow [37], were similar between PCOS and normal subjects. The data on endothelial progenitor cell counts also should be interpreted with caution, in that no uniform definition of such cells currently exists, and that endothelial progenitor cell function was not measured. Nevertheless, circulating endothelial progenitor cell counts usually correlate both with NO signaling [37] and with endothelial function [38].

The current study found no correlation between the extent of platelet NO resistance and either HOMA/QUICKI scores or plasma testosterone levels. Nevertheless, it is not possible, on the basis of lack of such correlations, to exclude completely the possibility that insulin resistance and/or hyperandrogenism contribute towards the pathogenesis of the observed NO resistance. As this subject

cohort did not meet criteria for metabolic syndrome, it remains uncertain whether the observed anomalies would have been accentuated in its presence.

The limited follow-up data in the current study establish that platelet NO resistance persists unchanged for a mean of 30 months post initial assessment. In the interests of further understanding the potential relationship between PCOS and cardiovascular risk in women [39], it is now of great interest to determine whether this anomaly remains undiminished as PCOS subjects approach the age of increased risk of cardiovascular events.

### Conflict of interest/disclosures

None.

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