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

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Declaration

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Signed

Amenah Jaghoori
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**Prizes and Awards during the course of Doctorate**

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.
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.
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**CHAPTER 6**

Table 1  
Summary of the observed female vascular hypersensitivity in response to specific agonists in different vascular beds.
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
PGI2  Prostacyclin
TXA2  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  Phosphatidylinositol 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
LTD  Lab-To-Balloon
WISE  Women’s Ischaemia 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 $\alpha_1$-adrenergic receptor stimulation, compared to males. Blocking eNOS and/or cyclooxygenase revealed that prostaglandins account for in the observed $\alpha_1$-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 $\alpha_1$-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 $\alpha_1$-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 $\alpha_1$-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 $\alpha_1$-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.