Sex-Dependent Differences in Vasomotor Responses of Older Male and Female Humans

A Thesis Submitted to The University of Adelaide as the requirement for the degree of Doctor of Philosophy

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Declaration

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Abstracts, Presentations and Awards derived from this thesis

Published Abstracts

1. **Jaghoori A**, Worthington M, Edwards J, Stuklis R, Wilson D, Beltrame J. Gender differences in internal mammary artery vasoconstrictor responses. Heart, Lung and Circulation. 01/2011; 20:S8-S9

2. **Jaghoori A**, Jakobczak R, Worthington M, Edwards J, Stuklis R, Wilson D, Beltrame J. Sex differences in vascular reactivity of internal mammary artery and subcutaneous microvessels. Heart, Lung and Circulation. 01/2012; 21:S8-S9

3. **Jaghoori A**, Jakobczak R, Wilson D, Beltrame J. Heterogeneous vasoconstrictor responses amongst men and women ASCEPT 2012

4. **Jaghoori A**, Jakobczak R, Worthington M, Edwards J, Wilson D, Beltrame J.Vascular hyper-reactivity in women with and without coronary artery disease. Global Heart. 9/2014; 1S:e28-e29

Conference proceedings

1. **Jaghoori A,** Worthington M, Edwards J, Stuklis R, Wilson D, Beltrame J. Gender differences in internal mammary artery vasoconstrictor responses**.** Cardiac Society of Australia and New Zealand 2011. **Poster Presentation.**

2. **Jaghoori A,** Worthington M, Edwards J, Stuklis R, Wilson D, Beltrame J. Gender differences in internal mammary artery vasoconstrictor responses**.** Postgraduate Conference, The University of Adelaide 2011. **Poster Presentation.**

3. **Jaghoori A,** Worthington M, Edwards J, Stuklis R, Wilson D, Beltrame J. Gender differences in internal mammary artery vasoconstrictor responses**.** The Queen Elizabeth Hospital Annual Research Day, Adelaide, Australia 2011

4. **Jaghoori A,** Jakobczak R, Worthington M, Edwards J, Stuklis R, Wilson D, Beltrame J. Sex differences in vascular reactivity of internal mammary artery and subcutaneous microvessels. Cardiac Society of Australia and New Zealand 2012. **Oral Presentation.**

5. **Jaghoori A,** Jakobczak R, Wilson D, Beltrame J. Heterogeneous vasoconstrictor responses amongst men and women. Australian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) conference 2012. **Poster Presentation.**

6. **Jaghoori A,** Jakobczak R, Wilson D, Beltrame J. Hyper-constrictive responses of female large and small arteries. The Queen Elizabeth Hospital Annual Research Day, Adelaide, Australia 2012. **Oral Presentation.**

7. **Jaghoori A,** Jakobczak R, Worthington M, Edwards J, Stuklis R, Wilson D, Beltrame J. Hypersensitivity to vasoconstrictors in the female internal mammary artery. The Queen Elizabeth Hospital Annual Research Day, Adelaide, Australia 2013. **Oral Presentation.**

8. **Jaghoori A,** Jakobczak R, Worthington M, Edwards J, Wilson D, Beltrame J. Vascular hyper-reactivity in women with and without coronary artery disease. Ralph Reader Prize (clinical sessions), World Cardiology Congress (WCC) 2014. **Oral Presentations.**

9. **Jaghoori A,** Jakobczak R, Worthington M, Edwards J, Wilson D, Beltrame J. Sex differences in vasomotor reactivity. State of Heart, annual scientific meeting of the International Society of Cardiovascular Pharmacology 2014. **Poster Presentation**

Prizes and Awards during the course of Doctorate

1. First prize for the best oral in Junior PhD student section in TQEH Research Day.2011
2. First prize for the best poster presentation in basic mechanisms section in Post Graduate conference 2011, University of Adelaide.
3. First prize for the best poster in school of Medicine in Post Graduate conference 2011, University of Adelaide.
4. First prize for the best poster presentation in cardiovascular special interest group prize, Australian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) conference 2012
5. Runner up for the International Society of Heart Research (ISHR) young investigator award in the Cardiac Society of Australia and New Zealand (CSANZ) conference 2012
6. Finalist for the Ralph reader prize (clinical sessions), World Cardiology Congress (WCC) 2014.
7. Awarded Cardiac Society of Australia and New Zealand (CSANZ) 2011 travel grants.
8. Awarded Faculty of health sciences, University of Adelaide, Postgraduate travelling fellowships 2012.
9. Awarded Ivan Delaland travel grant for outstanding PhD candidate in Cardiology and Pharmacology, The Queen Elizabeth Hospital 2012
10. Awarded Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists student travel grant 2012.

List of thesis figures and tables

List of abbreviations

CVD	Cardiovascular Disease
LDL	Low Density Lipoprotein
VCAM-1	Vascular Cell Adhesion Molecule-1
VLDL	Very Low-Density Lipoprotein
HDL	High-Density Lipoprotein
AII	Angiotensin II
AT1	Angiotensin II Type 1
VSMC	Vascular Smooth Muscle Cell
IVUS	Intravascular Ultrasound
FMD	Flow Mediated Dilation
CAD	Coronary Artery Disease
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
GC	Guanylate Cyclase
cGMP	cyclic Guanosine-3′,5′-monophosphate
PKG	Protein Kinase G
CAM	Calmodulin
eNOS	Endothelial Nitric Oxide Synthase
PI3K	Phosphatidylinositol 3-kinase
PKB	Protein Kinase B
nNOS	Neuronal Nitric Oxide Synthase
iNOS	Inducible Nitric Oxide Synthase
BH4	Tetrahydrobiopterin
ADMA	Asymmetrical Dimethylarginine
COX	Cyclo-Oxygenase
PGI_2	Prostacyclin
TXA_2	Thromboxane
cAMP	cyclic Adenosine Monophosphate
NSAID	Nonsteroidal Anti-Inflammatory Drugs
ET	Endothelin
PLC	Phospholipase C
IP3	Inositol triphosphate
DAG	Diacylglycerol
PKC	Protein Kinase C
EDHF	Endothelial Derived Hyperpolarizing Factor
PE	Phenylephrine
ROK	Rho-associated Kinase
5HT	5-Hydroxytriptamine
MLCK	Myosin Light Chain Kinase
MLCP	Myosin Light Chain Phosphatase
MYPT	Myosin Phosphatase Targeting subunit
PIP2	Phosphotidylinositol 4,5-biphosphate
GDP	Guanosine Diphosphate
GTP	Guanosine Triphosphate
GEF	Guanine nucleotide Exchange Factor
GAP	GTPase-Activating Proteins

ACS	Acute Coronary Syndrome
MI	Myocardial Infarction
AMI	Acute Myocardial Infarction
STEMI	ST-Elevation Myocardial Infarction
NSTEMI	Non- ST-Elevation Myocardial Infarction
ECG	Electrocardiogram
ACE	Angiotensin Converting Enzyme
ARB	Angiotensin Receptor Blocker
PCI	Percutaneous Coronary Intervention
CABG	Coronary Artery Bypass Graft
IMA	Internal Mammary Artery
SV	Saphenous Vein
CHD	Coronary Heart Disease
BMI	Body Mass Index
IHD	Ischaemic Heart Disease
PVD	Peripheral Vascular Disease
FRS	Framingham risk score
CRP	C-reactive Protein
PTD	Pain-To-Door
DTB	Door-To-Balloon
LTB	Lab-To-Balloon
WISE	Women's Ishaemia Syndrome Evaluation study
MVD	Microvascular Dysfunction
QOL	Quality of Life
NHT	Normal HEPES-Tyrode
KPSS	Potassium Physiological Salt Solution
KCl	Potassium Chloride
L-NAME	Ng-nitro-L-arginine methyl ester
DFP	Diisopropylfluorophosphate
DTT	Dithiothreitol
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
APS	Ammonium Persulfate
TEMED	N,N,N',N'-tetramethylethylenediamine
TBS-T	Tris buffered saline – Tween 20
IgG	Immunoglobulin G
ANOVA	Analysis of Variance

Thesis Abstract

Background and Aims: Sex differences have been observed in several cardiovascular diseases, in terms of mortality and morbidity. Female patients experience worse clinical outcomes than their male counterparts. Although multiple mechanisms may be involved, sex differences in vascular reactivity of large and small blood vessels have not been investigated. This thesis aims to assess sex-dependent difference in vasoconstrictor responses of human vessels isolated from a variety of vascular beds from older patients (mean age 68 years) with and without existing coronary artery disease. Specific aims include evaluation of :**(1a)** sex differences in vascular responses of internal mammary artery (IMA) and saphenous vein (SV) segments from male and female patients undergoing CABG and **(1b)** mechanisms underlying sex dependent vascular responses. **(2)** sex differences in microvascular reactivity of vessels isolated from mediastinal and peripheral subcutaneous areas in patients with CAD. **(3a)** sex difference in vascular reactivity of subcutaneous microvessels from patients with no known CAD, undergoing elective non-cardiac surgery. **(3b)** subcutaneous microvascular reactivity of males and females patients with CAD to those without known CAD.

Methods: This thesis used wire myography technique to assess functional changes in vasoconstrictor responses of isolated large conduit and small blood vessels. Concentration-response curves were formed for various vasoconstrictors including phenylephrine, serotonin, endothelin-1 and the thromboxane mimetic, U46619. Western blot analysis was employed to measure the biochemical parameters, including receptor abundance endothelin-1.

Summary of major findings: Female IMA segments display hypersensitive responses to serotonergic and α_1-adrenergic receptor stimulation, compared to males. Blocking eNOS and/or cyclooxygenase revealed that prostaglandins account for in the observed α_1-adrenergic mediated sex differences. Biochemical analysis revealed increased density of $5HT_{2A}$ receptors in the female IMA.

Similar sex differences were observed in the pericardial microvessels of the same patient cohort, with females showing increased sensitivity to serotonergic and α_1-adrenergic receptor stimulation. Interestingly, no sex differences were observed in the peripheral subcutaneous microvessels of patients with existing CAD.

In patients without known CAD, female subcutaneous microvessels were hypersensitive to serotonergic and α_1-adrenergic receptor stimulation, compared to matched males. When compared to subcutaneous microvessels of male and female patients without known CAD, male and female CAD patients exhibited increased sensitivity to α_1-adrenergic agonist. Male CAD patients were also hypersensitive to serotonin and the thromboxane A_2 mimetic, U46619, relative to those without known CAD.

Conclusions: For the first time, in a population cohort with a mean age of 68 years, female vascular hyper-reactivity in both large graft arteries (IMA) and microvessels has been demonstrated. Female vascular hypersensitivity is consistently seen in response to serotonergic and α_1-adrenergic receptor agonist. In part, this may be due to sex-differences in prostanoid activity. The IMA hyper-reactivity in the group of older women may contribute to their poorer outcomes following CABG and microvascular differences amongst patients without documented cardiovascular disease may pre-

dispose them to hypertension.