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

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January 2013

A thesis submitted to the University of Adelaide as the requirement for the degree of Doctor of Philosophy

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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 |
| $BH_4$ | Tetrahydrobiopterin |
| BMI | Body mass index |
| C | Celsius |
| $Ca^{2+}$ | Calcium |
| cAMP | Cyclic adenosine monophsophate |
| cGMP | Cyclic guanosine monophosphate |
| CVD | Cardiovascular disease |
| DAG | Diacylglycerol |
| DDAH | Dimethylarginine dimethlyaminohydrolase |
| 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 |
| $H_2O_2$ | 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 |
| $IP_3$ | Inositol 1,4,5-triphosphate |
| IR | Insulin resistance |
| ISDN | Isosorbide dinitrate |
| LDL | Low-density lipoprotein |
| L-NMMA | $N^G$-monomethyl-L-arginine |
| MBS | Metabolic syndrome |
| MDA | Malondialdehyde |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| nNOS | Neuronal nitric oxide synthase |
| NO | Nitric oxide |
| $NO^{\cdot}$ | Nitric oxide free radical |
| $NO^+$ | Nitrosonium |
| $NO^-$ | Nitoxyl |
| NOS | Nitric oxide synthase |
| NTG | Nitroglycerin |
| $O_2^-$ | Superoxide |

| | |
|---|---|
| OC | Oral contraceptive |
| PAI-1 | Plasminogen activator-1 |
| PCOS | Polycystic ovary syndrome |
| PG | Prostaglandin |
| $PIP_2$ | Phosphatidylinositol 4,5-bisphosphonate |
| PKC | Protein kinase C |
| $PLA_2$ | Phospholiapse $A_2$ |
| PLC | Phospholipase C |
| PRMT | Protein arginine methyltransferases |
| PRP | Platelet-rich plasma |
| PWA | Pulse wave analysis |
| ROS | Reactive oxygen species |
| SD | Standard deviation |
| SDMA | $N^G$,$N^{G'}$-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 |
| $TxA_2$ | 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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# Acknowledgements

I wish to take this opportunity to thank all the wonderful people who have been involved in making this thesis a reality for me.

Firstly, my heartfelt thanks to my principle supervisor, Professor John Horowitz, who has (over many cups of coffees) given me endless time, encouragement and support. Without his brilliance and enthusiasm, this thesis would not have been possible. Many thanks also to my co-supervisor, Dr Yuliy Chirkov, for taking on this project and sharing his knowledge on everything to do with platelets.

I am also grateful for my dear friends, Dr Aaron Sverdlov and Dr Doan Ngo, for their practical and emotional support. Thanks also to Dr Sharmalar Rajendran for convincing Prof to be interested in women's' health, and to my fellow PhD students, Ha Nguyen and Nathan Procter, for sharing the joys and hardships of research with me.

I would also like to thank all the Cardiology laboratory staff, Geraldine, Irene and Tamila, for helping me find my feet in the lab and running of various assays.

Finally, to my wonderful husband Alex, it would not have been possible to complete the PhD without your love, support and A.D. Rodgers funding. Thank you for believing in me. We were also blessed with the arrival of "the best things in our lives" during my studies, our two precious girls, Sophie and Maddie. Also thanks to Mom, Dad, Greg and Sylvia for "being there".

# Publications, presentations and awards

## Peer reviewed articles relating to this thesis

**Chan WP**, Sverdlov AL, Horowitz JD. Management of the metabolic syndrome in cardiovascular disease. Curr Treat Options Cardiovasc Med.2008 Feb;10(1):27-38.

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
*5th 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*
*(Nominated for Outstanding Research by an Early Career Investigator by Arteriosclerosis, Thrombosis and Vascular Biology Council, 2012)*
*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*

## Awards/grants relating to this thesis

South Australian Health Scholarship (Divisional Scholarship) 2006-2010

Cardio Vascular Lipid Research Grant 2009