

**Cardiovascular Risk  
Assessment in Women :  
Impact of Ageing,  
Polycystic Ovarian  
Syndrome and  
Menopause on Nitric  
Oxide Signalling**

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# Thesis Summary

Ageing represents an independent and strong risk factor for cardiovascular disease (Lakatta and Levy 2003), and in women, menopause appears to trigger a substantial increase in cardiovascular disease incidence (Castelli 1984). One potential basis for this observation is impairment of vascular endothelial function (Yasue, Matsuyama et al. 1990; Egashira, Inou et al. 1993). However no stratified comparisons of endothelial function or tissue responsiveness to nitric oxide (NO) with increasing age have previously been reported in either gender.

The objectives of the experiments contained in this thesis were therefore to:-

- 1) Characterise the putative variability in platelet and vascular responsiveness to NO in women of ages 18 to 60 years.
- 2) Compare this variability with that in vascular endothelial function and its biochemical determinants.
- 3) Compare the above-mentioned putative fluctuations to those present in age-matched patients with PCOS, a condition characterised by presence of impaired NO signalling in early adult life.
- 4) Determine the possible impact of menopause on NO signalling in vessels and platelets.

## **Methods**

In order to examine these objectives, we conducted a case-control study of women aged between 18 and 60 years, which allowed us to firstly, examine

NO signalling and various parameters in normal ageing women and then secondly, to compare these with women with PCOS. A subset of 40 perimenopausal women was studied prospectively to assess the relationship between menopause and platelet and vascular parameters.

PCOS women were selected based on Rotterdam criteria and women who were pregnant or on clopidogrel were excluded from the study. Inhibition of platelet aggregation by nitric oxide was the primary outcome measure. Vascular endothelial function utilizing applanation tonometry, plasma concentrations of  $N^G, N^G$ -dimethyl-L-arginine (ADMA) and endothelial progenitor cell count (EPC) were assessed as markers of endothelial dysfunction. High-sensitivity C-reactive protein (hs-CRP) was measured as a marker of inflammation.

## **Results**

The key findings from this thesis are:

- (1) With increasing age in normal women, there was progressive attenuation of platelet responses to NO (ANOVA,  $P < 0.0001$ ) with no significant changes in vascular NO responses.
- (2) There was also evidence of endothelial dysfunction with increasing age ( $p < 0.0001$ ) which was accompanied by elevation of ADMA concentrations with increasing age ( $p = 0.003$ ).
- (3) Irrespective of age, PCOS women exhibited greater impairment of platelet NO responses and endothelial function ( $p < 0.05$ , 2 way ANOVA) compared to normal women. Furthermore, these anomalies were evident in PCOS women from an early age but had a tendency to converge with normal women above the age of 40 years.

(4) The changes in platelet and endothelial function in normal women were not correlated with oestradiol levels.

### **Conclusions**

Normal ageing in women is associated with attenuation of NO-based signalling in platelets and blood vessels. In women with PCOS, these changes are present from early adult life, which may form the pathophysiological basis for premature atherogenesis seen in these individuals. The changes in NO signalling are not totally attributable to the onset of menopause.

## Glossary of abbreviations

Abbreviation	Definition
AA	Arachidonic Acid
ACEI	Angiotensin converting enzyme inhibitor
ASK-1	Apoptosis signal-regulating kinase 1
ADMA	Asymmetric $N^G, N^G$ -dimethyl-L-arginine
ADP	Adenosine diphosphate
Aix	Augmentation Index
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ATRA	Angiotensin II receptor antagonist
BH <sub>4</sub>	Tetrahydrobiopterin
BMI	Body mass index
C	Celsius
Ca <sup>2+</sup>	Calcium
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CVD	Cardiovascular disease
DAG	Diacylglycerol
DDAH	Dimethylarginine dimethylaminohydrolase
DM	Diabetes mellitus
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
EPC	Endothelial progenitor cell
FBF	Forearm blood flow
FA	Flavin adenine dinucleotide



FMD	Flow-mediated dilatation
FMN	Flavin mononucleotide
GP	Glycoprotein
GTP	Guanosine triphosphate
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HDL	High density lipoprotein
HOMA-IR	Homeostasis model of insulin resistance
HPLC	High performance liquid chromatography
HRT	Hormone replacement therapy
hs-CRP	High-sensitivity C-reactive protein
IL	Interlukin
iNOS	Inducible nitric oxide synthase
IP <sub>3</sub>	Inositol 1,4,5-triphosphate
IR	Insulin resistance
ISDN	Isosorbide dinitrate
LDL	Low-density lipoprotein
L-NMMA	N <sup>G</sup> -monomethyl-L-arginine
MBS	Metabolic syndrome
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NO <sup>·</sup>	Nitric oxide free radical
NO <sup>+</sup>	Nitrosonium
NO <sup>-</sup>	Nitoxyl
NOS	Nitric oxide synthase
NTG	Nitroglycerin
O <sub>2</sub> <sup>-</sup>	Superoxide

OC	Oral contraceptive
PAI-1	Plasminogen activator-1
PCOS	Polycystic ovary syndrome
PG	Prostaglandin
PIP <sub>2</sub>	Phosphatidylinositol 4,5-bisphosphate
PKC	Protein kinase C
PLA <sub>2</sub>	Phospholipase A <sub>2</sub>
PLC	Phospholipase C
PRMT	Protein arginine methyltransferases
PRP	Platelet-rich plasma
PWA	Pulse wave analysis
ROS	Reactive oxygen species
SD	Standard deviation
SDMA	N <sup>G</sup> ,N <sup>G'</sup> -dimethyl-L-arginine
SEM	Standard error of mean
sGC	Soluble guanylate cyclase
SNP	Sodium Nitroprusside
SOD	Superoxide dismutase
TGFβ	Transforming growth factor-beta
TRX	Thioredoxin
TSP-1	Thrombospondin-1
TxA <sub>2</sub>	Thromboxane A
TXNIP	Thioredoxin-interacting protein
vWf	Von Willebrand factor

## Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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## Publications, presentations and awards

### Peer reviewed articles relating to this thesis

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Rajendran S, Willoughby SR, **Chan WP**, Liberts EA, Heresztyn T, Saha M, Marber MS, Norman RJ, Horowitz JD. Polycystic ovary syndrome is associated with severe platelet and endothelial dysfunction in both obese and lean subjects. *Atherosclerosis* 2009 Jun;204(2):509-14.

Ngo DT, **Chan WP**, Rajendran S, Heresztyn T, Amarasekera A, Sverdlov AL, O'Loughlin PD, Morris HA, Chirkov YY, Norman RJ, Horowitz JD. Determinants of insulin responsiveness in young women: Impact of polycystic ovarian syndrome, nitric oxide, and vitamin D. *Nitric Oxide* 2011 Oct 30;25(3):326-30

Willoughby SR, Rajendran S, **Chan WP**, Procter N, Leslie S, Liberts EA, Heresztyn T, Chirkov YY, Horowitz JD. Ramipril Sensitizes Platelets to Nitric Oxide: Implications for Therapy in High-Risk Patients. *J Am Coll Cardiol* 60(10): 887-894

**Chan WP** Ngo DT, Sverdlov AL, Rajendran S, Heresztyn T, Stafford I, Chirkov YY, Horowitz JD. Premature ageing of cardiovascular/platelet function in polycystic ovarian syndrome. Accepted by *American Journal of Medicine* 16 Jan 2013, in press.

### Accepted presentations at international meetings

**Chan WP**, Ngo DT, Rajendran S, Horowitz JD. Implications of insulin sensitivity on inflammatory activation and atherogenic risk in normal and PCOS females  
*Heart, Lung and Circulation* 2010;19S:S58  
*Cardiac Society of Australia and New Zealand Annual Scientific Meeting, Adelaide 2010 and European Society of Cardiology Congress 2010, Stockholm, Sweden*

Ngo DT, **Chan WP**, Rajendran S, Sverdlov AL, Horowitz JD. Polycystic ovary syndrome is associated with insulin resistance independent of obesity, vitamin D status and inflammatory activation  
*Heart, Lung and Circulation* 2010;19S:S60

*Cardiac Society of Australia and New Zealand Annual Scientific Meeting, Adelaide 2010*

Ngo DT, **Chan WP**, Rajendran S, Heresztyn T, Amaresekera A, Sverdlov AL, Chirkov YY, Horowitz JD.

Insulin resistance in polycystic ovarian syndrome and obesity: correlations with ADMA and inflammatory activation

*5<sup>th</sup> International Symposium on ADMA, Chicago 2010*

**Chan WP**, Sverdlov AL, Ngo DT, Rajendran SR, Chirkov YY, Horowitz JD. Impact of ageing on the platelet and vascular responses in women with polycystic ovary syndrome

*Oral and poster presentation at European Society of Cardiology Congress 2011, Paris*

**Chan WP**, Sverdlov AL, Ngo DT, Chirkov YY, Horowitz JD.

Does oral contraceptive therapy induce platelet nitric oxide resistance?

*European Society of Cardiology Congress 2011, Paris*

**Chan WP**, Ngo DT, Sverdlov AL, Chirkov YY, Horowitz JD.

Effects of Ageing on Nitric Oxide Signalling in Women: Comparison with Polycystic Ovarian Syndrome.

*American Heart Association Scientific Sessions 2012, Los Angeles*

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*Cardiac Society of Australia and New Zealand Annual Scientific Meeting, Brisbane 2012*

*Awarded South Australian Cardiovascular PhD student/Cardiac Fellow Research Prize, SA Heart Education and Research Foundation June 2012*

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