EVALUATION OF ALTERATIONS IN CARDIOVASCULAR

STRUCTURE AND FUNCTION IN

END-STAGE RENAL FAILURE

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To Annabel, without whom

none of this would have been possible.
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ABSTRACT

Background:
Chronic renal dysfunction is associated with myriad alterations in cardiovascular structure and function, resulting in markedly elevated rates of cardiac and vascular morbidity and mortality. Utilising advances in cardiovascular magnetic resonance imaging (CMR), we evaluated the cardiovascular sequelae of arterio-venous fistula formation in advanced chronic kidney disease, and the impact of elective arterio-venous fistula ligation following successful renal transplantation. Furthermore, we undertook to evaluate the diagnostic accuracy of dobutamine-stress CMR in the detection of haemodynamically-significant coronary artery disease prior to renal transplantation. Finally, we invasively evaluated coronary endothelial function in the presence of advanced renal dysfunction, and compared this to subjects with preserved renal function.

Methods / Results:
Study 1: CMR was undertaken to evaluate cardiac structure and function, brachial artery endothelial function (as assessed by flow-mediated dilatation) and aortic distensibility in twenty-four subjects at baseline, and 6-months following, clinically indicated arterio-venous fistula creation in preparation for the commencement of haemodialysis for end-stage renal failure. Following arterio-venous fistula creation, mean cardiac output increased by 25.0% (p<0.0001), with substantial associated increases in left and right ventricular volumes, left and right atrial area and left ventricular mass (12.7% increase,
Peripheral endothelial function was significantly impaired at follow-up (9.0±9% vs. 3.0±6%, p=0.01). No significant change in aortic distensibility was identified.

**Study 2:** Cardiac and vascular function were similarly assessed utilising CMR in eighteen subjects prior to, and 6-months following, clinically indicated arterio-venous fistula ligation in the context of successful, stable renal transplantation. Following AVF-ligation, mean cardiac output fell by 15.6% (p=0.004), with significant attendant decreases in atrial and ventricular chamber dimensions. Notably, left ventricular mass fell by 9.7% (p=0.0001) at follow-up. Aortic distensibility was unchanged following AVF-ligation, though endothelial function improved significantly (2.5±6.5% vs. 8.0±5.9%, p=0.043).

**Study 3:** Dobutamine-stress CMR was performed in twenty-one subjects prior to clinically-induced invasive coronary angiography before potential renal transplantation. Dobutamine-stress CMR demonstrated 100% sensitivity and 93% specificity for the detection of angiographically significant coronary disease (≥70% stenosis severity). This compared favourably to results for the institutional-standard (SPECT: sensitivity 67%, specificity 38%; p<0.0001 compared to CMR).

**Study 4:** At invasive coronary angiography, endothelium-dependent and endothelium-independent coronary endothelial and microvascular function were evaluated amongst eight pre-renal transplant subjects with only minimal coronary artery disease (≤20% epicardial coronary stenoses). Utilising intra-coronary infusions of acetylcholine (10⁻⁷M and 10⁻⁶M), adenosine (48mcg) and glyceryl tri-nitrate (100mcg), results were compared to thirteen control subjects with minimal coronary artery disease but comparatively preserved renal
function. There was no significant difference in endothelium-dependent or endothelium-independent coronary endothelial function between the cohorts. Microvascular function (as assessed by coronary flow reserve following adenosine administration) was markedly impaired in subjects with advanced renal impairment compared to controls (1.9±0.4 vs. 3.0±1.1, p=0.01).

Conclusions:
Chronic kidney disease is associated with substantial alterations in cardiovascular structure and function. Arterio-venous fistulae, though necessary for the performance of haemodialysis, appear to contribute significantly to the high burden of cardiovascular maladaptation present in this condition. Recent advances in CMR and stress-CMR may play a significant role in improving the detection of sub-clinical cardiovascular disease in these high-risk patients.
DECLARATION

This body of work contains no material which has been accepted for the award of any other degree or diploma in any university or 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.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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ACKNOWLEDGMENTS

It was with no small amount of trepidation that I initially committed to the commencement of this PhD. Having never been exposed to meaningful research during my under-graduate education or post-graduate clinical training, as an advanced trainee in Cardiology the idea of removing myself from clinical medicine for three years seemed a path both onerous and of little lasting professional value – merely a hoop to be jumped through in order to “tick the research box”. It is with great gratitude and humility that I commit this Thesis to the numerous colleagues who have mentored, aided and supported me through an experience that has proven to be of immense professional and personal value.

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### Abbreviations

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<tr>
<td>Ach</td>
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</tr>
<tr>
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<td>Acute Coronary Syndrome</td>
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<tr>
<td>ADMA</td>
<td>Asymmetric dimethylarginine</td>
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<tr>
<td>AF</td>
<td>Atrial Fibrillation</td>
</tr>
<tr>
<td>AGE</td>
<td>Advanced Glycosylation End-products</td>
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<tr>
<td>Alx</td>
<td>Augmentation Index</td>
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<tr>
<td>ANP</td>
<td>Atrial Natriuretic Peptide</td>
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<tr>
<td>ANZDATA</td>
<td>Australian and New Zealand Dialysis and Transplant Registry</td>
</tr>
<tr>
<td>APV</td>
<td>Average Peak Velocity</td>
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<tr>
<td>ARVC</td>
<td>Arrhythmogenic Right Ventricular Cardiomyopathy</td>
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<td>ASCOT</td>
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<td>Cardiovascular Magnetic Resonance Imaging</td>
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<td>Cardiac Output</td>
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CrCL  Creatinine Clearance
CT    Computed Tomography
CV    Cardiovascular
CVD   Cardiovascular Disease
DBP   Diastolic Blood Pressure
eGFR  Estimated Glomerular Filtration Rate
eNOS  Endothelial Nitric Oxide Synthase
EPO   Erythropoietin
ESKD  End Stage Kidney Disease
ESRF  End-stage Renal Failure
ET-1  Endothelin-1
FAME  Fractional Flow Reserve versus Angiography for Multivessel Evaluation
FFR   Fractional Flow Reserve
FISP  Fast-imaging with Steady State Free Precession
FMD   Flow-mediated Dilatation
FOV   Field of View
GFR   Glomerular Filtration Rate
GTN   Glyceryl Tri-nitrate
Hb    Haemoglobin
HDx   Haemodialysis
HMG-CoA Hydroxyl-methylglutaryl coenzyme-A
HR    Heart Rate
ICA   Invasive Coronary Angiography
IHD   Ischaemic Heart Disease
<table>
<thead>
<tr>
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<tr>
<td>IMR</td>
<td>Index of Microvascular Resistance</td>
</tr>
<tr>
<td>IQR</td>
<td>Inter-quartile Range</td>
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<td>IVUS</td>
<td>Intravascular Ultrasound</td>
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<tr>
<td>K/DOQI</td>
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<tr>
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<td>New York Heart Association</td>
</tr>
<tr>
<td>oxLDL</td>
<td>Oxidised Low Density Lipoprotein</td>
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PAP  Pulmonary Artery Pressure
PCI  Percutaneous Coronary Intervention
PGI₂  Prostacyclin (Prostaglandin I₂)
PHT  Pulmonary Hypertension
PP  Pulse Pressure
PPV  Positive Predictive value
PTCA  Percutaneous Trans-luminal Coronary Angioplasty
PTH  Parathyroid Hormone
PWV  Pulse-wave Velocity
QALY's  Quality-Adjusted Life Years
QCA  Quantitative Coronary Angiography
RA  Right Atrial
RAAS  Renin-Angiotensin-Aldosterone System
RRT  Renal Replacement Therapy
RTx  Renal Transplantation
RV  Right Ventricular
SBP  Systolic Blood Pressure
SPECT  Single Photon Emission Computed Tomography
SSFP  Steady-State Free Precession
STEMI  ST-segment Elevation Myocardial Infarction
TIIDM  Type II Diabetes mellitus
TE  Echo Time
TR  Repetition Time
TTE  Trans-thoracic Echocardiography
TXA₂  Thromboxane
UVB  Ultraviolet B

25(OH)VinD3  25-hydroxyvitamin D₃
BACKGROUND
**Chronic Kidney Disease**

Chronic kidney disease (CKD) is defined as any condition associated with evidence of kidney damage and/or reduced renal function, present for at least 3-months. Classified according to the National Kidney Foundation, CKD has been stratified according to the degree of renal impairment, ranging from mild renal injury (Stage 1), which is generally asymptomatic, through to End-Stage Kidney Disease (ESKD or Stage 5). Classification is based around urine content and glomerular filtration rate (GFR).

<table>
<thead>
<tr>
<th>Stage</th>
<th>GFR</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>≥ 90</td>
<td>Normal renal function, but urine or other abnormalities indicative of renal dysfunction (e.g. proteinuria).</td>
</tr>
<tr>
<td>2</td>
<td>60-89</td>
<td>Mildly reduced renal function, with urine or other abnormalities indicative of renal disease.</td>
</tr>
<tr>
<td>3</td>
<td>30-59</td>
<td>Moderately reduced renal function.</td>
</tr>
<tr>
<td>4</td>
<td>15-29</td>
<td>Severely reduced renal function.</td>
</tr>
<tr>
<td>5</td>
<td>&lt;15</td>
<td>Very severe renal disease. Commonly referred to as end-stage kidney disease (ESKD)</td>
</tr>
</tbody>
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*Table 1: Classification of Stages of Chronic Kidney Disease according to the National Kidney Foundation Kidney Disease Outcome Quality Initiative (K/DOQI) Advisory Board Findings.*

**Glomerular Filtration Rate**

GFR is the volume of fluid filtered from the renal glomerular capillaries into Bowman's capsule, per unit time (minutes). GFR is a substantially more accurate measure of renal function than the routine evaluation of serum creatinine, as
serum creatinine will remain within most laboratories’ normal range until ≥50-60% of total kidney function is lost. Importantly, GFR is a measure of glomerular function, rather than renal tubular function, and as such, many of the homeostatic functions of the kidney may remain relatively intact despite declining GFR. Specifically, the tubular mechanisms maintaining water and sodium/potassium homeostasis, as well as acid-base balance, remain robust, even unto severe renal dysfunction. As such, many patients are unaware of the presence of significant renal dysfunction (as assessed by GFR) due to an absence of apparent symptoms or clinical signs (such as oedema due to reduced free water clearance). This tubular compensation for glomerular impairment/loss gives rise to the potential for the ‘silent’ advance of renal dysfunction, in the absence of surveillance urinalysis or blood tests in at-risk individuals (e.g. diabetic and/or hypertensive patients).

Numerous methods exist for the evaluation of GFR, with variable associated diagnostic accuracy and prognostic value. The “gold standard” method for evaluating GFR involves the administration of a chemical that maintains a steady pharmacokinetic profile within the serum, with no extra-renal excretion, and is freely filtered at the glomerulus, but undergoes neither reabsorption nor secretion within the renal tubules. A fixed dose is administered intravenously, with subsequent urine excretion quantified. Inulin is the classical agent used for this purpose, although radiopharmaceuticals are also routinely used in clinical practice.

Such ideal properties for assessing GFR are distinct from serum creatinine, which although freely filtered at the glomerulus, is known to undergo active tubular secretion, with substantial inter- and intra-individual variability due to the
saturable nature of the tubular secretory process. Furthermore, tubular secretion of creatinine may be impaired or blocked by various pharmaco-therapeutic agents (e.g. trimethoprim and cimetidine), and hence measurement of serum creatinine in patients receiving these agents may provide a spurious indication of altered renal excretory function relative to previous measurements.3

Furthermore, as renal function declines, there is a rise in extra-renal degradation of creatinine,4 potentially leading to under-estimation of the decline in renal function due to attenuation of the rise in serum creatinine arising from decreased renal clearance.5,6 Thus, serum creatinine, and derived creatinine clearance, may over- or under-estimate true GFR, depending on the stage of renal disease and concomitant therapies. Confusingly, these terms (‘creatinine clearance’ and ‘glomerular filtration rate’) are often used interchangeably in clinical practice.

Currently, the most commonly used estimate of renal excretory function is the ‘estimated GFR’ (eGFR), which is now routinely reported as a component of results from renal function serological assays. This measure was developed by the Modification of Diet in Renal Disease Study Group [MDRD], and is most commonly derived from four key variables: serum creatinine, age, gender and race.7

For estimation of eGFR for creatinine values in μmol/L:

\[
eGFR = 32,788 \times [\text{Serum Creatinine}]^{1.154} \times [\text{Age}]^{-0.203} \times [0.742 \text{ if female}]
\]

For individuals of African heritage, results from the above equation are then multiplied by 1.210, due to the relatively greater mean muscle mass within this racial group. Application of this equation to the Australian Aboriginal population
remains contentious and further work is required in this area – particularly in light of the relatively high prevalence of CKD in the Australian Indigenous population.

The MDRD equations have been validated in a variety of CKD cohorts, however these formulae (the common 4-variable eGFR, and the original 6-variable version which also includes blood urea nitrogen and serum albumin) underestimate GFR in healthy patients with GFR>60mL/min.\textsuperscript{9,10} As such, in clinical practice, values greater than this are generally reported as “eGFR >60mL/min/1.73m\textsuperscript{2}”.

Prior to the development of the MDRD eGFR, GFR was more commonly estimated utilizing the Cockcroft-Gault formula. First published in 1976\textsuperscript{11}, this equation estimates an individual’s “creatinine clearance” (eCrCL), as a measure of GFR.

\[
\text{eCrCL} = \frac{(140-\text{age}) \times \text{Body weight} \times \text{Constant}}{\text{Serum Creatinine (μmol/L)}}
\]

Where the constant is 1.23 for men and 1.04 for women. As this equation utilises serum creatinine, age, gender, and body mass (in kilograms) to estimate creatinine production, as well as clearance, it is widely agreed that the derived values provide a more individualised estimate of glomerular function than eGFR, as the eGFR methodology underestimates true GFR in heavy people, and overestimates glomerular function in underweight individuals. The requirement for clinical details such as body weight inhibits the use of this equation in the routine reporting of serum biochemistry however.
Disease Burden

Despite substantial advances in health care in recent decades, CKD accounts for a significant and growing burden of morbidity and mortality in Australia.\textsuperscript{12} Approximately 50\% of Australians aged 65 or over have significant renal impairment and an increasing number are progressing to End Stage Renal Failure (ESRF) requiring renal replacement therapy (RRT) (dialysis or transplantation).\textsuperscript{13} (Figure 1)

\textbf{Figure 1: Prevalence of ESRF patients in Australia in 2005, with relative contributions of dialysis (peritoneal and haemodialysis) and transplant renal replacement therapies (adapted from ANZDATA Annual Report, 2006).}\textsuperscript{14}

In fact, while the Australian population has grown by approximately 40\% over the last 25 years, the number of Australians treated with dialysis or kidney
transplantation has grown by more than 400% over the same period (Figure 2).\textsuperscript{15} With the burgeoning epidemics of obesity and Type II Diabetes Mellitus (TIIDM), the continuing impact of the glomerulonephritides and hypertension, and the progressive aging of the Australian population, it is anticipated that CKD and ESRF will continue to rise in prevalence for the foreseeable future.\textsuperscript{15}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure2.png}
\caption{Prevalence of End-Stage Kidney Disease (ESKD) in relation to the Australian population between 1980 and 2004.\textsuperscript{15}}
\end{figure}

**Financial Burden of CKD**

The growing prevalence of CKD has significant attendant costs – most directly to the individual patient and their family/carers, but also to the community. Australian health care expenditure on CKD was $898.7 million in 2004-5, with conservative estimates anticipating compounded rises in CKD expenditure of up
to 27% per year – more than double the rate of growth in total health care expenditure.\textsuperscript{16} The provision of RRT in the form of dialysis (both peritoneal and haemodialysis) and renal transplantation programs is highly cost intensive, and accounts for nearly 85% of the total CKD budget.\textsuperscript{16} Such expenditure is estimated to provide Australian RRT patients an overall benefit of 60,000 life-years over a period of 10-years from RRT commencement, or 30,000 quality-adjusted life years (QALY’s).\textsuperscript{15}

Although the aforementioned costs are substantial, these figures fail to include the additional costs associated with the management of the frequent co-morbid conditions endured by CKD patients, nor the indirect and non-health sector costs associated with the condition.

**Aetiology**

In Australia and New Zealand, population details of ESKD are diligently recorded by the participant renal medicine units of the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA). As a reflection of the universal commitment of local Nephrologists to ANZDATA’s *raison d’être* - to monitor treatment and perform analyses to improve the quality of care for people with kidney failure - 100% of all Renal Medicine Units in Australia and New Zealand participate in the data collection process. As such, ANZDATA provides an enviably comprehensive overview of CKD and ESKD in the Australian and New Zealand context.

The most contemporary ANZDATA Annual Report (2010)\textsuperscript{17} states that there were 18,243 people receiving RRT in Australia as at 31\textsuperscript{st} December, 2009. This figure comprises 7,902 individuals with a functioning renal transplant (RTx), and
10,341 individuals undergoing dialysis treatment – either peritoneal or haemodialysis. The provision of RRT occurred at a rate of 834 per million population, with 2,337 people commencing RRT in Australia during 2009 – an incidence of 107 per million population per year. The mean age at RRT commencement was reported at 60.8 years (median 63.6 years), with a rising tide of elderly Australians commencing RRT during the last three decades.18 (Figure 3)

**Figure 3:** Trends in patient age at commencement of Renal Replacement Therapy in Australia, 1981-2006.19

Conventional cardiovascular risk factors such as hypertension, hyperlipidaemia and TIIDM are recognized to be highly prevalent in the CKD cohort. Of the new patients commencing RRT in 2007, diabetic nephropathy was the most common
cause of ESKD, recorded as the primary cause in 31%. A further 25% attributed glomerulonephritis as the primary cause of ESKD, and 16% hypertensive renal disease.\textsuperscript{20} This finding is a relatively new phenomenon, with glomerulonephritis consistently listed as the most common primary cause of ESKD in Australia prior to 2005, when diabetic nephropathy became the most prevalent primary underlying aetiology for the first time (30%, vs. 25% glomerulonephritis).\textsuperscript{21} This growing emergence of diabetic nephropathy as the primary contributor to the need for RRT therapies in Australia is anticipated to continue to rise for the foreseeable future.\textsuperscript{20}

\textbf{Co-Morbid Conventional Cardiovascular Risk Factors}

Despite substantial technical improvements in the management of CKD and ESRF over the last 2-3 decades, minimal progress has been made on the impact of cardiovascular disease in affected patients. As previously mentioned, conventional CV risk factors are highly prevalent amongst CKD patients. The burden of CVD however, is disproportionate to the conventional risk factor burden.\textsuperscript{22}

Notably, the prevalence of CKD and ESRF increase with advancing age, peaking in the 65-74 year age group for ESRF. Furthermore, a significant proportion of advanced CKD and RRT patients have pre-existing coronary artery, peripheral artery and cerebrovascular disease. Such factors contribute significantly to the risk of future cardiovascular events in these cohorts, independent of other contributory risk factors such as diabetes and hypertension.
Diabetes Mellitus

TIIDM contributes substantially to the burden of pre-ESRF CKD in the Australian community.\textsuperscript{12,23} It is well appreciated that the prevalence of TIIDM in Australia has risen steadily over the last two decades, rising from <1.5\% of the population in 1989, to almost 3.5\% by 2005.\textsuperscript{19} This growing burden of diabetes is over-represented amongst older Australians, with a peak incidence in the 65-74 years age group.\textsuperscript{19} This skewed prevalence ensures a rising tide of CKD and ESRF amongst elderly Australians, with the greatest prevalence of diabetes diagnosis coinciding with the greatest population burden of CKD also (Figure 4).\textsuperscript{19}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image1.png}
\caption{Age-specific prevalence of diabetes diagnosis, by gender, 2004-5.\textsuperscript{19}}
\end{figure}

\textbf{NOTE:}
This figure/table/image has been removed to comply with copyright regulations. It is included in the print copy of the thesis held by the University of Adelaide Library.
**Hypertension**

Hypertension is known to be a potent risk factor for the development of renal disease and may hasten progression of renal dysfunction in early CKD.\(^{24}\) As mentioned previously, hypertensive renal disease accounted for 16% of ESRF cases in Australia in 2008, with rates steadily increasing over the last two decades.\(^{20}\) Additionally, hypertension is a common consequence of advancing CKD (if it is not already present), further contributing to the ‘pro-CVD milieu’ in this condition. Hypertension contributes significantly to the burden of cardiac and vascular disease of CKD and ESRF and will be discussed in greater detail below.

**Smoking**

Smoking is known to be associated with an increased risk of renal impairment and proteinuria.\(^ {25}\) Although the prevalence of smoking in the Australian community has steadily fallen in recent times (Figure 5)\(^ {26}\), smoking prevalence amongst individuals commencing RRT has remained remarkably stable since 2001, but at a relatively lower rate (12% of patients entering RRT during 2001-2007 vs. 19.8% of the general Australian population aged ≥14 in 2007).\(^ {19,20}\) This persistence of smoking as a contributing risk factor to CVD disease progression in a population already at markedly elevated risk of cardiovascular morbidity and mortality remains a significant challenge to clinicians managing CKD and ESRF.
Hyperlipidaemia

Hyperlipidaemia is a common co-morbid condition in CKD patients, and appears to increase in prevalence with increasing renal dysfunction. Although numerous studies have demonstrated substantial prognostic benefits with low-density lipid (LDL) lowering utilizing hydroxyl-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors (“statins”) in the general community and various higher-risk cohorts, there remains no convincing evidence of prognostic advantage to these agents in the ESRF context, despite similar LDL-lowering efficacy. Although substantial controversy persists regarding the use of statin therapy in ESRF patients, and the generalizability of the AURORA study results in particular, it is supposed that the causal relationship between LDL and the generation of cardiovascular events is altered in dialysis patients. It is well appreciated that, although vascular disease is common in ESRF, only one quarter...
of cardiac mortality in ESRF is attributable to acute myocardial infarction\textsuperscript{41}, while other common causes of death such as cardiac arrest, arrhythmia and heart failure could not be expected to be affected by LDL-lowering. Notably, the larger \textit{Study of Heart and Renal Protection} (SHARP) involving combination therapy with ezetimibe and simvastatin in the management of hyperlipidaemia in advanced CKD recently demonstrated a significant reduction in major atherosclerotic events\textsuperscript{42}. This study has provided greater confidence in the utility of lipid lowering in the CKD context, although questions still remain regarding the pathobiology of atherosclerosis in this condition.

\textbf{Obesity}

The prevalence of obesity in Australia has more than doubled over the last 20 years\textsuperscript{43}, with significant corresponding increases in the prevalence of overweight Australians during this period also. Obesity is a known risk factor for cardiovascular disease and contributes to deleterious vascular remodelling and hypertension.\textsuperscript{44-47} Although indices of malnutrition, such as low BMI, albumin and total cholesterol, have previously been associated with increased mortality in ESRF\textsuperscript{48,49}, excess visceral adiposity and the associated metabolic syndrome remain significant contributors to morbidity and mortality in CKD and ESRF\textsuperscript{50}.

\textbf{Disease-Specific Cardiovascular Risk Factors}

\textbf{Anaemia}

Anaemia is a common complication of declining renal function, and is strongly predictive of adverse cardiovascular prognosis in CKD and ESRF\textsuperscript{51,52}.
kidneys are responsible for the production of endogenous erythropoietin (EPO),
progressive destruction of the renal parenchyma inevitably leads to reduced red
cell haematopoiesis, and resultant anaemia due to EPO deficiency. This
underproduction of red blood cells is further complicated by reduced red cell
lifespan in advancing CKD, and dilutional anaemia associated with progressive
derangement of extracellular fluid homeostasis as renal function declines.\textsuperscript{53,54}
Numerous observational studies support the role of exogenous EPO
supplementation in ESRF for the management of anaemia and the minimization
of deleterious cardiovascular remodelling and premature mortality.\textsuperscript{51,55-61}
Despite this, normalisation of haemoglobin (Hb) levels has failed to demonstrate
convincing improvements in left ventricular geometry, or overall survival in
prospective studies.\textsuperscript{62-64} Furthermore, recent evidence disputes the use of EPO
supplementation to prevent cardiovascular events in pre-dialysis CKD\textsuperscript{65,66}, with
evidence actually suggesting an increase in adverse outcomes if Hb>13.5g/dL is
targeted.\textsuperscript{67,68} Despite these conflicting findings, EPO administration remains a
mainstay of international guidelines regarding the management of anaemia in
CKD.\textsuperscript{60,69}
Iron deficiency is also common in the CKD and ESRF populations, contributing
further to the development of anaemia. Due to the not-infrequent development of
relative EPO resistance in the uraemic context\textsuperscript{70}, supra-normal ferritin levels are
often required to ensure sufficient red cell haematopoiesis. Substantial work has
focused on the optimal delivery and serum levels of iron in CKD, with evidence
strongly supporting the role of regular intravenous iron administration in
haemodialysis patients in particular.\textsuperscript{71} Evidence supporting a role for aggressive
iron supplementation in pre-dialysis CKD is less convincing however.\textsuperscript{71}
**Disordered Calcium / Phosphate Metabolism**

Calcium and phosphate homeostasis relies upon a complex, tightly controlled system of hormones and serum ions that influence calcium and phosphorus regulation at the level of the kidney, intestine and bone.\(^{72}\) Derangement of the renal contribution to this complex regulatory system leads to a number of compensatory, but ultimately pathological responses.

**Vitamin D / Parathyroid Hormone**

Vitamin D is poorly named, as its synthesis and actions more closely represent the behaviour of a hormone than a true vitamin, as with sufficient exposure to sunlight, no dietary supplementation is necessary.\(^{73}\) Once bio-transformed to its active metabolite (1,25-dihydroxyvitamin D\(_3\)), Vitamin D influences homeostatic mechanisms within the kidney, intestines, bone, and parathyroid glands, with strong influence over calcium and phosphorus physiology. Vitamin D is initially derived from 7-dehydrocholesterol (provitamin D\(_3\)), transformed within the epidermis following exposure to ultraviolet B radiation (UVB). Vitamin D\(_3\) continues to be produced for many hours after epidermal exposure to UVB (most commonly from sunlight), and is then transported to the liver by vitamin D-binding protein, where it is hydroxylated to the still-inactive 25-hydroxyvitamin D\(_3\) [25(OH)VitD\(_3\)]. 25(OH)VitD\(_3\) is then further hydroxylated by renal tubular mitochondria to the active 1,25-dihydroxyvitamin D\(_3\). This activation is indirectly enhanced by hypocalcaemia, which triggers increased production of parathyroid hormone (PTH). PTH acts to restore serum calcium levels, and contributes to
increased 1,25-dihydroxyvitamin D₃ synthesis within the renal tubular cells, which in turn provides negative feedback to further PTH production. 1,25-dihydroxyvitamin D₃ assists PTH in increasing circulating calcium levels through the enhancement of intestinal calcium and phosphate absorption, as well as stimulation of increased bone turnover. Excess phosphate is normally deposited in bone by osteoblasts (predominantly as hydroxyapatite), or excreted by the renal tubules under the influence of PTH.²²,²³

Renal parenchymal destruction leads to relative 1,25-dihydroxyvitamin D₃ deficiency, reduced gastro-intestinal calcium absorption, excess PTH secretion and commonly, compensatory secondary hyperparathyroidism. This maladaptive secondary hyperparathyroidism leads to progressive depletion of calcium stores within the bone reservoir, leading to a condition known as renal osteodystrophy. This derangement of calcium homeostasis is further complicated by the progressive decline in renal phosphate excretion, leading to a state of chronic hyperphosphataemia – an independent risk factor for vascular calcification and subsequent arterial stiffness. Arterial stiffness leads to increased cardiac work and reduced coronary blood flow, which, particularly in this renal failure cohort, may contribute to excess CV mortality.²⁴

Phosphate Restriction

Vitamin D supplementation has been routinely undertaken in advancing CKD, to prevent hypocalcaemia and secondary hyperparathyroidism. Although the enhancement of gastrointestinal calcium absorption mitigates the need for increased PTH secretion and restricts the development of renal osteodystrophy, as previously mentioned, Vitamin D (commonly administered in the form of oral
calcitriol) also leads to increased gastrointestinal phosphate absorption, exacerbating the decline in renal excretion of this pro-calcific anion.

As the capacity for phosphate excretion falls, restriction of phosphate absorption becomes an essential component of CKD management. Even following the commencement of dialysis in ESRF, the removal of circulating phosphate often fails to adequately compensate for dietary phosphate intake. The removal of phosphate during dialysis is impaired by the complex interaction kinetics of intracellular and extracellular phosphate within the human body, and by dialysis’ inability to remove large amounts of phosphate from the serum using conventional dialysis methodologies (both peritoneal and haemodialysis).75 Historically, control of hyperphosphataemia was achieved through the administration of oral aluminium-hydroxide at meal times to bind dietary phosphates prior to absorption. Although effective, this led to progressive aluminium intoxication in ESKD patients, most notably in the form of osteomalacia and dementia.73 Calcium-based phosphate binders are used widely, as they are inexpensive and effective, increasing gastro-intestinal phosphate excretion through the intra-luminal formation of insoluble calcium-phosphate. In combination with vitamin D supplementation however, it became apparent that calcium-based phosphate binders may contribute to the excess vascular calcification of CKD by inducing a strongly positive calcium balance.76 Non-calcium-based phosphate binders have now been developed (e.g. sevelamer-HCL), with evidence of reduced risk of hypercalcaemia and cardiovascular calcification.77-79 There remains uncertainty, however, whether these newer therapies lead to improved clinical outcomes in CKD and ESRF patients.80
As hyperphosphataemia and hyperparathyroidism have been independently associated with cardiovascular morbidity and mortality, as well as all-cause, cardiovascular and fracture-related hospitalisation in ESRF patients\textsuperscript{74}, efforts to control these entities remains an important goal for renal clinicians and researchers.

**Uraemia**

As glomerular and tubular function progressively decline, there is a gradual accumulation of numerous organic by-products of metabolism usually excreted by the intact kidneys. This accumulation is associated with the development of the clinical syndrome of “uraemia”, characterised by a complex constellation of symptoms and signs, including fatigue, anorexia, nausea, pruritis, muscle cramps, restless legs, reduced mental acuity, peripheral neuropathy and ultimately seizures and coma.\textsuperscript{81} Additionally, uraemia is associated with the development of a number of significant physiological derangements that contribute directly to the promotion of CVD. These include insulin resistance, platelet dysfunction, increased oxidative stress and the promotion of a pro-inflammatory milieu.\textsuperscript{81}

**Insulin Resistance**

Insulin resistance is defined as the inadequate blood glucose response to physiological levels of circulating insulin. Insulin resistance, and the associated metabolic syndrome, have been strongly implicated in the development of CVD in the general population, and contribute significantly to the burden of premature mortality in the Australian community.\textsuperscript{19,82,83} Similarly, insulin resistance has also
been established as an independent predictor of cardiovascular mortality in non-diabetic patients with ESRF.\textsuperscript{84} Significantly, uraemia \textit{per se} is known to contribute to the development of insulin resistance, through induction of tissue insensitivity to insulin.\textsuperscript{85} This uraemia-associated insulin insensitivity is known to correlate linearly with declining renal function, and has been demonstrated to manifest early in the course of renal dysfunction.\textsuperscript{86,87} The resultant compensatory hyper-insulinaemia has long been known to be associated with glucose intolerance and dyslipidaemia\textsuperscript{88-90} – further potential contributors to CVD progression. Furthermore, the hyper-insulinaemia of CKD is implicated in the development of muscle wasting in advancing CKD and ESRF, potentially contributing to the commonly described fatigue and exercise intolerance in uraemia patients.\textsuperscript{91,92} Although multi-factorial, this uraemia-induced sedentariness of advancing CKD has also been implicated in the 'pro-CVD milieu' of this condition.\textsuperscript{93}

\textit{Platelet Dysfunction}

Uraemia is the most common systemic disorder associated with clinically important platelet dysfunction.\textsuperscript{94} Moderate thrombocytopenia is a common finding in uraemia, with the mean platelet volume of remaining platelets often reduced – a measure inversely proportional to bleeding time.\textsuperscript{95} Although reduced in number, thrombocytopenia sufficiently severe to cause bleeding is rare. Despite this, numerous other factors are known to impair platelet function in uraemia whether by impacting platelet granule composition\textsuperscript{96,97}, platelet-platelet interactions\textsuperscript{98-100} or platelet-vessel interactions.\textsuperscript{101-103} Moreover, the presence of anaemia may further impair platelet function in uraemia.\textsuperscript{104-106} Within the circulation, red blood cells increase platelet vessel wall contact by displacing
platelets away from the axial flow of blood towards the vessel wall.\textsuperscript{95} Red blood cells further enhance platelet function through the release of ADP and inactivation of PG\textsubscript{I\texttwoheadvector{2}}.\textsuperscript{95,107} Additionally, Hb acts as a scavenger for nitric oxide (NO), which along with PG\textsubscript{I\texttwoheadvector{2}} is increased in uraemia and are known to inhibit platelet function.\textsuperscript{95,108} Dialysis is associated with improvement in platelet function, through the removal of a variety of circulating factors associated with platelet functional abnormalities.\textsuperscript{109-111} Despite this, haemodialysis may be associated with an increase in bleeding risk, due to inappropriate platelet activation induced by interaction of circulating platelets with the dialysis membrane, as well as through the use of heparin anti-coagulation during dialysis. Peritoneal dialysis has been demonstrated to be more effective in correcting uraemic platelet dysfunction than haemodialysis\textsuperscript{112}, though this RRT methodology is not suitable for all patients. Finally, platelet function may be further impaired by the administration of aspirin, a common therapy in CKD and ESRF due to the high rate of CV morbidity and mortality in this condition. It is recognised that aspirin may have a more significant impact on bleeding time in uraemia than in non-uraemic patients, and may further be associated with increased risk of gastro-intestinal ulceration and subsequent bleeding.\textsuperscript{113,114} The management of the competing interests of CVD prevention and bleeding minimisation provide an ongoing clinical quandary in the management of CKD and ESRF patients, due to the paradoxical association of impaired haemostasis and increased risk of atherothrombosis in uraemia.\textsuperscript{115}
Oxidative Stress

Oxidative stress is a widely used term that describes the compensatory biological response to noxious oxidative stimuli – a process that is most commonly self-limiting. In CKD and a variety of other chronic inflammatory conditions however, this oxidative stress exceeds biologically optimal levels, leading to disadvantageous physiological sequelae.

Oxidative processes predominantly occur within the mitochondria, with the mitochondrial cytochrome oxidase enzyme responsible for 90% of the oxygen metabolised by human cells. This enzyme transfers four electrons to oxygen in a direct redox reaction that creates two molecules of water in the process. This reaction generally proceeds without any intervening steps, however a small percentage of this reaction (<5%) proceeds via an intermediate step that results in the formation of “free radicals”. To counteract the production of these potentially injurious free radicals, numerous antioxidant responses have developed to interact with these by-products of oxygen metabolism and render them inert before they can cause oxidative damage to cellular components and function.

Phagocytes within the human immune system specifically utilise reactive oxygen species in the host defence against pathogens. Four enzymes are predominantly responsible for the creation of reactive oxidative products: NADPH oxidase, superoxide dismutase, nitric oxide synthase and myeloperoxidase. These enzymes produce superoxide anion, hydrogen peroxide, NO and hypochlorous acid respectively, often utilised in the destruction of invading microorganisms. Oxidative stress is achieved when the ongoing production of these free radicals exceeds the available anti-oxidant capacity of surrounding tissue – leading to the
generation of a local chronic inflammatory state and perpetuating local tissue injury.

Uraemia is associated with an increase in oxidative stress.\textsuperscript{118,119} This increased oxidative burden leads to an increase in oxidative modification of proteins, lipids and carbohydrates that progressively increases as renal function declines, along with inactivation of the ‘vascular protective’ molecule nitric oxide and subsequent formation of peroxynitrite.\textsuperscript{116} The effect of oxidative stress on dynamic vascular function will be discussed later in this Background section.

\textit{Oxidative Stress in the Promotion of CVD}

Atherosclerosis is now widely regarded as an immune / inflammatory response to endothelial injury\textsuperscript{120,121}, and that this injury is initiated by the accumulation of sub-intimal lipid.\textsuperscript{122} Native lipoprotein particles (most notably LDL) freely enter the intima under normal circumstances, and are utilised by vascular cells via LDL-receptor mediated endocytosis without precipitating an immunological or inflammatory response. These native LDL particles, thus, are not phagocytosed by local macrophages and do not initiate atherosclerotic changes in the vessel wall.\textsuperscript{120,122,123} Oxidative transformation of lipoproteins (LDL in particular), however, markedly alters the biological activity of these particles. Furthermore, smaller, denser LDL particles are particularly susceptible to oxidative modification, leading to the recognition of this subtype of LDL as being particularly atherogenic.\textsuperscript{124,125} Following oxidative transformation, the oxidised LDL (oxLDL) particles become chemotactic for monocytes and T lymphocytes, initiate a local pro-inflammatory response, induce phenotypic endothelial alterations and trigger macrophage differentiation.\textsuperscript{120,126} Subsequently, these pro-
inflammatory molecules are avidly taken up by intimal macrophages utilising scavenger receptors, rather than the traditional LDL-receptors present ubiquitously on the surface of mammalian cells.\textsuperscript{120,127} This adaptive response to potentially injurious oxLDL eventually overwheels the macrophage's capacity for handling the intracellular lipid, leading to the formation of heavily cholesterol-laden foam cells within the vessel wall – cells known to be critical components of the ‘fatty streak’ and an initiating step in the atherosclerotic process. Critically, the uptake of these modified LDL particles into differentiated macrophages is also believed to initiate a chronic pro-inflammatory milieu within the vessel wall, perpetuating the creation of local oxidative molecules and advancing the atherosclerotic process further.\textsuperscript{127}

Distinct from the oxidative process precipitated by conventional cardiovascular risk factors such as smoking and obesity, and the hyper-atherogenic properties of numerous chronic inflammatory conditions (e.g. rheumatoid arthritis), ESKD further contributes to the promotion of CVD through the accumulation of oxidative end-products – most notably aldehyde (carbonyl) species. Reactive aldehydes may be formed by numerous oxidative reactions.\textsuperscript{128} Generally excreted via renal clearance, many such oxidative end-products are present in uraemic serum (and in the serum of haemodialysis patients in particular), at substantially higher concentrations than are present in healthy individuals.\textsuperscript{129,130} These aldehydes are implicated in the promotion of advanced glycosylation end-products (AGE) – formed through the irreversible, non-enzymatic interaction of reactive carbonyl compounds (i.e. aldehydes) and a variety of human proteins.\textsuperscript{131} These AGE’s are known to be potent promoters of the atherosclerotic process\textsuperscript{132}, have been recognised as contributors to the oxidisation of LDL\textsuperscript{133}, and are
believed to contribute substantially to the excess cardiovascular morbidity and mortality of CKD and ESKD.\textsuperscript{134}

\textit{Immune System Dysregulation}

After CVD, infectious disease is the second most common cause of death in ESKD patients. This elevated risk of infection reflects a state of immune system dysregulation, further demonstrated through a higher prevalence of autoimmune disease and neoplasms, cutaneous anergy in delayed-type hypersensitivity reactions to common antigens, and impaired response to vaccination.\textsuperscript{135} This state of immunodeficiency predates the commencement of dialysis, but is enhanced by haemodialysis in particular due to immune cell activation by serum contact with dialysis membranes.\textsuperscript{135,136} The underlying causes behind this immune system dysregulation have not yet been comprehensively characterised, but are believed to originate from an imbalance between pro-inflammatory cytokines and their inhibitors in the context of the uraemic milieu.\textsuperscript{135} Intercurrent infections, co-morbidities, dialysis-related triggers and even renal failure itself have been implicated in the genesis of the pro-inflammatory state of uraemia. Regardless of the myriad underlying causes, the chronic inflammatory activation of CKD is known to further promote atherosclerotic progression, endothelial dysfunction, haematopoietic resistance and cardiac failure – all independent risk factors for reduced survival in this clinical context.\textsuperscript{137}
Cardiovascular Sequelae of CKD

Cardiovascular disease (CVD) is the leading cause of death amongst ESRF patients.\textsuperscript{20,138,139} Mortality rates amongst ESRF patients are widely accepted to be 15- to 30-times that of the age-matched mortality in the general population.\textsuperscript{20,140} Strikingly, according to the US Renal Data System\textsuperscript{141} only 35% of haemodialysis patients can expect to live for a five year period.\textsuperscript{93} Cardiovascular mortality rates are similarly raised across all age groups, with CV mortality amongst the youngest age groups (age 25 to 34 years) particularly excessive – up to 500 times that of age-matched peers in the general community.\textsuperscript{142}

This increased risk of CVD extends to less severe stages of renal dysfunction also.\textsuperscript{139} Alan Go and colleagues presented striking data in the \textit{New England Journal of Medicine} in 2004, demonstrating the rising risk of CV death with advancing renal dysfunction.\textsuperscript{143} This study of 1,120,295 ambulatory adults within the San Francisco Bay area revealed a dramatic inverse association between incremental risk of all-cause mortality, risk of CV events and risk of all-cause hospitalisation with declining renal function, as measured by eGFR (Figure 6).

Furthermore, it has been shown to be more likely that patients with early CKD will die of CVD, than develop ESRF.\textsuperscript{144} The explanation for this rising risk of hospitalization, CV events and mortality with declining renal function most likely finds its genesis in the myriad conventional and disease-specific CV risk factors previously described. In particular, declining renal function is associated with complicated neurohormonal and biochemical derangements that strongly promote the development of hypertension, vascular stiffening, endothelial dysfunction and various cardiac abnormalities. Although briefly alluded to above,
the cardiac and vascular manifestations of CKD and ESRF will be discussed further below.

Figure 6: Age-standardised Rates of Death from Any Cause (Panel A), Cardiovascular Events (Panel B) and Hospitalisation (Panel C) according to eGFR among 1,120,295 ambulatory adults. (Reproduced from Go AS, et al, N Engl J Med, 2004 with permission. Copyright © [2004] Massachusetts Medical Society. All rights reserved.)

Left Ventricular Disease in CKD

The prevalence of cardiac abnormalities in CKD is substantial. In a landmark study, Parfrey and colleagues evaluated 432 ESKD patients with trans-thoracic echocardiography at the initiation of dialysis therapy and demonstrated that less than 16% of patients studied had normal left ventricular size and systolic function. In this study, 41% of patients had concentric left ventricular hypertrophy (LVH), 28% LV dilatation, and 16% demonstrated systolic dysfunction. Significantly, these abnormalities appeared to be closely related to patient prognosis, both with regards time from dialysis initiation to development of heart failure, and overall survival from index echocardiogram (Table 2).
<table>
<thead>
<tr>
<th>Echocardiography Findings</th>
<th>Prevalence of Finding % (n)</th>
<th>Time to Development of Heart Failure (median)</th>
<th>Significance vs. Patients with Normal LV</th>
<th>Overall Survival (median)</th>
<th>Significance vs. Patients with Normal LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal LV Size and Function</td>
<td>15.6% (64)</td>
<td>n/a</td>
<td>-</td>
<td>&gt;66 months</td>
<td>-</td>
</tr>
<tr>
<td>LV Dilatation</td>
<td>28.0% (117)</td>
<td>38 months</td>
<td>p = 0.002</td>
<td>56 months</td>
<td>p=NS</td>
</tr>
<tr>
<td>Concentric LVH</td>
<td>40.7% (168)</td>
<td>38 months</td>
<td>p&lt;0.001</td>
<td>48 months</td>
<td>p=NS</td>
</tr>
<tr>
<td>LV Systolic Impairment</td>
<td>15.6% (64)</td>
<td>19 months</td>
<td>p&lt;0.001</td>
<td>38 months</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of echocardiographic findings of abnormalities in left ventricular structure and function at dialysis commencement (Reproduced from Parfrey PS, et al. Nephrol Dial Transplant, 1996;11:1277-1285, with permission of Oxford University Press).

In this Study, the relative risk of developing heart failure in patients with LV abnormalities (independent of age, gender, diabetes and ischaemic heart disease [IHD]) was 3.0 (p<0.001) for LVH, 2.74 (p=0.002) for LV dilatation, and 3.66 (p<0.001) for LV systolic impairment.145

Other authors have previously demonstrated LVH to be an independent predictor of increased mortality in CKD.146 As suggested by the study by Go and colleagues143, the cardiac and vascular abnormalities of CKD have been shown to begin early in the course of the disease.147,148 Furthermore, the development of
LVH continues to have prognostic significance in ESRF, with reduced survival noted amongst patients developing LVH following the commencement of dialysis. In particular, it is a significant finding that routine dialysis does not achieve stabilisation of the progressive increase in left ventricular mass (LVM), indicating repeated imaging assessments may be useful to monitor this important correlate of clinical outcomes periodically after dialysis commencement.

Recent evidence suggests that more intensive haemodialysis programs may also play a role in ameliorating the progression of LVH in dialysis-dependent ESRF. Left ventricular dilatation and hypertrophy are fundamental cardiac adaptive responses to increases in volume and pressure loads. Increased LVM is frequently seen in athletes (e.g. cyclists, long distance runners), during pregnancy and as a physiological adaptation to growth from infancy to adulthood. In all of these cases, the development of increased LVM is a normal physiological compensation that is reversible with attenuation of the situational trigger.

The development of LVH in CKD appears to be multi-factorial, but the prevalence of LVH appears to be directly related to the severity of renal dysfunction. As in the general population, LV dilatation and hypertrophy are fundamentally adaptive responses designed to maintain cardiac output and prevent excessive increases in myocardial wall stress. In particular, increased LVM in CKD has previously been demonstrated to be closely linked to increased cardiac stroke work index – a factor of stroke volume and changes in left ventricular mean systolic pressure – and exacerbated by other factors such as age, gender, anaemia, oxidative stress, renin-angiotensin-aldosterone system (RAAS) activation.
In CKD however, LVH is not simply a factor of cardiomyocyte hypertrophy compensating for increased physiological demand. Hypertensive and, in particular, uraemic LVH is further characterised by significant expansion of the supporting extracellular architecture of interstitial fibrous tissue.\textsuperscript{155} This increase in extracellular matrix leads to a progressive increase in the relative proportion of interstitial myocardial fibrosis to cardiac myocytes, causing progressive ventricular stiffening and resultant abnormalities of diastolic filling. Additionally, in experimental models, this fibrotic process has been coupled to a decline in vascular capillary density, increasing the distance required for O$_2$ diffusion to contracting myocytes.\textsuperscript{156} Thus, even in the absence of obstructive epicardial coronary artery disease, LVH may be associated with a relative cellular ischaemia that may contribute further to ventricular diastolic and systolic impairment. Significantly, this substantially altered myocardial architecture, in combination with perpetuating neurohormonal and vascular triggers, ensures that the capacity for meaningful regression of uraemic LVH is markedly reduced.

Given LVH has been determined to be probably the most powerful indicator of mortality and CV events in patients with advanced CKD\textsuperscript{157}, numerous studies have evaluated the capacity for LVH regression utilising aggressive management of associated triggers – most notably anaemia and hypertension.\textsuperscript{63,158-161} These studies have demonstrated mixed results. Although the development of anaemia has been strongly associated with findings of LVH in epidemiological studies of CKD patients\textsuperscript{162}, anaemia correction alone appears not to lead to significant reductions in LVM.\textsuperscript{63,158,159} Such findings suggest that the relationship between anaemia and development of LVH is likely not directly causal.\textsuperscript{158}
Significantly however, in 2001, Gérard London’s prolific research group published a landmark Study in the *Journal of the American Society of Nephrology* evaluating whether a concerted interventional study specifically targeting LVM reduction through dialysis optimisation and tight control of Hb and blood pressure could impact on patient survival amongst 153 haemodialysis patients. Blood pressure, trans-thoracic echocardiography and aortic pulse wave velocity were performed at baseline and then again at follow-up (54±37 months). The intervention led to significant reductions in systolic and diastolic blood pressure (DBP) and cardiac output across the cohort, and a significant associated reduction in overall LVM of approximately 10% (290±80g vs. 264±86g, p<0.01). This striking demonstration of LVM regression through targeted therapeutic optimisation was particularly significant, as Cox proportional hazard analyses demonstrated a strong, independent association between LVM reduction and overall survival (after correction for age, gender, diabetes, history of CVD, and “all non-specific CV risk factors”) (Figure 7). This study demonstrated that a 10% change in LVM was associated with a relative risk of 0.78 (0.63-0.92, p=0.0012) for all cause mortality, and 0.72 (0.51-0.90, p=0.0016) for CV event-free survival across the cohort. This relative risk reduction was particularly notable amongst subjects with no prior history of CVD (RR 0.69 [0.52-0.83, p=0.0182] for all cause mortality, and 0.52 [0.23-0.75, p=0.0204] for CV event-free survival).
Figure 7: Probability of overall survival (A) and cardiovascular event-free survival (B) amongst successful responders to the LVM reduction intervention, and non-responders (response defined by a >10% change in LVM) (p<0.001 for both). (Reproduced from London et al, J Am Soc Nephrol, 2001, with permission from the American Society of Nephrology).

Although oft-debated, this Study’s results demonstrate the potential for improvement in patient prognosis in ESRF associated with therapeutic efforts that reduce LVM, and reflects the known association of worsened prognosis with increases in LVM. These findings mirror Studies in the general population\textsuperscript{163-165}, but are particularly pertinent in the high-risk CKD context. Such observations form an important basis for the Research Studies outlined herein.

\textbf{Congestive Cardiac Failure}

Congestive cardiac failure (CCF) is known to be portentous of increased morbidity and mortality in the general population\textsuperscript{166,167} Renal function however,
is a potent risk factor for death and hospitalisation amongst patients with clinical features of heart failure. In CKD, and in ESRF in particular, salt and water retention due to failing renal excretory capacity or insufficient dialysis is often indistinguishable from ‘true’ heart failure due to cardiac dysfunction. Myocardial hypertrophy and fibrosis contributes substantially to diastolic left ventricular impairment, and are important contributors to the development of heart failure in CKD. Furthermore, excess volume loading, myocardial ischaemia and uraemic cardiomyopathy are important predisposing factors in the development of left ventricular dilatation and systolic myocardial dysfunction. Additionally, arterio-venous fistulae (AVF) created for the purpose of haemodialysis place a substantial increased volume load on the left ventricle in ESRF, necessitating corresponding increases in baseline cardiac output to compensate. In the stiff, hypertrophied ventricle, or the dilated ventricle with regional systolic impairment, physiological reserve is rapidly overwhelmed by further increases in cardiac demand, or acute insult to the ventricular myocardium. Dialysis enables the periodic removal of excess extracellular fluid, and the maintenance of an ideal, ‘dry-weight’, theoretically preventing fluid overload contributing to CCF in ESRF. Despite this, within 6-months of commencing dialysis, approximately one-third of patients will exhibit clinical features of CCF. A further 7% of dialysis patients will develop CCF each year, promoted by systolic impairment, anaemia, hypertension, hypoalbuminaemia and advancing age. Thus, although dialysis may provide an effective method for the periodic removal of excess fluid and solutes in ESRF, advancing CKD creates a “perfect storm” for the development of CCF. It is therefore clear that a substantial burden
of the myocardial injury predisposing to the development of CCF is present prior to dialysis commencement.

Heart failure is a common cause of death in CKD and ESRF, and is an independent risk factor for mortality in affected patients.\textsuperscript{173} Dialysis patients hospitalised for heart failure, features of fluid overload or pulmonary oedema have an abysmal 5-year survival rate – 12.5\%, 20.2\% and 21.3\% respectively.\textsuperscript{174} Even lesser forms of CCF may have a dramatic impact on survival. Regardless of aetiology, New York Heart Association (NYHA) classification of exertional dyspnoea severity has been proven to be a potent measure of survival in ESRF (Figure 8).\textsuperscript{175} Cardiac morphological features may further influence prognosis in this condition, with increases in LVM, LV dilatation and echocardiographic evidence of LV systolic impairment adding incremental risk prior to, and following, the clinical manifestation of CCF.\textsuperscript{173} Therefore, the focus of preventative care in CKD and ESRF has moved to the prevention of LVH and LV dilatation development. Therapeutic options in this regard remain limited, however.
Figure 8: Kaplan-Meier survival analysis for all cause mortality by NYHA Classification for 1322 patients with ESRF. (Reproduced from Postorino M, et al, Nephrol Dial Transplant., 2007, by permission of Oxford University Press).

It has been proposed that intermittent 3x/week haemodialysis may complicate, rather than assist in, the management of ESRF patients with CCF. Haemodialysis-induced myocardial dysfunction has recently received attention as an under-appreciated clinical phenomenon that may have longer-term implications for cardiac function. It has been associated with an acute reduction in global and regional myocardial blood flow utilising positron emission tomography (PET) and repetitive haemodialysis-induced myocardial stunning may contribute to the development of longer term regional left ventricular dysfunction, regardless of epicardial coronary artery patency.
Peritoneal dialysis, rather than haemodialysis, has been proposed as a more effective means of managing ESRF patients with CCF, allowing ‘continuous’ ambulatory dialysis that may more evenly manage fluid retention and the associated neuro-hormonal activation in ESRF patients with impaired cardiac function.\textsuperscript{179} The potential disadvantage of this periodic cardiac overload of haemodialysis has been further supported by the finding that clinical features of inter-dialysis session fluid retention are associated with a markedly elevated risk of all-cause and cardiovascular death in affected patients.\textsuperscript{180}

CCF poses a difficult management dilemma in the care of CKD patients, with prevention through aggressive risk reduction appearing to offer the greatest potential for therapeutic success. Further studies to identify contributors to cardiac dysfunction in CKD and potential disease-specific therapeutic options in this cohort are urgently needed.

\textbf{Atrial Disease in CKD}

Atrial fibrillation (AF) is the most common sustained arrhythmia in the general population and is known to be associated with an increased risk of morbidity and mortality.\textsuperscript{181} Recent studies have confirmed AF as a significant negative prognostic factor in ESRF.\textsuperscript{182-184} The prevalence of AF is increased in ESRF compared to the general community, and contributes disproportionately to morbidity and mortality in affected patients.\textsuperscript{183,185} Although haemodialysis in particular is a known risk factor for the development of AF\textsuperscript{182,186}, it remains unclear whether declining renal function contributes independently to this risk, or whether the high prevalence of AF risk factors (e.g. advancing age,
hypertension, CCF, LVH, coronary artery disease and valvular heart disease) amongst CKD patients is responsible for the high prevalence of this condition in CKD.

It is increasingly appreciated that AF initiation and perpetuation relies upon the interaction of arrhythmia triggers and supporting atrial substrate.\textsuperscript{187,188} Specifically, ectopic electrical activity within the pulmonary veins commonly provides the initial trigger, with left (and right) atrial structural and electrical remodelling providing the necessary substrate for longer-term persistence of the arrhythmia.\textsuperscript{185,188}

Left atrial (LA) volume is commonly increased in ESRF, in comparison to the general community.\textsuperscript{189} Factors such as hypertension, myocardial ischaemia and anaemia contribute substantially to the development of LVH and diastolic LV impairment in CKD patients, resulting in a chronic increase in left ventricular end-diastolic pressure (LVEDP). This increased LVEDP leads to chronic LA stretch and compensatory LA (and often pulmonary vein) dilatation.\textsuperscript{190-192} Additionally, LA dilatation is accompanied by the development of patchy interstitial fibrosis within the LA myocardium, delaying local conduction velocities and providing rich substrate for the perpetuation of multiple re-entrant circuits and subsequent AF.\textsuperscript{187,188,193}

CKD is known to be associated with an increase in cardiac volume and pressure load, contributing to atrial, as well as ventricular, sequelae. It is unsurprising therefore that LA and right atrial (RA) dilatation are common findings in CKD and ESRF.\textsuperscript{189} LA dilatation, in particular, has been associated with increased serological evidence of inflammation, and greater burden of atherosclerosis at coronary angiography.\textsuperscript{194} Importantly, atrial dilatation has been identified as an
independent risk factor for the development of AF in ESRF and may contribute to the poor CV prognosis of patients with CKD.\textsuperscript{183,195} Recent evidence confirms an association between LA dilatation and increased mortality in ESRF patients with LVH.\textsuperscript{196} Certainly, there is evidence supporting the prognostic benefits of LVH regression in the general community\textsuperscript{197} and such regression has been associated with reductions in risks relating to AF.\textsuperscript{198} As mentioned, it appears that at least partial regression of LVH can be achieved in ESRF with aggressive targeting of the relevant risk factors, such as HT, anaemia and markers of calcium/phosphate balance.\textsuperscript{199} It remains to be seen whether the deleterious health outcomes associated with atrial dilatation can be reversed in CKD and ESRF, however regression of LVH and the associated chronic elevation of LVEDP may play an important role in this relationship.

**Valvular Disease in CKD**

Valvular calcification is common in CKD.\textsuperscript{200} Valvular sclerosis and calcification of the aortic valve (in particular) is common in the general population with advancing age, affecting 20-30\% of the population over the age of 65 years, with clinically significant aortic stenosis in 2\%.\textsuperscript{201-203} In ESRF, aortic valve calcification occurs in 28-55\% of patients, but occurs 10-20 years earlier than in the general population.\textsuperscript{204-206} Mitral annular and valvular calcification is also common in CKD\textsuperscript{207,208} and has been linked to the presence of coronary atherosclerosis and reduced survival in affected patients\textsuperscript{209,210}, although not all studies of this association have been confirmatory.\textsuperscript{211} In comparison to aortic valvular calcification however, mitral calcification is less commonly associated with
clinically relevant valvular dysfunction requiring surgical correction.\textsuperscript{204,206,212,213}

Mitral regurgitation (rather than mitral stenosis) is the more common outcome of mitral valvular and para-valvular calcification, with mitral incompetence contributing further to left ventricular volume loading, and advancing the development of CCF in this population.\textsuperscript{206,212}

Although patient age remains a strong risk factor for the development of valvular calcification in advancing CKD\textsuperscript{213,214}, progression to clinically significant aortic stenosis occurs more rapidly in ESRF, potentially evolving from mild, subclinical disease to haemodynamically compromising valvular dysfunction within only a few years.\textsuperscript{206,214,215} In fact, it has been demonstrated that the mean annual decrease in aortic valve area amongst ESRF patients with calcific aortic stenosis is more than four-fold greater than observed in non-uraemic patients (0.23cm\(^2\)/yr vs. 0.05cm\(^2\)/yr).\textsuperscript{200,214}

Prevention remains the mainstay of therapy. Although advancing age is a potent contributor to valvular calcification in CKD, hypertension, vitamin D3 levels and calcium / phosphate product are modifiable risk factors for this condition.\textsuperscript{204,214-216} Recent evidence supports the use of aggressive phosphate restriction and the use of the binding agents such as sevelamer in the attenuation of valvular calcification in ESRF.\textsuperscript{78,217} Furthermore, although often difficult to control, the high-output state of renal failure is believed to contribute substantially to valvular deterioration, due to high trans-valvular flow rates, and the associated promotion of injury-related valvular inflammation and calcification.\textsuperscript{200,218}

Following the development of severe calcific aortic stenosis, conservative medical management is associated with high mortality rates. Due to the rapid progression of aortic stenosis in this condition, more frequent echocardiographic evaluation is
warranted in ESRF patients than is usual in the general population. Haemodialysis may also be problematic in this condition, particularly where significant fluid removal is necessary, as the combination of aortic stenosis and a non-compliant, hypertrophied LV may precipitate an acute reduction in cardiac output with aggressive ultrafiltration. Prompt and aggressive treatment of arrhythmias such as AF, is also recommended to maintain cardiac output, particularly in the context of compensatory LVH and associated diastolic dysfunction necessitating preservation of the atrial contribution to LV filling. Additionally, ESRF patients with valvular disease are at an increased risk for endocarditis, and appropriate prophylactic measures should be considered where clinically appropriate.

Surgical aortic valve replacement is associated with higher procedural and post-procedural mortality in ESRF than in the general population, but is similarly associated with improvement in overall survival in symptomatic stenotic disease. Mitral valve replacement is less common than aortic valve surgery in ESRF, and some groups promote mitral valvular repair in congestive uraemic cardiomyopathy, despite the known risks of accelerated calcification and delayed repair failure in ESRF. Surgical practice guidelines have historically preferred the use of mechanical prosthetic valves over bioprostheses in ESRF, due to the greater presumed risk of bio-prosthetic calcification and recurrent valvular dysfunction with biological xenografts. Retrospective research in this area disputes this contention however, and promotes the use of bio-prostheses in ESRF, due to the reduced risk of haemorrhage from anticoagulation, and the similar medium-term post-surgical prognosis. In the absence of prospective studies, and with large
observational studies demonstrating high mortality rates for ESRF patients following valve replacement regardless of prosthesis type, current guidelines suggest bioprosthetic valves may be appropriate in many patients.\textsuperscript{227} Progressive valvular calcification and dysfunction are highly prevalent in CKD and ESRF and represent a unique challenge to physicians treating affected patients. In the context of an aging Australian population, and the provision of dialysis to increasingly elderly patients, valvular calcification and dysfunction will inevitably become more common in the management of Australian CKD patients.

**Endothelial Dysfunction in CKD**

It is widely acknowledged that endothelial dysfunction is an important initiating factor in the development of atherosclerosis.\textsuperscript{228} Endothelial dysfunction represents a failure of the balance between endothelial injury and the capacity for repair and involves the fundamental alteration of vaso-protective mechanisms into pro-atherosclerotic tendencies, with the hallmark change of impaired NO bioavailability. Dysfunctional endothelium is characterised by a reduction in vaso-dilatory molecules (most notably NO), and an increase in vaso-constrictive factors.\textsuperscript{229} This imbalance is largely quantified by dynamic changes in conduit vessel size, mediated by these endothelium-derived vasoactive molecules, described as ‘endothelium-dependent’ vasodilatation.\textsuperscript{230} Additionally, dysfunctional endothelium becomes pro-coagulant (promoting clinically relevant athero-thrombotic events) and pro-inflammatory (releasing cytokines that are chemo-attractant for chronic inflammatory cells).\textsuperscript{231} As previously mentioned, the development of this local pro-inflammatory milieu leads to local tissue injury,
fibrotic / calcific repair and the development of atherosclerotic plaques. Thus, endothelial dysfunction provides a unique marker of the systemic tendency towards the development of future, clinically significant CV events.\textsuperscript{232}

Pathobiology of Endothelial Dysfunction in CKD

Numerous vasodilatory and anti-aggregatory factors are released by the intact endothelium. The principal smooth muscle cell relaxing factors are NO, prostacyclin (PGI\textsubscript{2}) and endothelial derived hyperpolarising factor (EDHF) which also exert anti-aggregatory effects on local platelets.\textsuperscript{233} Endothelin-1 (ET-1) and thromboxane (TXA\textsubscript{2}) are prominent examples of endothelial-derived vasoconstrictive factors that have been implicated in diseases of the vascular endothelium.\textsuperscript{234} In the evaluation of endothelial function, substantial work has focussed on NO as a critical factor, due to the experimental capacity for NO to protect against atherosclerosis, and for NO inhibition to promote experimental atheroma formation.\textsuperscript{235} Clinically, endothelial dysfunction is almost universally characterised by reduced NO bioavailability, and NO bioavailability is known to be reduced in CKD.\textsuperscript{235}

NO is formed \textit{in vivo} by transformation of the amino acid L-arginine through the action of endothelial NO-synthase (eNOS). Once produced, NO is susceptible to further transformation by local oxygen free radicals in situations of high oxidative stress (such as CKD), with resultant loss of its vaso-protective activity. Specifically, superoxide anion (O\textsubscript{2}•) scavenges NO, yielding peroxynitrite (ONOO•), which may be further transformed to form nitrate and the highly reactive OH• - a potent contributor to local tissue injury.\textsuperscript{233,236,237}
In addition to increased NO degradation, NO activity may be further affected by reduced production in CKD.\textsuperscript{238,239} eNOS may be inhibited by a variety of circulating factors, many of which accumulate with declining renal function and have been proposed as pathogenic in CKD. Principal among the factors implicated in the endothelial dysfunction of uraemia is asymmetrical dimethylarginine (ADMA) – a member of a family of guanidine compounds known to be present in uraemic serum in sufficient concentrations to inhibit eNOS.\textsuperscript{240} The relationship between ADMA and the promotion of endothelial dysfunction and CVD in CKD has been extensively investigated since the initial proposal of causation in 1992.\textsuperscript{241,242} ADMA has been associated with impairment in measures of endothelial function\textsuperscript{243}, and has been found to be a strong predictor of CV events and death in patients with early CKD, ESRF and in the general population.\textsuperscript{241,244,245} Furthermore, as a small, water-soluble molecule, ADMA is freely removed with dialysis\textsuperscript{246}, and acute reduction in ADMA following dialysis have been associated with acute improvements in endothelial function\textsuperscript{247}, although these findings are contentious.\textsuperscript{248-251}

Thus, the combined effects of increased oxidative stress within the pro-inflammatory milieu of CKD, and the endothelium-toxic effects of uraemic serum, contribute to the inhibition of endothelial vaso-protective properties and the promotion of the clinical phenotype of endothelial dysfunction of CKD. This phenotype is believed to be predominantly responsible for the promotion of atherosclerosis in advancing CKD\textsuperscript{241,252} – a process known to be profoundly accelerated in this condition.\textsuperscript{253}
Assessment of Endothelial Function

In the research and clinical settings, endothelial function may be measured utilising a variety of different methodologies, and in a variety of vascular beds. Numerous circulating biomarkers have been associated with endothelial dysfunction, both in the general community and in CKD.\textsuperscript{254-256} Evaluation of flow-mediated arterial dilatation (FMD) is the most commonly utilised approach for the evaluation of regional endothelial function. This method relies upon the normal endothelial cell response to increased mechanical shear-stress – the release of endothelium-derived factors responsible for vascular smooth muscle cell relaxation and subsequent vaso-dilatation.\textsuperscript{254} Utilising this principle, a standardised method for evaluating conduit artery dilatation in response to increased flow was developed.\textsuperscript{257} Evaluating the calibre of the brachial artery (most commonly), FMD is measured in response to an increase in arterial flow, typically induced by a period of distal circulatory bed ischaemia (e.g. forearm).\textsuperscript{254} Ultrasound methodologies are most commonly used, but suffer from the potential for investigator-initiated inaccuracies – particularly in inexperienced hands.\textsuperscript{254,258} Newer imaging methodologies, such as computed tomography (CT) and cardiovascular magnetic resonance imaging (CMR), are increasingly being utilised to enhance diagnostic accuracy and reproducibility, and potentially contribute additional functionality in this field (see below).\textsuperscript{259,260}

Endothelial Dysfunction and Cardiovascular Risk in CKD

FMD is mediated, in part, by the release of NO from endothelial cells – a response that is reduced in patients with CV risk factors or clinically significant
atherosclerosis (peripheral, cerebral and coronary). Evaluation of endothelial dysfunction in the forearm (i.e. peripheral) is used as a surrogate for endothelial function in clinically relevant arterial systems such as the coronary circulation and hence has been used to provide a non-invasive method for predicting the presence of coronary artery atherosclerosis, and future coronary events in at-risk patients. Additionally, improvements in peripheral endothelial function have been associated with therapeutic interventions that improve cardiovascular risk. Almost all traditional and unconventional risk factors for atherosclerosis and CV morbidity and mortality have been found to be associated with endothelial dysfunction. The mechanisms by which smoking, hypertension, diabetes mellitus and hypercholesterolaemia contribute to the development of endothelial dysfunction are complex and heterogenous. CKD provides a particularly complex precipitant for endothelial dysfunction, resulting from multiple circulating factors injurious to the endothelium and promotional of the necessary pro-inflammatory setting for endothelial dysfunction and accelerated atherosclerosis. Endothelial dysfunction has been identified in pre-dialysis CKD patients, haemodialysis and peritoneal dialysis patients and following renal transplantation and has similarly been implicated in the promotion of cardiovascular structural and functional alterations promoting CVD. Notably, impairment in forearm endothelial function has been shown to be associated with an increase in all-cause mortality in ESRF.
Coronary Artery Disease in CKD

Coronary artery disease (CAD) is a leading cause of morbidity and mortality in CKD patients.\textsuperscript{20,141} As with the systemic circulation, the coronary arteries may be affected by a wide range of pathological processes, including endothelial dysfunction, intimal / medial calcification, atherosclerosis, and atherothrombosis precipitating acute coronary syndrome (ACS). Disease within the coronary arteries, however, plays a significantly greater role in CV morbidity and mortality, and hence evaluating and understanding the patho-physiology of CAD is of critical importance in attempts to ameliorate the risks of CVD in CKD patients.

Coronary Endothelial Function

The coronary endothelium is responsible for the maintenance of blood flow within the coronary circulation, and the regulation of inflammation, thrombosis and platelet activation in this territory.\textsuperscript{254} Imbalance between local vaso-dilatory, anti-inflammatory substances (such as NO) and vaso-constrictive, pro-inflammatory mediators (such as ET-1) provides the basis for the development of coronary endothelial dysfunction and clinically significant CAD. Significantly, coronary endothelial dysfunction has been demonstrated to be predictive of increased risk for subsequent cardiac events, even in the presence of only minor coronary atherosclerosis.\textsuperscript{262,269}

As previously mentioned, peripheral measures of endothelial function (i.e. FMD) have been associated with invasively assessed coronary endothelial function, although the absolute correlation was found to be modest.\textsuperscript{270} Peripheral arterial
applanation tonometry has also been utilised to estimate coronary vasomotor function, though such indirect measures remain surrogates for actual coronary endothelial function.\textsuperscript{254}

“Gold standard” evaluation of coronary artery endothelial function involves the introduction of a Doppler-based coronary flow wire into the coronary circulation, and evaluating coronary flow and diameter characteristics in response to a range of stimuli – both direct pharmacological agents and remote (e.g. cold pressor\textsuperscript{271}) stimuli. Classically, acetylcholine (Ach) is introduced into the coronary artery utilising an intra-coronary infusion catheter during invasive coronary angiography (ICA). The coronary flow wire is repositioned within the proximal to mid-portion of the coronary artery avoiding nearby bifurcations, until a stable Doppler signal is achieved. Coronary artery diameter (by quantitative coronary angiography [QCA], or more recently by intravascular ultrasound [IVUS]\textsuperscript{272}), is assessed 5mm distal to the tip of the coronary flow wire. Alterations in coronary blood flow (CBF) and coronary vascular resistance are observed in response to infusion of previously validated doses of intra-coronary Ach.\textsuperscript{273,274}

The response to Ach provides a marker for the local availability of NO, and hence can evaluate both macrovascular and microvascular coronary endothelium-dependent vasodilator function.\textsuperscript{254} In the presence of dysfunctional endothelium, the normal vaso-dilatory stimulus of Ach can become vaso-constrictive, with subsequent changes in macrovascular and microvascular function measured by changes in coronary luminal diameter and CBF respectively. Endothelium-independent microvascular coronary vasomotor function is also assessed utilising the administration of intracoronary adenosine boluses (18-48µg) to
achieve maximal hyperaemia, with endothelium-independent macrovascular function measured in response to intra-coronary glycercylnitrate (GTN). Coronary blood flow is calculated from the widely used formula:

\[
\pi \times 0.125 \times (\text{Coronary Diameter})^2 \times (\text{Coronary Blood Flow APV}) \times 60
\]

where Diameter is measured in cm, Average Peak Velocity (APV) of coronary blood flow is measured in cm/sec and CBF is measured in mL/min.

A normal response is considered to have occurred if endothelial-dependent CBF increases by more than 50% in response to Ach, and the coronary flow reserve (CFR) – the ratio of maximal to baseline CBF - is greater than 2.5 following adenosine.

Remarkably little work has focussed on the evaluation of coronary endothelial function in CKD. Although the invasive nature of the procedure has inherent risks, it is likely that the potential nephrotoxicity of angiographic contrast agents has impeded assessment of pre-dialysis patients in particular. It is widely presumed that the extensive literature demonstrating peripheral endothelial dysfunction in CKD and ESRF applies similarly to the coronary circulation. A single study evaluating non-endothelium-dependent coronary endothelial function amongst diabetic patients (a cohort known to have a high prevalence of coronary endothelial dysfunction) with ESRF but angiographically normal coronary arteries was published in the American Heart Journal in 2004. This study compared invasive coronary endothelial function indices in 11 diabetic patients with preserved renal function (serum creatinine < 1.0mg/dL [88µmol/L]) and no evidence of microvascular complications (i.e. retinopathy or neuropathy), with 21
patients with ESRF from diabetic nephropathy. A control group of 32 subjects without diabetes or renal impairment was also evaluated. CFR was found to be impaired (defined in this Study as CFR<2.0) in 9% of normal subjects and 18% of diabetic, non-ESRF patients, compared to 57% of patients in the diabetic nephropathy cohort (p<0.001). Interestingly, APV increased in all cohorts in response to maximal hyperaemia (with intravenous adenosine 140µg/kg/min), and peak APV was not significantly different between the 3 groups.277 The reason for the reduced CFR amongst the ESRF cohort (1.6±0.5 vs. 2.7±0.7 for non-ESRF diabetics vs. 2.8±0.8 for normal controls, p<0.001 for ESRF vs. other cohorts) was an elevated basal APV in 67% of abnormal CFR cases.277 That is, ESRF appeared to be associated with an elevation in basal coronary blood flow velocity, resulting in a blunted response to hyperaemic stimulus (adenosine), rather than the presence (solely) of microvascular disease which might be expected to cause blunting of the hyperaemic response, rather than elevation of baseline APV. This finding has recently been affirmed non-invasively utilising PET.278 Published in 2009, this Study evaluated basal and hyperaemic (post-dipyridamole) myocardial perfusion in twenty-two patients with moderate to severe CKD (divided into three cohorts according to eGFR), and compared these results to ten healthy control subjects with preserved renal function. Although this Study found preserved CFR amongst the CKD patients (as distinct from the previously discussed invasive study), basal myocardial blood flow was significantly elevated amongst the CKD patients vs. controls (p<0.001), with a negative correlation between eGFR and myocardial blood flow seen (Spearman correlation coefficient=0.63, p=0.0001).278 An ultrasound-based FMD protocol was also undertaken in this Study, with FMD (% change in brachial artery diameter)
significantly reduced amongst the CKD cohorts compared to controls (p<0.03). The authors conclude that “coronary and peripheral vascular function are disturbed by different mechanisms in patients with CKD”, although direct comparison of the two methodologies utilised in this Study is questionable. FMD is a measure of endothelium-dependent vasodilatation in response to an increase in endothelial shear-stress (flow) resulting from induction of distal vascular bed ischaemia.\textsuperscript{25,27}\textsuperscript{9} This evaluation of large-vessel endothelial-dependent function in the brachial artery is distinct from the evaluation of coronary endothelium-independent, microvascular function evaluated following the administration of dipyridamole in this study. Although previous studies have demonstrated a link between peripheral microvascular function (as assessed by ‘gold-standard’ venous occlusion plethysmography) and peripheral arterial FMD responses\textsuperscript{280}, the two are not equivalent and comparison of coronary microvascular responses to dipyridamole with FMD as a surrogate marker of peripheral microvascular function might perhaps be considered disingenuous.\textsuperscript{281}

A further Study evaluated invasive CFR amongst 124 patients with GFR<60mL/min/1.73m\textsuperscript{2}, compared to 482 control subjects with GFR>60mL/min/1.73m\textsuperscript{2}, finding reduced GFR to be predictive of impaired CFR by univariate, but not multivariate, analysis.\textsuperscript{282} Although the mean CFR was reduced in subjects with mildly impaired renal function (compared to controls), only increasing age, female gender, rising BMI and co-existent hypertension were significantly associated with impairment of CFR by multivariate analysis.\textsuperscript{282} No comment was made on differences in basal APV values between the two cohorts.

Although the non-invasive Study, in particular, has limitations, these three studies highlight abnormalities in coronary microvascular homeostasis in
patients with declining renal function. Although uraemia per se may directly influence microvascular function, coexistent factors such as diabetes mellitus, hypertension and LVH may play a significant role in the genesis of elevated basal CBF, and impairment in hyperaemic reserve in CKD patients. The metabolic syndrome and hypertension (+ associated myocardial diastolic dysfunction) have been implicated in the genesis of CFR impairment in the general population.\(^{283-288}\)

Numerous studies identifying impairment in coronary microvascular function in LVH have also been published\(^{289-296}\), however only a few have demonstrated an increase in basal CBF.\(^{291,293}\) Furthermore, LVM alone may not be responsible for this impairment in CFR on a background of increased basal CBF, as previously demonstrated by the differing CFR between athletes with compensatory LVH, and hypertensive patients with secondary LVH.\(^{292}\)

Numerous other factors associated with CKD have also been associated with coronary microvascular dysfunction. Mitral annular and aortic valve calcification – common findings in CKD - have also been associated with findings of reduced CFR in the general population.\(^{297,298}\) Furthermore, systemic inflammation in patients with systemic lupus erythematosis and rheumatoid arthritis has previously been identified as a potential contributor to coronary microvascular dysfunction\(^{299}\), and this may have implications for the microvascular dysfunction of the pro-inflammatory uraemic state of CKD also.

Anaemia, expansion of the extracellular fluid compartment and iatrogenic arterio-venous fistulae (AVF) for the performance of haemodialysis may all contribute substantially to the increase in systemic blood-flow of CKD and ESRF patients. Combined with compensatory LVH and increased myocardial metabolic demand caused by elevated systemic blood pressure, systolic myocardial wall
stress, diastolic LV pressure and myocardial mass, an increase in basal coronary blood flow in this condition appears likely.\textsuperscript{295} Uraemic microvascular dysfunction and obstructive coronary microvascular disease also appear potential contributors to the blunted coronary microvascular response to dipyridamole and adenosine in studies to date. Thus, in combination with an increase in basal oxygen demand, CKD and ESRF patients likely suffer from an inability to sufficiently increase myocardial oxygen delivery during periods of increased metabolic demand (e.g. exercise / obstructive coronary disease), potentially contributing substantially to the elevated rate of symptomatic CAD in this condition.

Remarkably, endothelial-dependent coronary vasomotor function appears not to have been evaluated in this condition. This observation forms the basis for the invasive component of this PhD.

**Coronary Atherosclerosis**

CKD is associated with altered coronary plaque composition, including a higher calcific burden and more diffuse atherosclerotic disease.\textsuperscript{253,300-302} These differences in plaque morphology may play a significant role in the genesis of clinically significant cardiac events. In particular, plaque calcification is more extensive within coronary plaques of uraemic patients than amongst CAD patients with preserved renal function.\textsuperscript{204,302} Strikingly, this calcification of coronary plaques is evident in ESRF patients from a young age – much younger than is usual within the general population.\textsuperscript{303} Furthermore, the process of atherosclerotic plaque calcification appears to increase progressively with
declining renal function, greatest amongst ESRF patients requiring dialysis.\textsuperscript{204} This calcification has been demonstrated to be almost entirely calcium phosphate, rather than calcium oxalate or other conjugates.\textsuperscript{302} This finding has particular relevance in the context of the altered calcium \textit{}/phosphate handling of advancing CKD, and the control of calcium \textit{x} phosphate product in ESRF patients in particular, although this link remains contentious.\textsuperscript{304} Another striking finding regarding CAD in the context of declining renal function is the propensity for progression, once initiated.\textsuperscript{305} Not only is the prevalence of coronary atherosclerotic plaques amongst autopsy and angiographic studies of ESRF patients high\textsuperscript{301,306,307} – approximately 30-40\% of most series – but the presence and severity of coronary atherosclerosis plays a significant role in patient survival, particularly following dialysis commencement.\textsuperscript{308,309} The traditional assessment of CV disease severity based on symptoms and evidence of end-organ damage may not adequately represent the true risk of CVD in CKD patients.\textsuperscript{256} As previously stated, the inverse relationship between renal function and CVD-risk implicates disease-specific mechanisms in the development of the accelerated atherosclerosis, arteriosclerosis and myocardial disease seen of CKD.\textsuperscript{143,310} Not only is the burden of coronary atherosclerotic disease greater, but the propensity for clinically relevant coronary atherothrombotic events is increased further by the uraemic milieu.

Over the past three decades, enormous advances have been made in the treatment of acute coronary syndrome (ACS). Invasive revascularisation and adjunctive pharmacotherapy, in addition to standard anti-ischaemic therapy, has led to marked reductions in CV mortality in the general population.\textsuperscript{19} Unfortunately, the prognosis for ESRF patients following ACS remains
unacceptably high however, and has not appreciably improved over the same time period. Following acute myocardial infarction (MI), patients with ESRF have been reported to have an inpatient mortality of 31.8\% \textsuperscript{312} and longer term mortality rates of 59.3\% at 1 year, and 89.9\% at 5 years.\textsuperscript{313} This abysmal prognosis is not only confined to dialysis patients however, with a progressive gradient of declining prognosis following MI in advancing CKD.\textsuperscript{312} In a recent evaluation of 19,029 patients presenting to US hospitals with ST-segment elevation myocardial infarction (STEMI), in-hospital survival amongst patients with Stage 3a (eGFR=45-59mL/min/1.73m\textsuperscript{2}), Stage 3b (eGFR=30-44mL/min/1.73m\textsuperscript{2}) and Stage 4 (eGFR=15-29mL/min/1.73m\textsuperscript{2}) CKD was 8.8\%, 17.9\% and 27.3\% respectively (p value for interaction<0.0001).\textsuperscript{312} This compared to in-hospital mortality of 2.3\% for patients with preserved renal function.\textsuperscript{312}

Although less profound, CKD patients also suffer a markedly increased risk of death following hospitalisation for non-STEMI ACS.\textsuperscript{312,314,315} Amongst 30,462 patients presenting with non-ST-segment elevation myocardial infarction (NSTEMI) to US hospitals in 2007, in-hospital mortality increased from 1.8\% for patients without renal dysfunction, to 13.4\% and 12.4\% for patients with Stage 4 and Stage 5 CKD respectively.\textsuperscript{312}

The are numerous reasons for this increased risk. CKD patients have been systematically excluded from the majority of clinical trials assessing new therapies in CVD.\textsuperscript{316,317} Thus, concerns exist regarding the applicability of clinical guidelines based on studies from non-CKD patients, to the CKD population. Significantly, CKD patients presenting to hospital with ACS tend to be ‘undertreated’, with lower rates of ‘routine’ medication utilisation (e.g. aspirin,
clopidogrel, beta-blockers, statins) and lower rates of coronary revascularisation.\textsuperscript{312,318,319} This discrepancy may result from considered omission rather than neglect, given previous findings of equivocal benefit for statin therapy in ESRF\textsuperscript{35}, and concerns regarding the nephrotoxicity of an invasive management strategy in pre-dialysis patients (Stages 3-4 ± 5). In fact, it appears that dialysis patients may receive more aggressive interventional therapy than pre-dialysis patients for this reason, with the Study by Fox et al\textsuperscript{312} demonstrating increased utilisation of cardiac catheterisation, percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) in Stage 5, versus Stage 4, CKD in NSTEMI patients. In STEMI patients however, the utilisation of invasive management strategies declined with increasing CKD severity.\textsuperscript{312} Despite the increased overall mortality in Stage 5 CKD, bleeding rates are high in advancing CKD, particularly in the context of the anti-thrombin and anti-platelet agents commonly employed in the management of ACS. This excess risk of major bleeding may stem from the intrinsic platelet dysfunction of uraemia, or from inappropriate dosing in the context of reduced renal clearance.\textsuperscript{320-322} Furthermore, the high rate of pre-existing cardiac dysfunction (both diastolic and systolic), and the reduced physiological reserve induced by advancing CKD appear to contribute substantially to the poor prognosis of CKD patients following hospitalisation with acute myocardial ischaemia.

Even with aggressive invasive management of CAD, historical 2-year survival rates following CABG and PCI have been reported to be 56.4±1.4% and 48.4±2.0% respectively.\textsuperscript{323} This poor prognosis also highlights a potential advantage of CABG over PCI for the revascularisation of ESRF patients, with the relative benefit of CABG over PCI confirmed by other studies.\textsuperscript{324} This advantage
appears to stem from the very high re-stenosis rates in ESRF patients following percutaneous trans-luminal coronary angioplasty (PTCA) and bare-metal stent PCI, although differences in patient complexity have been blamed.\textsuperscript{325-327} Recent results for PCI in the drug-eluting stent era have been more promising however\textsuperscript{328}, though renal insufficiency remains a potent predictor for increased risk of late stent thrombosis.\textsuperscript{329}

**Vascular Disease in Chronic Kidney Disease**

CKD is characterised by complex alterations in vascular structure and function. Although atherosclerotic disease is highly prevalent in CKD and ESRF, contributing substantially to the burden of cardiovascular morbidity and mortality in this condition, non-atheromatous vascular remodelling, calcification and stiffening play a major role in the evolution of CVD in this population.

**Arterial Function**

The systemic arterial bed has two distinct, inter-related functions: to deliver an adequate blood-supply to peripheral tissues (conduit function) and to modulate pressure variations arising from intermittent left ventricular ejection (cushioning function).\textsuperscript{330} The conduit function relies on transmission of blood via large, low-resistance central arteries, to smaller, less elastic peripheral arteries and arterioles. In the absence of atherosclerotic obstruction, the conduit function can accommodate significant changes in blood-flow, depending on distal tissue requirements (e.g. skeletal muscle beds may receive up to 10-times resting blood-flow during exertion).\textsuperscript{331} The arterial conduit adaptation to increased
physiological demand is mediated through acute changes in blood-flow velocity, and by arterial diameter (resistance) changes – a process necessitating intact endothelium-dependent vasomotor function.\textsuperscript{331}

The arterial cushioning effect arises predominantly from the large, central elastic arteries (such as the aorta and carotid arteries), and lessens progressively towards the periphery, as arteries become more muscular.\textsuperscript{332} This arterial elastance allows for the accommodation of up to 50% of ventricular stroke volume within the larger central arteries, with elastic recoil in diastole ensuring continuous tissue perfusion across the cardiac cycle.\textsuperscript{331} The capacity for the arterial system to cushion the impact of ventricular systolic ejection depends on the ‘visco-elastic’ properties of the arterial wall.\textsuperscript{331} The elasticity of the central arteries is largely a function of the absolute and relative representation of elastin and collagen within the vessel wall.\textsuperscript{331} The collagen to elastin ratio increases progressively towards the periphery, contributing to progressively stiffer “resistance” arteries distally. Additionally, turnover of extracellular matrix proteins by metalloproteinases and other proteolytic enzymes contributes to arterial stiffness over time.\textsuperscript{332} Finally, vascular calcification – as a function of atherosclerosis, age and other disease states (such as CKD) – may also contribute to larger artery stiffness.\textsuperscript{333}

As arterial elasticity declines, the elastic recoil during ventricular diastole progressively lessens, contributing to lower diastolic blood pressure. Additionally, central arterial stiffness leads to an increased velocity of the forward pressure wave (from left ventricle to the periphery). In an elastic arterial system, this forward (incident) pressure wave is reflected from sites of structural or functional inhomogeneity within the peripheral arterial tree, creating a
backward (reflected) pressure wave (from periphery towards ascending aorta) that contributes to the maintenance of diastolic blood pressure.\textsuperscript{334,335} The incident pressure wave and the reflected pressure waves interact, with timing and amplitude of each wave contributing to the final arterial waveform for each cardiac cycle.\textsuperscript{336} The appearance and amplitude of these waveforms also depend entirely on the site of assessment as it relates to the sum of the component waves, as pressure waveforms within the central arteries (distant from sites of reflection) will be different to those of the peripheral arteries (closer to sites of wave reflection).

Increased vascular stiffness is associated with increased incident wave velocity, and hence earlier wave reflection from the periphery. Thus, the reflected waves may eventually arrive within the ascending aorta during mid-systole, rather than diastole, contributing substantially to left ventricular afterload, and detracting from diastolic blood pressure. Thus, aging and disease states that increase vascular stiffness contribute to an increase in central systolic blood pressure, a decline in diastolic blood pressure, and a resultant elevation of pulse pressure (PP) at the expense of coronary artery diastolic perfusion pressure.\textsuperscript{337-340}

**Atherosclerosis**

In advancing CKD, atherosclerosis poses a significant threat to arterial conduit function and tissue perfusion. Atherosclerosis is often well-advanced prior to the commencement of dialysis in ESRF\textsuperscript{341-343}, but appears to be accelerated in haemodialysis patients in particular.\textsuperscript{253} Although CAD contributes substantially to the burden of overall CVD in CKD patients, atherosclerosis within the peripheral,
cerebral and renal vascular beds plays a major role in overall patient morbidity and mortality.\textsuperscript{22,344,345} As with the coronary system, atherosclerosis in CKD patients exhibits a higher calcific burden, and less lipidic plaque as is usual in the general population. Given the dearth of evaluable benefit for statins in ESRF\textsuperscript{34,35} (potentially related to the more advanced fibrotic/calcific nature of atherosclerotic plaques in ESRF), atherosclerotic disease prevention benefits greatest from earlier intervention in the course of CKD disease progression.

**Arteriosclerosis**

Significant arterial structural remodelling occurs in CKD. Many of the alterations identified in CKD patients are similar to alterations known to occur with aging – specifically dilatation, intimal-medial hypertrophy and stiffening of the aorta and major arteries.\textsuperscript{346} In advancing CKD, anaemia, arterio-venous fistulae, hypertension and fluid retention contribute to a perpetual state of “volume/flow overload” that promotes compensatory systemic arterial remodelling.\textsuperscript{331} The result of this remodelling, however, is highly disadvantageous to end-organ and cardiac function.

CKD is characterised by a stepwise increase in vascular stiffening corresponding to the stages of declining renal function.\textsuperscript{347} This progressive decline in aortic and elastic artery distensibility is associated with progressive increases in incident (and reflected) wave velocity and resultant patho-physiological complications. Intima-media thickening and calcification, particularly within the larger elastic arteries, contribute substantially to this increased vascular stiffening.\textsuperscript{346} The resultant increases in systolic and pulse pressures, LV afterload and
compensatory LVH represent core components of the clinically relevant cardiovascular pathology of CKD.

This combination of arterial and ventricular stiffening in CKD leads to a progressive decline in cardiac reserve, as the reduced arterial compliance leads to disadvantageous physiological responses to increased circulatory demand. Exertion is generally associated with increases in heart rate (HR) and LV stroke volume (LVSV) (unless diastolic dysfunction is severe, where LVSV may fall with rising HR). In CKD, the increased arterial stiffness is associated with magnified blood pressure and LV afterload changes with any increase in LV stroke volume, due to the reduced arterial capacitance reserve. Significantly, coronary perfusion may be markedly altered during these conditions, even in the absence of obstructive coronary atherosclerosis.

**Vascular Calcification**

Vascular calcification primarily refers to the process of calcium phosphate deposition within the intima and/or media of arteries and arterioles, and is a common finding with advancing age and atherosclerosis. In CKD, vascular calcification is a potent contributor to increased arterial stiffness. As previously described, destruction of the renal parenchyma leads to a pro-calcific milieu that enhances and promotes premature cardiac and vascular calcification, contributing significantly to the excess burden of CV morbidity and mortality in this condition. Vascular calcification may be intimal or medial, with differing aetiologies and clinical implications. Intimal calcification is primarily associated with atherosclerotic plaques, and is most commonly identified as diffuse,
punctate crystals that may progressively aggregate.\textsuperscript{349} It has been proposed that atherosclerotic calcification develops initially within more metabolically active plaques and may represent healing of previously disrupted plaques.\textsuperscript{350} Furthermore, intimal calcification may be promoted by vascular smooth muscle and macrophage cell death, lipoproteins (particularly oxidised forms) and the presence of increased extracellular calcium and phosphate concentrations.\textsuperscript{349} IVUS studies, however, indicate that vulnerable coronary plaques in the general population are most commonly not calcified, and that increased calcification is associated with more stable, fibrotic plaques.\textsuperscript{351-354} Thus, in the general population, progressive calcification may represent an adaptive mechanism stabilising atherosclerotic plaques at a later stage of their temporal development.\textsuperscript{355} In CKD however, the presence of excessive vascular calcification, may represent an acceleration of this adaptive response, in relation to a greater burden of atherosclerosis and a more amenable systemic milieu.

Medial calcification however, occurs independently of atherosclerosis, most commonly in the form of linear deposits along elastic lamellae and increases progressively with age, contributing substantially to vascular stiffening.\textsuperscript{356} At it’s most severe, it may form dense circumferential sheets within the media, bounded by vascular smooth muscle cells on either side.\textsuperscript{349} Most commonly identified in the peripheral vessels of elderly individuals as the appearance of vascular “rail-tracking” on plain radiographs\textsuperscript{357}, medial calcification is also prevalent in younger patients with diabetes and CKD.\textsuperscript{349}

As previously discussed, although the mechanisms behind the pro-calcific milieu of CKD are numerous and remain to be definitively delineated, the pathophysiological implications are widely appreciated. Vascular calcification is
associated with significant morbidity and mortality.\textsuperscript{333,358-363} Arterial calcification contributes to significant vascular structural and functional alterations that are subsequently associated with decreased DBP and increased PP\textsuperscript{364} - haemodynamic indices closely linked with reduced survival in ESRF patients.\textsuperscript{365,366} In ESRF, vascular calcification is strongly influenced by the prescribed dose of calcium-based phosphate binders and the duration of haemodialysis.\textsuperscript{364} Currently-available therapies to reduce the burden of vascular calcification, once present, have failed to dramatically alter the poor prognosis of ESRF patients however.\textsuperscript{40,367} Further work in this field is urgently required.

Non-invasive Evaluation of Arterial Stiffness

\textit{Pulse Wave Velocity}

Vascular stiffening may be evaluated by various methodologies. Perhaps the simplest and best-validated measure is pulse wave velocity (PWV) – the assessment of the velocity of incident wave travel between two points in the arterial system. Thus, to calculate PWV, the distance travelled between two points is divided by the time taken by the incident wave to traverse the points (PWV = Distance / Time). There is a linear correlation between the speed of travel of the pulse wave and arterial stiffness over the distance traversed.\textsuperscript{348} Carotid-femoral PWV is considered the ‘gold standard’ for the evaluation of central arterial stiffness.\textsuperscript{368} Widely used in epidemiological and interventional studies in CKD, ESRF and a variety of other disease-states, carotid-femoral PWV relies on an assumption of distance between the aortic arch and the site of evaluation at the carotid artery, and on the course of the aorta (measured as a
straight line over the ventral thorax and abdomen) between the right carotid and right femoral sites of evaluation.\textsuperscript{368} Although numerous methods have been utilised to reduce the potential for inaccuracy based on inaccuracies in surface distance measurement (e.g. related to factors such as abdominal obesity or prominent female bust), or partial obstruction to flow induced by aortic or iliac artery atherosclerosis.\textsuperscript{369} Generally, transit-time is measured from foot-to-foot of the waves at the two measurement sites (Figure 9), and may be enhanced further by electrocardiographic gating.\textsuperscript{368} Although differences in distance measurement arising from body habitus or ectatic aortic degeneration (common in the elderly and hypertensives) are less important in serial measurements (assuming the same inaccuracies are made at each evaluation), establishment of normal population values, or comparison of results between populations may be severely impaired by such methodological inaccuracies.\textsuperscript{368}
Figure 9: Measurement of carotid-femoral PWV, utilising the foot-to-foot method, where \((\Delta t)\) represents the change in time, and \((\Delta L)\) represents the distance between waveform measurement sites. (Reproduced from Laurent S. et al. Eur Heart J, 2006;27:2592, with permission from Oxford University Press).

Regardless of the known limitations of the methodology, PWV is independently predictive of future cardiovascular events, cardiovascular death and all-cause mortality in a variety of patient cohorts – including ESRF, pre-dialysis CKD, diabetes, established CAD, essential hypertension, healthy elderly and younger, normal populations. The simplicity, reproducibility and prognostic significance of PWV ensures it will remain the
non-invasive gold standard for the evaluation of central arterial stiffness for the foreseeable future.

**Arterial Distensibility**

Although PWV provides an “overall” assessment of central (aortic/carotid/iliac) arterial stiffness, methods for the assessment of regional arterial stiffness may provide alternative, or incremental information not provided by PWV (e.g. specific evaluation of carotid artery stiffness in the assessment of cerebrovascular risk). Distensibility of superficial arteries such as the carotid, brachial, femoral, and more distal arteries have been evaluated utilising ultrasound techniques (Figure 10). Such techniques require a higher level of training, and are more time consuming than evaluation of PWV, and hence are poorly suited to epidemiological studies and better suited to mechanistic / interventional studies.\(^{368}\) Magnetic resonance imaging has more recently been utilised to evaluate non-superficial arteries (such as the aorta) in a variety of physiological and disease states\(^ {259,386-393}\), and has been shown to correlate well with traditional PWV.\(^ {394}\) Very recently, CMR-derived aortic distensibility and volumetric arterial strain have been found to be predictive of cardiovascular clinical end-points and overall survival in ESRF on multivariate analysis.\(^ {395}\)
Figure 10: Schematic representation of the method for determining arterial distensibility by measuring changes in arterial lumen cross-sectional area across the cardiac cycle ($\Delta A =$ Maximal change in lumen area during cardiac cycle; $D =$ diameter), as they relate to local pulse pressure. (Reproduced from Laurent S. et al. Eur Heart J, 2006;27:2593, with permission from Oxford University Press).

In addition to the evaluation of arterial stiffness through assessment of PWV and arterial distensibility, evaluation of the central impact of peripheral wave reflection may also be performed non-invasively.\textsuperscript{368} This may be performed through the evaluation of the arterial waveform at the common carotid artery, or peripherally (conventionally the radial artery) through the application of a ‘transfer function’.\textsuperscript{396-398} Both Millar strain gauge transducers, and arterial applanation tonometry have been used for this purpose.\textsuperscript{396-400} Whether directly evaluated (at the carotid), or derived from peripheral (radial) artery applanation tonometry, the resultant central pressure waveform can be interrogated to determine the augmentation index (AIx) – the difference between the second and first systolic pressure peaks ($P2 - P1$; Figure 11), expressed as a percentage of the
Central arterial AIx has been demonstrated to provide independent prognostic value in the prediction of all-cause mortality in ESRF patients\textsuperscript{401,402}, as well as the prediction of CV events in a variety of other disease states.\textsuperscript{403-405}

\textbf{Figure 11:} Representation of an illustrative central pressure waveform, demonstrating identification of the Augmentation Pressure; determined as the difference between the second (P1) and first (P2) systolic pressure peaks. AIx may then be calculated by evaluation of the augmentation pressure in relation to the pulse pressure. (Reproduced from Laurent S. et al. Eur Heart J, 2006;27:2595, with permission from Oxford University Press).
**Arterial Stiffness as a Modifiable Risk Factor**

Arterial stiffness provides a clinically relevant measure of longitudinal vascular injury and is predictive of future cardiovascular events independent of conventional CV risk factors.\(^{372,375,379,380,382,383,406}\) Although risk factors such as hypertension and hyperlipidaemia may be effectively reduced by pharmacologic intervention, the vascular injury induced by such risk factors over time is not immediately negated by such therapies. Thus arterial stiffness has been postulated to provide a more relevant surrogate for future CV risk than conventional risk factors.\(^{370}\) Until recently, however, this premise had received little attention.

The *Conduit Artery Function Evaluation (CAFÉ)*\(^404\) sub-study drew on 2,073 patients from the larger *Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT)*\(^407\) to assess this issue. CAFÉ demonstrated that despite similar reductions in brachial systolic blood pressure (SBP) and PP, the combination of amlodipine + perindopril was more efficacious in reducing central SBP and PP than the atenolol + thiazide combination\(^404\), corresponding to the demonstration of reduced cardiovascular events in the amlodipine + perindopril cohort.\(^407\) Similarly, losartan was shown to reduce arterial stiffness and central PP to a greater degree than the comparator (atenolol) with an associated reduction in stroke in the *Losartan Intervention for Endpoint Reduction in Hypertension (LIFE)* study.\(^408\) The differing impact of alternative anti-hypertensive agents on central vascular stiffness has thus been proposed as a potential explanation for the differential effects of these agents on CV events.\(^404,409\)

Of particular relevance was the finding that the CV benefits of blood pressure lowering in ESRF were attenuated in patients where PWV was not improved by
anti-hypertensive therapies.\textsuperscript{371} Thus, though the conventional risk factor was moderated by standard therapies, the modifiability of the associated arterial alterations was a more useful predictor of future CV prognosis.\textsuperscript{370} Such findings highlight the potential prognostic impact of therapeutic interventions that improve central arterial structure and function, and provide the basis for the vascular research studies described herein.

**Arterio-Venous Fistulae and Cardiovascular Structure and Function**

**Arterio-venous Fistula Creation**

As CKD advances towards end-stage, patient co-morbidities and preference play a significant role in determining the optimal mode of RRT. Although live-donor renal transplantation has increased in prevalence and popularity in eligible patients in recent years, in Australia most advanced CKD patients also undergo surgical creation of a radial or brachial AVF (where surgically feasible). The surgical anastomosis of the radial (or brachial) artery to an adjacent vein (e.g. cephalic vein) creates a low-resistance iatrogenic channel, diverting arterial pressure and flow directly into the systemic venous system. Once matured, such fistulae provide a ‘fail-safe’ method for the performance of haemodialysis if/when the metabolic derangements of decompensated ESRF intervene. The haemodynamic consequences of AVF creation are not benign however. Immediately following creation, AVF are associated with a 10-20\% increase in systemic cardiac output, achieved predominantly through a reduction in systemic vascular resistance, increased myocardial contractility (through sympathetic
nervous system activation) and an increase in stroke volume and heart rate. Over the following week, circulating blood volume increases in conjunction with increases in atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). These alterations are associated with early increases in LV filling pressure, and associated increases in LA volume and LV end-diastolic volume (LVEDV). AVF blood-flow increases progressively over the initial 6-12 weeks following creation, diverting a substantial burden of overall cardiac output to service the AVF (commonly 0.5-2L/minute). To maintain sufficient systemic cardiac output, LV CO must increase proportionately, resulting in longer-term implications for cardiac and vascular structure and function. In fact, AVF creation has been demonstrated to be associated with worsening of LVH in ESRF patients, and echocardiographic features of diastolic dysfunction including left atrial dilatation. In some patients, giant AVF may lead to progressive high-output cardiac failure, despite preserved systolic LV function. Such cases may require AVF banding or ligation to limit flow and reduce systemic cardiac demand.

Many of the earliest studies evaluating the cardiac effects of AVF creation were performed prior to the institution of routine erythropoietin supplementation when significant anaemia may have contributed to the haemodynamic effects witnessed. Despite this, the increased cardiac demand associated with AVF creation is undisputed, and is believed to be a significant contributor to the excess burden of CV morbidity and mortality of ESRF. Specifically, AVF creation has been discouraged in ESRF patients with significantly depressed cardiac function (LVEF<30%), due to fears of reduced physiological reserve precipitating fulminant cardiac decompensation. Patients with pre-existent
coronary stenoses may not have the coronary flow reserve to manage this increase in cardiac work, with an associated deterioration in sub-endocardial perfusion and worsened anginal symptoms.\textsuperscript{421} Furthermore, patients with prior CABG utilising internal thoracic artery grafts may develop coronary steal phenomena from ipsilateral AVF creation/haemodialysis.\textsuperscript{422,423} Given the prognostic advantage of internal thoracic artery grafting in CABG\textsuperscript{424-426}, such findings may have deleterious implications for the management of CKD patients requiring coronary revascularisation.

Despite these issues, AVF may still be created in elderly ESRF patients, following appropriate selection to avoid cardiovascular complications.\textsuperscript{427} The alternative to AVF creation for the performance of haemodialysis, is the insertion of a large-bore a central venous catheter (e.g. Permacath). Such catheters have a substantial morbidity and mortality, related to vascular complications (bleeding, thrombosis, central venous stenosis) and infection.\textsuperscript{428} The use of AVF, despite the disadvantageous cardiovascular adaptations, is associated with lesser cardiovascular mortality than catheter-based haemodialysis.\textsuperscript{428}

**Pulmonary Hypertension in Patients with Arterio-venous Fistulae**

Pulmonary hypertension (PHT) refers to an elevation of mean pulmonary artery pressure (PAP) beyond normal levels (>25mmHg). PHT may be caused by cardiac, pulmonary, vascular or systemic conditions, but regardless of aetiology, the development of PHT portends increased morbidity and mortality, regardless of the underlying condition.\textsuperscript{429-431}
PHT is a common finding amongst haemodialysis patients with iatrogenic arterio-venous access.\textsuperscript{432-434} The causes for this elevation are multi-factorial, with factors such as local vascular tone, pulmonary vascular calcification, reduced left ventricular diastolic compliance, increased left atrial pressure and elevated pulmonary blood flow induced by AVF maturation considered contributory.\textsuperscript{433,435-437} Significantly, this failure of the pulmonary circulation to compensate for the increased right ventricular output is associated with reduced survival for affected ESRF patients.\textsuperscript{435} Importantly though, the acute and chronic affects of AVF creation and, furthermore, AVF-ligation on right atrial, right ventricular and pulmonary arterial decompensation have not been systematically studied.

\textbf{Arterio-Venous Fistula Ligation}

Following successful RTx, AVF may persist indefinitely, despite the absence of ongoing clinical necessity. The longer-term cardiac impact of AVF persistence following successful RTx is not known, however there is mounting evidence that AVF contribute significantly to persistence of LVH (and potentially, associated diastolic left ventricular dysfunction) in post-transplant patients. The published data in this field is contradictory however\textsuperscript{438-440}, with some evidence that only larger AVF are responsible for disadvantageous cardiac remodelling.\textsuperscript{441} A recent study further suggests that the AVF, rather than the necessity for haemodialysis, is the propagating factor in LVH, demonstrating a marked reduction in adverse cardiac indices following failure of AVF, but ongoing intermittent haemodialysis via a central venous catheter.\textsuperscript{442} In addition to the ongoing uncertainty regarding the benefits of routine AVF ligation with regards left ventricular geometry and
function, the effect of AVF ligation on right ventricular and atrial structure and function as well as central and peripheral vascular structure and function remain uncertain.

Ongoing uncertainty in this arena forms the foundation for the Studies evaluating alterations in cardiovascular structure and function with AVF creation and AVF ligation within this PhD.

**Effects of Renal Transplantation on Cardiovascular Structure and Function**

RTx is associated with dramatic alterations in QOL, morbidity and mortality for ESRF patients. In fact, successful cadaveric RTx has been shown to more than triple the life-expectancy of ESRF patients on dialysis, when compared to patients remaining on the waiting list. A substantial component of this benefit arises from a reduction in cardiovascular deaths. Despite this, CVD remains a leading cause of death amongst RTx recipients. Significantly, despite marked improvements in post-transplant care, longer-term survival amongst successful RTx recipients has not appreciably improved in Australia over the last 1-2 decades. Furthermore, RTx recipients experience an incidence of CV mortality that is many times that of the age-matched controls from the general population. Thus renal transplantation is associated with substantial CV benefits, but a significant burden of CVD persists in this vulnerable patient cohort.

LV dilatation and hypertrophy are highly prevalent amongst RTx recipients. Increased LVM has been shown to be present in 40-60% of new RTx recipients.
and only the minority of RTx patients have normal left ventricular structure and systolic function as measured by standard trans-thoracic echocardiography (TTE).\textsuperscript{454} Despite this, RTx has been shown to be associated with significant reductions in LVM and LV volumes following transplantation, though cardiac structure and function do not universally normalise.\textsuperscript{454-463} Thus, despite restoration of renal excretory function, RTx does not completely abrogate the trigger to disadvantageous cardiac remodelling. As previously mentioned, the role of AVF persistence in this context remains to be definitively elucidated.

Mounting evidence has demonstrated that the rigorous screening processes involved in selecting ESRF patients suitable for RTx is relatively successful in selecting patients at lower risk of ischaemic CV events. Amongst patients free of major cardiac events 1 year following transplantation, the subsequent incidence of a major ischaemic event has been reported to be 1.2-1.5\% per year\textsuperscript{453,464,465} – a rate similar to that experienced by the general community within the Framingham Heart Study.\textsuperscript{465,466} Despite this, the rate of new CCF was found to be 3-4 times that seen in the general population\textsuperscript{467-469}, with only \~30\% of CCF events in post-RTx patients appearing to be associated with an ischaemic precipitant.\textsuperscript{465} Compared to RTx patients who remain free of new ischaemic events or CCF, affected RTx patients have a 1.5- to 2-fold higher rate of death, independent of each other, patient age, gender or the presence of TIIDM.\textsuperscript{465} Unlike the more common association of IHD and CCF in the general community, CCF following RTx appears to be aetiologically distinct from IHD, but is associated with significant mortality risk.\textsuperscript{465} The role of pre-existing, sub-clinical LV pathology, and the potential contributions of immunosuppressive therapies,
hypertension, anaemia, graft dysfunction and persistent AVF remain to be determined.

Vascular structure and function appear also to be favourably influenced by RTx. Endothelial dysfunction is a common finding in ESRF, contributing substantially to the premature development of CVD in affected patients. Although generally improved, endothelial dysfunction commonly persists following RTx. Many of the immunosuppressive agents utilised following RTx contribute to this persistent endothelial dysfunction, and may contribute further to overall CVD risk through unfavourable effects on lipid profiles, blood pressure and glucose intolerance. Thus, endothelial dysfunction post-RTx remains a potential trigger to the development of clinically significant CVD, and hence remains a potential target for therapeutic interventions, whether by improved blood pressure and lipid control, smoking cessation or by other novel means.

Large artery structure and compliance is also adversely affected by ESRF and long-term dialysis, with improvement but persistence of increased arterial stiffness following transplantation. The calcineurin inhibitors have been implicated in the promotion of arterial stiffness following RTx, although the process is clearly multi-factorial. The role of ventriculo-arterial coupling in the promotion of deleterious LV and arterial remodelling post-RTx remains to be determined.

Such observations contribute to the foundation underlying the Study evaluating alterations in cardiovascular structure and function following elective AVF ligation within this PhD.
Evaluation of Cardiovascular Risk Prior to Renal Transplantation

CVD is the leading cause of death in ESRF and RTx cohorts, however the risk of CVD morbidity and mortality in ESRF patients is markedly reduced by RTx. RTx is an expensive and highly intensive intervention however, utilising a scarce community resource (i.e. organs). Furthermore, RTx is not without risk, as it is associated with an early increase in all-cause and cardiovascular mortality for recipients. In fact, the survival advantage of RTx appears not to be apparent until beyond 250 days post-transplantation, making survival to this point a necessary pre-condition to RTx benefit. Furthermore, according to a large Scandinavian study, between the second and third years post-RTx, 41.4% of failed transplants are lost due to chronic rejection, but 42% are lost due to patient death with a functioning graft. A substantial burden of this early mortality is due to CVD. To reduce peri-operative CV morbidity and mortality, or early graft loss due to CVD, potential RTx recipients undergo rigorous screening processes and these pre-RTx screening investigations need to consider not only peri-operative risk, but also must be designed to provide information regarding CV risk in the early years post-RTx to ensure recipients are most likely to enjoy the mortality advantages associated with the RTx process. Although there is widespread support for CV evaluation prior to RTx, there is no universally accepted screening process and hence each RTx centre may have different routines depending on local experience and expertise.

Given that CVD is a leading cause of morbidity and mortality in CKD and ESRF patients, and that conventional CV risk factors such as T1IDM and HT are highly prevalent in this population, exclusion of coronary artery disease (CAD) comprises a substantial component of CV screening in higher risk RTx candidates.
The presence or absence of exertional chest pain is a poor predictor of the presence of significant CAD in CKD and ESRF patients.\textsuperscript{493} It has been shown that angiographically severe CAD may be present in asymptomatic patients with CKD, potentially related to the reduced exertional capacity of uraemia and the potential for autonomic neuropathy related to both uraemia and TIIDM.\textsuperscript{494} Furthermore, up to 75\% of HDx patients with CAD may not experience typical angina\textsuperscript{495}, with silent ischaemia three times more prevalent than in the Framingham study population.\textsuperscript{496} Additionally, up to 50\% of ESRF patients with angina have been shown not to have significant CAD at coronary angiography.\textsuperscript{301,497,498} Reasons postulated for this phenomenon include the presence of anaemia, reduced coronary vasodilatory reserve, microvascular disease and supply-demand mismatch associated with the presence of LVH - a common echocardiographic finding in this context (as previously discussed).\textsuperscript{487} Thus, the presence or absence of chest pain provides little clinical certainty in distinguishing between the presence and absence of significant CAD in potential RTx recipients.

There is substantial debate regarding the necessity to perform ICA to exclude significant CAD prior to RTx. Numerous studies have been published demonstrating the advantages and limitations of non-invasive investigations in the ESRF context.\textsuperscript{494,499-513} In particular, in ESRF it appears that vasodilator stress is inferior to tachycardic stress in the non-invasive detection of significant CAD.\textsuperscript{513} This may result from the requirement for dipyridamole, adenosine and the recently FDA-approved regadenoson (commonly utilised pharmacological vasodilators in non-invasive ischaemia imaging) to achieve maximal myocardial hyperaemia through coronary microvasculature vasodilatation.\textsuperscript{514-516} ESRF is
associated with significant endothelial dysfunction, and theoretically, the coronary microvasculature of affected patients may not maximally vasodilate in response to endothelium-dependent stimuli. Hence, stressors that require detection of differential myocardial perfusion based on regional hyperaemia in non-obstructed coronary territories, as compared to relatively under-perfused territories unable to achieve maximal hyperaemia due to epicardial coronary obstruction, may be less accurate in ESRF.

**Tachycardia Stress Methodologies**

Exercise-based stress testing is widely accepted as the preferred, and most accurate, methodology for the non-invasive exclusion of coronary ischaemia. Uraemia, however, is commonly associated with reduced exertional capacity, frequently preventing ESRF patients from achieving the necessary level of tachycardia required to minimise the occurrence of a false negative result. Previous studies have demonstrated a higher risk of future CV events amongst patients unable to complete exercise stress tests, regardless of the presence of a negative result.\(^{517}\) Thus combinations of exercise and vasodilatory agents, or dobutamine infusion have been utilised to optimise non-invasive imaging results. Conventionally, exercise (+/- dipyridamole or adenosine) or dobutamine single photon emission computed tomography (SPECT) myocardial perfusion imaging (MPI) have been the most commonly utilised investigation in the non-invasive exclusion of ischaemia prior to RTx. Dobutamine stress echocardiography (DSE) is a commonly utilised alternative, with some results indicating a higher
specificity attributable to this investigation, but similar overall accuracy in non-renal failure patients.\textsuperscript{518-522}

Results of a meta-analysis of eleven smaller studies in ESRF patients indicate that a negative MPI or DSE study was associated with a 1.8% annualised risk of cardiac death, compared to a 6.3% risk following a positive study.\textsuperscript{511} A positive study of course, may be associated with more intensive, or invasive intervention to minimise the risk of MI, so the relatively low rate of MI following a positive MPI study is obviously not representative of an untreated group. Regardless, current non-invasive imaging techniques have an enviable evidence base supporting their prognostic value, and in particular the favourable prognosis associated with a negative study in the non-CKD population.\textsuperscript{523-530}

Despite such results in the general community, the presence of a negative study does not entirely exclude the presence of haemodynamically significant coronary artery disease, hence the preference within some RTx centres to rely solely upon ICA for the evaluation of coronary risk in this context.\textsuperscript{501} Importantly, however, it is now widely appreciated that ICA provides only a 2-dimensional “lumenogram”, and provides only limited insight into the potential haemodynamic and clinical significance of identified lesions in respect to therapeutic decision-making in the general community.\textsuperscript{531,532} Invasive assessment of fractional flow reserve (FFR) has become the new “gold standard” for the assessment of coronary lesion haemodynamic significance, particularly in determining potential benefit with regards coronary revascularisation, although ESRF patients have routinely been excluded from studies of this technology.\textsuperscript{531,533-536} Invasive studies, however are associated with procedural risk. The risk of nephrotoxicity related to iodinated contrast administration during ICA remains a concern, however femoral artery /
retroperitoneal bleeding and coronary or cerebrovascular complications, albeit uncommon, should be considered prior to performing these investigations as routine screening tests, regardless of the high prevalence of CAD in this cohort. Ideally, pre-RTx risk would be assessed non-invasively, but with a high degree of accuracy and lower rate of false positives without compromising sensitivity.

Dobutamine stress CMR (DS-CMR) has recently been demonstrated to provide a very high level of accuracy in the non-invasive detection of significant CAD, with low procedural risk, and avoidance of nephrotoxic contrast agents.\textsuperscript{537-540} Additionally, longer-term prognostic data has begun to emerge demonstrating very low rates of CV events following a negative DS-CMR study.\textsuperscript{541} Although access to CMR units with experience in dobutamine stress imaging is an issue preventing universal uptake, this highly accurate non-invasive imaging technique has the potential to improve specificity and hence reduce unnecessary invasive angiograms in pre-RTx candidates.

**Non-invasive Imaging of Cardiovascular Structure and Function in CKD**

The evaluation of cardiac structure and function is the cornerstone of non-invasive cardiac imaging. LV ejection fraction (LVEF), LV chamber dimensions and LVM are all closely associated with future CV morbidity and mortality in the general community and amongst CKD and ESRF patients. Additionally, measures of left and right atrial size and right ventricular size and function are known to provide incremental, clinically relevant information during cardiac imaging, with
recent evidence supporting an association between left atrial size and mortality amongst ESRF patients referred for RTx.196

Numerous methodologies exist to evaluate cardiac structure and function, though few are also capable of evaluating vascular structure and function simultaneously. The available methodologies are briefly discussed.

**Trans-thoracic Echocardiography**

TTE provides a widely available, relatively inexpensive, portable and universally safe method for evaluation of LV structure and function. Quantitative TTE imaging requires the acquisition of parasternal and trans-apical images of the heart via variable imaging windows in-between adjacent ribs. As a result, TTE is highly operator and acoustic window dependent.542-544 Pulmonary parenchymal over-expansion (such as in COPD), thoracic obesity (or profound cachexia where insufficient tissue remains to allow optimal probe contact), and myriad musculoskeletal and co-morbid medical conditions may impair image quality, and hence the reliability of quantitative TTE results.

Utilising 2-dimensional TTE, LVEF is conventionally evaluated by a method known as Simpson’s Bi-plane Method of Discs (MOD). Utilising the apical long-axis images in the 4-chamber and 2-chamber projections, 3-dimensional volumes are calculated based on geometric assumptions arising from the two long axis areas outlined in diastole and systole. Unfortunately, the LV apex is frequently foreshortened by echocardiography, and regional impairment of endocardial definition (particularly affecting the anterior wall) contribute to the relatively high-degree of intra-observer and inter-observer variability described when
measuring LVEF by TTE – often greater than 20%.\textsuperscript{545-548} TTE may also be used to estimate LVM, with robust prognostic data published in this area in a variety of patient subsets including CKD and post-RTx cohorts. TTE estimates of LVM have been shown to vary significantly, with accuracy declining as distance from the transducer increases.\textsuperscript{543} Thus, despite the numerous publications utilising TTE to evaluate LVM, concerns remain regarding the accuracy of this methodology.\textsuperscript{542,543,549,550}

Distinct from LV assessment, TTE allows for the reproducible assessment of left and right atrial chamber dimensions, with results comparable to 3-dimensional TTE and CMR measures.\textsuperscript{551-553} Quantitative right ventricular assessment is suboptimal by echocardiography however, owing to the non-uniform geometry of the right ventricle and limited number of available imaging planes by TTE. There is increasing recognition, however, of the importance of this chamber in the development of exertional limitation in health and disease.\textsuperscript{554,555} Real-time 3-dimensional TTE may provide superior accuracy in this field in the future.\textsuperscript{556} TTE does however provide valuable information regarding valvular structure and function, and LV diastolic function that is superior to that provided by other available methodologies. Furthermore, recent advances in real-time 3-dimensional TTE have enhanced the capabilities and reproducibility of this ubiquitous technology. Of course, TTE is limited to assessment of cardiac structure and function only, with alternative ultrasound techniques necessary for evaluation of peripheral artery structure and function. Acoustic window limitations also prevent evaluation of central arteries (e.g. aorta) in the determination of alterations in vascular structure and function in the CKD context.
Single Photon Emission Computed Tomography

ECG-gated myocardial perfusion tomography has been validated in the assessment of LV volumes and LVEF. As has previously been demonstrated with TTE, SPECT has reasonable reliability in the normal heart, but accuracy declines in the presence of LV impairment and prior myocardial infarction. SPECT also suffers from poor spatial and temporal resolution, and necessitates exposure to ionising radiation (10-20mSv doses have been reported), lessening its appeal as a routine screening investigation, particularly for serial studies.

Cardiovascular Computed Tomography

Substantial advances have been made in the field of CV CT in recent years. The availability of wider detector arrays (256-row and 320-row), able to image the whole heart in a single (or partial) gantry rotation has markedly improved cardiac image acquisition and speed. This further allows most studies to be completed within a single heartbeat, and with retrospective gating, to acquire both systolic and diastolic images for ventricular (and atrial) volume and LVEF assessment. The information provided by such imaging is comparable to that derived from CMR, and superior to standard TTE. CT however has significantly inferior temporal resolution to TTE and CMR, and spatial resolution during the systolic phases is frequently reduced to minimise radiation dose. CT is able, by widening the imaging field, to also image the aorta during cardiac assessment. Due to the capacity for unlimited image reconstructions following CT image acquisition, evaluation of aortic compliance is possible (with retrospective
gating) at any level within the field of imaging.\textsuperscript{571} Evaluation of peripheral arterial structure and function is more difficult, however.

Despite its numerous imaging advantages, the most significant limitation to imaging with CT is the escalating radiation exposure for repeated imaging (routinely <2 mSv per scan). Thoracic (and mammary) radiation exposure during cardiac CT largely precludes use of this investigation for routine cardiovascular imaging in research studies, or repeated serial studies, particularly in young patients/subjects (especially women), where the potential for triggering delayed malignancy is greatest.\textsuperscript{572-574}

\textbf{Cardiovascular Magnetic Resonance Imaging}

\textit{Cardiac Structure and Function}

CMR has been recognised as the modern non-invasive gold standard for the evaluation of cardiac structure and function. It has numerous advantages over TTE, SPECT and the emerging technology of ECG-gated multi-detector CT. Without exposure to contrast or ionising radiation, CMR is able to provide high quality epicardial and endocardial border definition for the left and right ventricles, with the capacity to evaluate structure and function with unrestricted imaging planes during image acquisition. Hence, CMR does not require any geometric assumptions in determining volumetric assessments of cardiac chambers.

LV and right ventricular function and mass can be measured with high degrees of accuracy and reproducibility by CMR.\textsuperscript{568,575-578} In fact, TTE has less than 50% of
the precision of CMR in evaluation of LVM – a strong independent predictor of CV events in ESRF and post-RTx patients.\textsuperscript{549,550}

In this regard, CMR has demonstrated the lowest standard deviation in differences between inter-study measurements for left ventricular volumetric indices and LVEF.\textsuperscript{579} Such improvements in inter-study standard deviations allows for a squared reduction in required sample sizes for research studies – i.e. halving of the standard deviation allows for a quartering of the required sample size. It has been determined that to undertake a study evaluating an intervention aiming to achieve a 10\% reduction in left ventricular mass (with 80\% power, and p=0.05), TTE would require a sample size of 505. For the demonstration of a statistically significant reduction in LVM of 10\%, CMR would require a sample size of only 14,\textsuperscript{544,549}

Right ventricular (RV) size and function is also readily assessable by CMR.\textsuperscript{578} CMR has become the non-invasive gold-standard investigation for this purpose also, providing markedly improved assessment of alterations in RV structure and function in healthy subjects and a variety of disease states, including congenital heart disease states, arrhythmogenic right ventricular cardiomyopathy (ARVC), atrial septal defects and primary PHT.\textsuperscript{555,556,578,580-583} RV mass measurements are also possible by CMR, with validation previously performed against \textit{ex vivo} calf hearts, with a high level of accuracy demonstrated.\textsuperscript{581} Although potentially more technically challenging due to the highly trabeculated nature of the right ventricle, such assessments may have clinical utility in disease states that contribute to the development of PHT.

Atrial size and function can be readily assessed by CMR during a conventional cardiac study. Both area and volumes may be calculated, with atrial function
assessable by atrial ejection fractions. In this regard, the atria may be interrogated in the horizontal long axis, short axis or vertical long axis to derive a true 3-dimensional volume in atrial diastole and systole, although horizontal long axis imaging is most commonly utilised.\textsuperscript{584} Furthermore, left and right ventricular diastolic function may also be estimated by mitral and tricuspid inflow characteristics during phase-contrast CMR sequences, deriving waveforms similar to that demonstrated by Doppler echocardiographic techniques.\textsuperscript{585-588} Even pulmonary vein flow characteristics may be assessable by CMR, in imitation of echocardiographic assessments.\textsuperscript{589}

Hence, CMR not only provides superior accuracy to other available imaging techniques, but allows a more comprehensive assessment of cardiovascular structure and function, and enables research into potentially beneficial therapeutic interventions to be undertaken with more readily achievable subject sample sizes.

\textit{Vascular Structure and Function}

Unlike the aforementioned technologies, CMR is also able to evaluate dynamic aortic, carotid and peripheral arterial structure and function in the same setting. Measures of aortic flow and compliance have been widely published utilising CMR techniques\textsuperscript{147,259,389-391,394,395}, with growing evidence supporting a link between declining aortic distensibility and increased morbidity and mortality in ESRF patients.\textsuperscript{333,371,395,406} Furthermore, there is evidence that aortic distensibility can be favourably modified by therapeutic interventions.\textsuperscript{590} Brachial artery FMD, as previously discussed, is a widely utilised and prognostically relevant investigation\textsuperscript{257,266,591}, most commonly performed using
ultrasound techniques. Impairment of endothelial function as measured by FMD has also been associated with increased central vascular stiffness, providing a link between functional and structural vascular changes in the initiation and perpetuation of disease. Brachial artery FMD has been demonstrated to improve following RTx, and thus provides a valuable, modifiable marker of vascular structure and function in response to therapeutic interventions in ESRF and post-RTx.

FMD is able to be reliably assessed utilising CMR. CMR allows for assessment of brachial artery area, with three-dimensional reconstructions allowing for a truly perpendicular imaging plane – a factor that is difficult to reliably achieve with ultrasound techniques. Furthermore, edge detection and image reproducibility are critical to detecting small changes in vessel diameter by ultrasound. For example, an 11.7% increase in brachial artery diameter from 3.65mm to 4.08mm with hyperaemia requires the reliable detection of a 0.43mm change in vessel diameter, unaffected by altered imaging plane or vessel deformation. CMR provides for the non-invasive, reproducible evaluation of cross-sectional vessel area, vessel compliance over the cardiac cycle and even quantification of brachial artery flow, if desired. Although the prognostic value of brachial artery flow and shear stress calculations remains to be determined, recent work suggests a stronger association between vessel flow and CV risk factors than traditional FMD measures. Such observations potentially open a new avenue of research in this area.

Thus, CMR is able to provide accurate, reproducible and comprehensive assessments of cardiac and vascular structure and function within a single investigation. Such utility provides the basis for the Projects outlined in this PhD.
Aims of this Thesis

The aim of the Studies outlined within this Thesis is to provide insights into alterations in CV structure and function in the context of routine therapeutic interventions and investigations in ESRF and following RTx.

Specifically, the broad aims of the Studies outlined include:

1. To evaluate alterations in CV structure and function following elective AVF-creation in pre-dialysis ESRF.
2. To evaluate alterations in CV structure and function following elective AVF-ligation following stable, successful RTx.
3. To evaluate the diagnostic accuracy of DS-CMR in the detection of haemodynamically significant CAD prior to RTx.
4. To evaluate the impact of ESRF on dynamic coronary endothelial function.
METHODS
Research Ethics Considerations

All patients involved in the research studies outlined herein provided written informed consent following approval of the study protocols by the Research Ethics Committee of the Royal Adelaide Hospital.

Study 1: Evaluation of Alterations in Cardiovascular Structure and Function Following Elective Arterio-Venous Fistula Creation in Pre-Dialysis End-Stage Renal Failure

Subjects

Inclusion Criteria

Patients aged over 18-years under the care of the Royal Adelaide Hospital Renal Medicine Unit for advanced, progressive CKD were eligible for the study. Patients were enrolled prior to undergoing clinically-driven, elective surgical AVF formation in preparation for anticipated haemodialysis commencement in the subsequent 6-12 months, as clinically indicated.

Exclusion Criteria

Patients with acute, or rapidly deteriorating renal dysfunction anticipated to require haemodialysis within the next 6 months.

CKD, ESRF or post-RTx patients with a history of previous AVF creation.

Solid organ or haematological malignancy in previous five years.

Current or planned pregnancy (within next 9 months)
Presence of contraindications to CMR:

Cardiac Pacemaker / Defibrillator or retained pacemaker leads

Cerebral aneurysm clips

Implanted neuro-stimulators or electronic devices including Cochlear Implant

History of penetrating eye injury, or presence of ocular metallic fragments

Presence of shrapnel

Claustrophobia

Presence of Specific Contra-indications to the use of Glyceryl-trinitrate:

Hypertrophic obstructive cardiomyopathy

Haemodynamically significant aortic or mitral stenosis

Recent cerebral haemorrhage / head trauma

Concomitant sildenafil, tadalafil or vardenafil therapy

Study Investigations

Following enrolment and informed consent, all patients were scheduled for elective surgical AVF-formation as per the clinical practice of the RAH Renal Medicine and Vascular Surgery Units.

Clinical history and study CMR evaluation of baseline CV structure and function were scheduled to occur two weeks prior to the elective surgical date (CMR protocol detailed below [Page 94]).

At six-months following elective AVF-formation, repeat clinical history and identical CMR study were performed to evaluate alterations in CV structure and function related to AVF-formation and maturation in the context of declining renal function. The CMR protocol is summarized below [Page 94].
Study 2: Evaluation of Alterations in Cardiovascular Structure and Function Following Elective Arterio-Venous Fistula Ligation Following Successful, Stable Renal Transplantation

Subjects

Inclusion Criteria

Patients aged over 18-years under the care of the Royal Adelaide Hospital Renal Medicine Unit following stable, successful RTx, with a persistently functioning AVF were considered for enrolment prior to clinically-indicated, surgical AVF ligation.

Exclusion Criteria

Patients with unstable or deteriorating post-RTx renal function anticipated to require reinstitution of haemodialysis within the subsequent 12-24 months.

Solid organ or haematological malignancy in previous five years.

Current or planned pregnancy (within next 9-months)

Presence of contraindications to CMR:

Cardiac Pacemaker / Defibrillator or retained pacemaker leads

Cerebral aneurysm clips

Implanted neuro-stimulators or electronic devices including Cochlear Implant

History of penetrating eye injury, or presence of ocular metallic fragments

Presence of shrapnel

Claustrophobia
Presence of Specific Contra-indications to the use of Glyceryl-trinitrate:

Hypertrophic obstructive cardiomyopathy

Haemodynamically significant aortic or mitral stenosis

Recent cerebral haemorrhage / head trauma

Concomitant sildenafil, tadalafil or vardenafil therapy

Study Investigations

Following enrolment and informed consent, all patients were scheduled for elective surgical AVF-ligation as per the clinical practice of the RAH Renal Medicine and Vascular Surgery Units.

Clinical history and study CMR evaluation of baseline CV structure and function were scheduled to occur two weeks prior to the elective surgical date (CMR protocol detailed below).

At six-months following elective AVF-ligation, repeat clinical history and identical CMR study were performed to evaluate alterations in CV structure and function related to AVF-ligation in the context of continued stable renal function following successful RTx.
Cardiovascular Magnetic Resonance Protocol – Studies 1 and 2

All CMR studies were performed utilising the Siemens Avanto 1.5 Tesla magnetic resonance imaging scanner (Siemens Medical Imaging, Erlangen, Germany) located within the Cardiovascular Investigation Unit of the Royal Adelaide Hospital.

Cardiac Protocol

All CMR studies were performed with the subjects in a supine position, with a phased-array surface coil positioned over the thorax. Initial long axis reference views were performed to enable optimal placement of the 7 to 12 parallel short-axis slices from the mitral annulus to beyond the left ventricular apex (Figure 12). Horizontal and vertical long axis views were also taken of the left atrium and left ventricle, as well as a long axis view of the left ventricular outflow tract. All images were acquired during expiratory breath-hold (approximately 8-12 seconds) with retrospectively ECG-gated True-FISP (Fast Imaging with Steady State Free Precession) sequences. Section thickness was 6mm with short-axis intersection intervals of 4mm. Acquisition time was determined to include 90% of the R-R interval, image matrix 256 x 150, Field of View (FOV) 380mm, Repetition Time (TR) 52ms, Echo Time (TE) 1.74ms, flip angle 70° with 8-15 cardiac cycles required per image.
Figure 12: Representation of CMR methodology for the use of the long axis reference images (horizontal long axis image shown on left) to enable acquisition of sequential, parallel ventricular short-axis images from the atrio-ventricular plane to the ventricular apices (end-diastolic images shown).

Offline analysis was performed to determine LA, RA, LV and RV dimensions in diastole and systole. Diastolic LVM was also concurrently determined. Image analysis was performed utilising proprietary software (Argus software, Siemens Medical Imaging, Erlangen, Germany). Ventricular short axis images were utilized for ventricular assessment, with epicardial and endocardial LV contours and endocardial RV contours manually traced at diastole (start of the ECG R-wave) and at end-systole (determined by the timing of the smallest ventricular cavity size). As per standard CMR protocol, myocardial trabeculations and unattached papillary muscles were ascribed to the left ventricular cavity. The basal LV slice was determined to be that slice where at least 50% of the cavity was surrounded
by ventricular myocardium, and the apical slice was defined as the most apical slice still containing intra-cavity blood-pool.\textsuperscript{596,597} A similar assessment of basal LV slice was then undertaken at end-systole, with the overall number of LV short-axis images generally 1-2 slices less at end-systole, due to the well recognized phenomenon of ventricular shortening associated with systolic contraction.\textsuperscript{544,598}

If the aortic valve appeared in the basal slice at diastole or systole, only the intra-cavity volume up to the level of the aortic valve was included as a component of the ventricular volume, as per accepted protocol.\textsuperscript{597}

For the right ventricle, intra-cavity volumes below the level of the pulmonary valve were included in the analysis. For the RV inflow tract, intra-cavity volumes were excluded if the surrounding myocardium was thin and not trabeculated, indicative of presence within the RA cavity.\textsuperscript{596}

By measurement of diastolic and end-systolic volumes for both left and right ventricles, and tracing of the epicardial LV contour at end-diastole, left and right ventricular end-diastolic volumes, end-systolic volumes, stroke volumes, ejections fractions, cardiac output and LVM were all able to be derived.

Left and right atrial area were assessed in the horizontal long-axis in atrial end-diastole (largest area) immediately prior to mitral valve opening.

Owing to the reduced accuracy in contour tracing by untrained, inexperienced observers\textsuperscript{599}, all study CMR images were evaluated by a single experienced operator blinded to the name and date of the Study.
Vascular Protocol

Aortic Distensibility

Immediately following completion of the cardiac protocol, ECG-gated True-FISP cine sequences were acquired of the ascending aorta, descending thoracic aorta and proximal abdominal aorta in a sagittal oblique orientation. Utilising the level of the right pulmonary artery as the reference level in each patient, a perpendicular cross-sectional True-FISP cine image was then taken at the ascending aorta and the proximal descending aorta. A final True-FISP cine image was then acquired at the level of the distal descending aorta, 5-10cm distal to the diaphragm (Figure 13), as previously described.394

Brachial artery blood pressure was taken concurrently using an MRI compatible automated non-invasive sphygmomanometer, with the results of three measurements averaged, and any outliers discarded and repeat blood pressure taken as required.
Figure 13: Sagittal oblique image of the aorta (left) with pictorial representation of the reference levels used in the acquisition of cross-sectional aortic images at the Ascending Aorta (AA), Proximal Descending Aorta (PDA) and Distal Descending Aorta (DDA) (right).

Aortic distensibility was then determined offline by manually tracing aortic cross-sectional images at the level of the ascending aorta, proximal descending aorta and distal descending aorta in end-diastole (minimum lumen area) and peak-systole (maximum lumen area). Aortic distensibility was derived by the following equation:

\[
\text{Aortic Distensibility} = \frac{\text{Area}_{\text{Systole}} - \text{Area}_{\text{Diastole}}}{\text{Pulse Pressure} \times \text{Area}_{\text{Diastole}}}
\]
where pulse pressure was derived from the non-invasive brachial artery blood pressure during image acquisition. Aortic area was measured in mm$^2$ and pulse pressure measured in mmHg, with the final unit of aortic distensibility represented as mmHg$^{-1}$, by standard convention. Measures of aortic distensibility were recorded at each location individually, and then the three measurements were averaged for an indicative average aortic distensibility for each subject. Our research group has previously published robust intra- and inter-individual reproducibility for this technique (1% and 2% respectively).  

*Brachial Artery Blood Flow – Arterio Venous Fistula Arm*

Patients were then removed from the scanner briefly, to allow removal of the thoracic phased-array surface coil and placement of a single four-channel Machnet phased-array surface coil (Machnet, Netherlands) overlying the brachial artery ipsilateral to the site of AVF surgery. Scout “time of flight” images were acquired of the brachial artery to allow positioning of the cross-sectional plane of brachial artery imaging, proximal to the brachial bifurcation. A single, velocity encoded sequence was acquired during free-breathing, with sequence acquisition individually optimized for peak brachial artery flow velocity. Analysis of brachial artery flow was performed utilizing proprietary software (Argus software, Siemens Medical Imaging, Erlangen, Germany), following manual tracing of the brachial artery in each of twenty-five phases over the cardiac cycle. Brachial artery flow was evaluated prior to AVF-surgery and at 6-months post-surgery.
**Brachial Artery Flow Mediated Dilatation**

Patients were again briefly removed from the scanner, to allow repositioning of the single four-channel Machnet phased-array surface coil (Machnet, Netherlands) to overlay the brachial artery contralateral to the site of AVF surgery. A manual sphygmomanometer cuff was then applied to the forearm ipsilateral to the imaging coil prior to imaging. Scout “time of flight” images of the brachial artery were then acquired to allow optimal positioning of the cross-sectional plane of brachial artery imaging, proximal to the brachial bifurcation.

At baseline, the brachial artery cross-sectional area was evaluated utilising True-FISP cine imaging at a single location at end-diastole. In a sub-group within each study, baseline brachial artery flow was also evaluated utilising velocity-encoded imaging. The forearm sphygmomanometer cuff was then inflated to 50mmHg above systolic blood pressure and pressure maintained for 5-minutes, as per validated protocol.\(^{254,258,594}\) One minute following release of the occlusive cuff, brachial artery endothelium-dependent vascular function was assessed utilizing repeat True-FISP cine imaging at the same location as the baseline image.

Ten minutes following release of the cuff pressure, repeat baseline cine imaging was performed (again at the same cross-sectional location within the brachial artery), prior to administration of 400mcg of sublingual GTN. Three minutes following GTN administration, True-FISP cine sequences were repeated, for evaluation of endothelium-independent vascular function.\(^{254}\)

Offline analysis was performed using proprietary software (Argus software, Siemens Medical Imaging, Erlangen, Germany), with manual tracing of the brachial artery lumen area in each of the cine and velocity encoded acquisitions. Endothelium-dependent responses were determined by percentage change in
vessel area following ischaemic stimulus, and endothelium-independent responses were determine by percentage change in vessel area following GTN administration.

Summary of CMR Investigations for Arterio-Venous Fistula Studies

The CMR protocol outlined was performed 1-2 weeks prior to planned elective AVF surgery, and the identical protocol was repeated 6-months following surgery for comparison with pre-surgical results.

A 6-month follow-up period was chosen for the AVF studies following evaluation of the available literature in this field. Studies utilising relatively short follow-up (<2-months) periods have demonstrated alterations in cardiac output, cardiac chamber size, and neurohormonal measures following AVF creation. Such studies were of insufficient follow-up duration to enable evaluation of adaptive changes in LVM however. Longer follow-up studies following either spontaneous or deliberate AVF closure have evaluated echocardiographic alterations in cardiac structure and function at 6-months, 10-months and 21-months with similar signals regarding the potential for advantageous CV remodeling following AVF-ligation. Thus, a 6-month follow-up period was chosen to ensure sufficient time to allow acute haemodynamic alterations to induce any relevant adaptive cardiac remodeling, but for the follow-up duration to not be so lengthy as to prevent completion of the Studies within the allotted PhD period, or prior to the commencement of dialysis in the majority of subjects. On the basis of the available literature, we believed 6-months to be an appropriate duration to allow firm conclusions to be determined regarding the
potential longer-term impact of AVF creation / ligation on CV structure and function, and to be of sufficient length to determine that where alterations were not seen, such parameters, perhaps, were either less malleable, or could not be influenced by the presence or absence of an AVF.
Study 3: Evaluation of the Diagnostic Accuracy of Dobutamine-Stress Cardiac MRI in the Detection of Angiographically-significant Coronary Artery Disease prior to Renal Transplantation

Subjects

Inclusion Criteria
Patients aged over 18-years under the care of the Royal Adelaide Hospital Renal Medicine Unit being considered for acceptance onto the South Australian RTx waiting list, for the treatment of advanced CKD / ESRF and who were clinically deemed to be at high-risk of significant CAD by the RTx assessment team.

Exclusion Criteria
Standard, clinically driven contraindications to RTx

Presence of contraindications to CMR:
Cardiac Pacemaker / Defibrillator or retained pacemaker leads
Cerebral aneurysm clips
Implanted neuro-stimulators or electronic devices including Cochlear Implant
History of penetrating eye injury, or presence of ocular metallic fragments
Presence of shrapnel
Claustrophobia

Presence of Specific Contra-indications to the use of Dobutamine:
Hypertrophic obstructive cardiomyopathy
Haemodynamically significant aortic or mitral stenosis
Recent cerebral haemorrhage / head trauma

*Presence of Specific Contra-indications to Invasive Coronary Angiography:*

Absence of suitable femoral arterial access to allow the performance of ICA

Allergy or other non-renal intolerance to iodinated contrast media

**Study Investigations**

*Dobutamine Stress Cardiac MRI*

Patients will be required to refrain from anti-anginal medications including beta-blockers, calcium channel antagonists and nitrate medications for a period of 48-hours prior to the scheduled procedure. On the day of the procedure, subjects were required to fast for 4-hours prior to the DS-CMR and blood sugar readings were taken in diabetic subjects. A single 18-gauge intra-venous cannula was inserted and body weight taken to determine the appropriate dobutamine dose.

All DS-CMR studies were performed with the subjects in a supine position, with a phased-array surface coil positioned over the thorax. Initial long axis reference views were performed to enable optimal placement of the three ventricular short axis and three ventricular long axis views (horizontal long axis, vertical long axis and longitudinal left ventricular outflow tract) evaluating all 17 American Heart Association myocardial segments.$^{600,601}$

Resting images were acquired during expiratory breath-hold (approximately 5-8 seconds) with retrospectively ECG-gated True-FISP sequences (25 phases per cardiac cycle; TR 2.7ms, TE 1.4ms, flip angle 60°). Typical in-plane spatial resolution was 1.8 x 1.8mm with section thickness of 6mm, similar to previously published work in this field.$^{539}$
Dobutamine infusion was undertaken as per standard protocol – progressive 3-minutely intravenous infusion of 10mcg/kg/min, 20mcg/kg/min, 30mcg/kg/min and 40mcg/kg/min ± atropine up to 1.2mg (in 600mcg increments) until achievement of ≥85% of maximal expected heart rate (220 – age [beats per minute]). Standard termination criteria for dobutamine cessation were applied. Regional wall motion was assessed at each dose increment, utilising the aforementioned views, with segmental analysis of the cine scans performed online throughout the procedure as a component of the procedural safety monitoring. Offline analysis was subsequently performed for the purposes of clinical and research reporting by consensus of two experienced observers blinded to the results of the subsequent coronary angiography. A standard segmental wall motion scoring system was utilised (1=normokinesis, 2=hypokinesis, 3=akinesia, 4=dyskinesis). For dobutamine studies, ischaemia was defined as ≥1 segment showing inducible regional wall motion abnormality as defined by deterioration in segmental wall motion score of ≥1 grade.539

**Invasive Coronary Angiography**

On a separate day following performance of the DS-CMR, patients were scheduled to undergo ICA. On the day of the procedure, subjects were required to fast for 6-hours prior to the procedure and blood sugar readings were assessed in diabetic subjects. A single 18-gauge intra-venous cannula was inserted and intravenous saline administered at a rate of 80mL/hour to a maximum of 500mL pre-procedure in non-dialysis-dependent patients with significant residual urine output.
Standard coronary angiography was performed via femoral arterial access. Standard Judkins catheters were used to engage the left main and right coronary arteries, with dedicated views taken of the left main, left anterior descending, left circumflex and right coronary arteries and their branches utilizing iodinated contrast (Iohexol-350).

Offline analysis of angiographic stenosis severity was performed by two experienced observers, with clinical significance determined for coronary stenoses of ≥70% in luminal diameter, by visual estimation.

Comparison of correlation of DS-CMR and invasive coronary angiographic findings was determined following independent reporting of each investigation by observers blinded to the results of the alternative investigation. Correlation of significant inducible wall motion abnormalities during DS-CMR with territories supplied by epicardial coronary arteries was based on individual coronary dominance.
Study 4: Evaluation of Dynamic Coronary Endothelial Function in End-Stage Renal Failure

Subjects

Inclusion Criteria

Subjects – Chronic Kidney Disease Study Cohort

Patients aged 18-75 years under the care of the Royal Adelaide Hospital Renal Medicine Unit being considered for RTx for the treatment of advanced CKD / ESRF and who are clinically deemed to require ICA prior to listing as a candidate by the South Australian RTx assessment team.

Subjects – Non-Chronic Kidney Disease Study Cohort

Patients aged 18-75 years undergoing clinically indicated ICA at the Royal Adelaide Hospital Cardiovascular Investigation Unit, and who are found to have only minor epicardial coronary artery stenoses and CrCL≥60mL/min.

Exclusion Criteria

Presence of significant co-morbidity that, in the opinion of the treating Cardiologist, may be expected to cause death or significant disability in the following 12-months.

Presence of Specific Contra-indications to Invasive Coronary Angiography:

Absence of suitable femoral arterial access to allow the performance of ICA

Allergy or other non-renal intolerance to iodinated contrast media
Presence of Specific Contra-indications to Coronary Endothelial Function Assessment:

Coronary anatomy unsuitable for Doppler Flo-wire instrumentation – e.g. excessive coronary tortuosity or heavy calcification

Presence of epicardial coronary artery stenoses in the target vessel of >20% luminal diameter

Haemodynamic instability as to prevent cardiac catheterisation.

Presence of Clinical Characteristics Unfavourable for Accurate Assessment of Coronary Endothelial Function:

Presence of cardiomyopathy

Presence of diffuse atherosclerosis within the Study vessel as determined by invasive angiography

Prior CABG

Study Investigations

Coronary Endothelial Function

At the time of Coronary Angiography, a 0.014-inch Coronary Doppler Flo-wire (Volcano Therapeutics, CA, USA) was advanced into the study vessel (the LAD was chosen preferentially) via an intra-coronary infusion catheter, following the administration of therapeutic heparin. The Flo-wire was positioned in the proximal or mid-segment of the Study vessel so as to obtain stable Doppler flow velocity signals. Resting coronary flow velocities were recorded and epicardial coronary diameter was determined 5mm distal to the tip of the coronary guidewire by QCA.
Protocol consisted of the following interventions:

- 5% dextrose control infusion for 2-minutes (Control 1)
- Acetylcholine (3-minute infusions of Ach $10^{-7}$M [1.6mcg/min], then $10^{-6}$M [16mcg/min])
- 5% dextrose control infusion for 5-minutes (Control 2)
- bolus dose of GTN (50mcg)
- bolus dose of Adenosine (48mcg)

as per validated protocol. At the completion of each intra-coronary infusion, a coronary angiogram was performed using 9mL of non-ionic contrast (Omnipaque, GE Healthcare, Waukesha, USA) injected through a Medrad infusion pump at 5mL/s, with images saved for offline analysis. Doppler indices, haemodynamic data (systemic blood pressure and heart rate) and ECG rhythm strip were recorded continuously throughout the study for off-line analysis.

Coronary endothelial function data were presented as response to Ach $10^{-6}$M for CBF and vessel diameter. Values for Ach $10^{-7}$M used if severe epicardial constriction response to $10^{-6}$M precluded data collection. Values for ESRF and non-CKD cohorts were then compared for evaluation of coronary endothelial dysfunction in ESRF vs. non-CKD control subjects.

**Quantitative Coronary Angiography**

Following acquisition of standardised coronary angiography during the Study protocol, offline analysis was performed using proprietary edge-detection software (QCA-CMS™, Medis Medical Imaging Systems, Leiden, Netherlands). Mean coronary diameter was assessed 5mm distal to the tip of the Doppler
guidewire over a 5mm segment. Changes in vessel diameter in response to Ach and GTN were analysed as measures of epicardial endothelium-dependent and – independent function. As previously described, subjects experiencing a >5% reduction in vessel diameter in response to Ach $10^{-6}$M were designated as having endothelium dysfunction.\textsuperscript{602}

**Coronary Blood Flow / Microvascular Function**

Coronary velocity measurements were analysed by a technician blinded to the results of the QCA analysis. Coronary blood flow (CBF) was then calculated from the formula:\textsuperscript{275}

$$[\pi \times 0.125 \times (\text{Coronary Diameter})^2 \times (\text{Coronary Blood Flow APV})] \times 60$$

where Diameter is measured in cm, Average Peak Velocity (APV) of coronary blood flow is measured in cm/sec and CBF is measured in mL/min. Coronary flow velocity reserve (CFR) is determined as a ratio of:

$$\text{APV (post-adenosine) / APV (baseline)}$$

A normal response is considered to have occurred if endothelial-dependent CBF increases by more than 50% in response to Ach, and the coronary flow reserve (CFR) – the ratio of maximal to baseline CBF - is greater than 2.5 following adenosine.\textsuperscript{276}
**Statistical Methods**

All measures were tested for normality using the Kolmogorov-Smirnov / Lilliefors Test and Shapiro-Wilk W test. All normally distributed values are represented as mean±standard deviation, with all column graphs represented as mean with standard error bars. Parameters that were deemed not normally distributed were reported as median and inter-quartile range (IQR). Paired parameters were analysed by two-tailed paired Student T-test. Where normally distributed, comparison of non-paired measures was performed by unpaired t-test (with Welch’s correction for samples of unequal variance), or one-way ANOVA as appropriate. Where parameters were not normally distributed, paired analyses were performed utilising the Wilcoxon matched pairs test, and non-paired analyses were performed using the Mann-Whitney U test. Correlation between parameters was performed utilising Pearson’s correlation for normally distributed samples, and Spearman’s correlation for non-parametric data.

In Study 1 and Study 2, comparison of binary test outcomes pre- and post-surgery was performed using Fisher’s exact test.

In Study 3, sensitivity, specificity, accuracy, predictive values (positive and negative) and likelihood ratios were calculated according to standard definitions and compared between groups ($\chi^2$ or Fisher’s exact test for small samples). Diagnostic performance of DS-CMR vs. stress-SPECT was performed utilising an identity binomial generalized estimating equation as per University Statistician advice. Accuracy of SPECT assessment of LVEF compared to non-invasive gold-
standard CMR was performed utilising paired Student T-test and Bland-Altman comparison.

In Study 4, comparison of haemodynamic and invasive coronary indices was undertaken with an unpaired T-test (with Welch correction for samples with different variances) where values were normally distributed and Mann-Whitney U test where values were non-parametrically distributed. Comparison of within-group haemodynamic measures was performed using one-way repeated-measures ANOVA with Tukey post-hoc multiple comparison test.

Statistical significance was determined by 2-tailed tests, with statistical significance determined at p=0.05.

Analyses were performed with Prism 5 for Mac OS X (GraphPad Software, Inc, CA, USA). The identity binomial generalized estimating equation was performed utilizing validated University software.
RESULTS
Study 1: Evaluation of Alterations in Cardiovascular Structure and Function following Elective Arterio-Venous Fistula Creation in Pre-dialysis End-Stage Renal Failure

Introduction

End-stage renal failure (ESRF) necessitates provision of alternative excretory capacity and pharmacological replacement of endogenous renal endocrine function for the maintenance of life. Although peritoneal dialysis provides many ESRF patients with a more flexible alternative, haemodialysis (HDx) remains at the core of renal replacement therapies (RRT) for ESRF patients worldwide. It is well appreciated that HDx performed via an implanted central venous catheter (e.g. Permacath, Vascath, etc) is associated with a marked increase in morbidity and mortality, predominantly related to vascular complications (bleeding, thrombosis, central venous stenosis) and infection.\(^{428}\) Thus, HDx necessitates the creation of an iatrogenic arterio-venous fistula (AVF) – most commonly involving the radial or brachial arteries and an adjacent large vein – with the subsequent low-resistance channel diverting arterial pressure and flow directly into the systemic venous system. Once matured, such fistulae provide a ‘fail-safe’ method for the performance of haemodialysis if/when the metabolic derangements of decompensated ESRF intervene. The haemodynamic consequences of AVF creation are not benign however.

Immediately following creation, AVF are associated with a 10-20% increase in systemic cardiac output, achieved predominantly through a reduction in systemic vascular resistance, increased myocardial contractility (through sympathetic
nervous system activation) and an increase in stroke volume and heart rate. Over the following week, circulating blood volume increases in conjunction with increases in ANP and BNP. These alterations are associated with early increases in LV filling pressure, and associated increases in LA volume and LVEDV. AVF blood-flow increases progressively over the initial 6-12 weeks following creation, diverting a substantial burden of overall cardiac output to service the AVF (commonly 0.5-2L/minute).

To maintain sufficient systemic cardiac output, LV CO must increase proportionately, resulting in longer-term implications for cardiac and vascular structure and function. In fact, AVF creation has been demonstrated to be associated with worsening of LVH in ESRF patients, and echocardiographic features of diastolic dysfunction including LA dilatation. In some patients, giant AVF may lead to progressive high-output cardiac failure, despite preserved systolic LV function. Such cases may require AVF banding or ligation to limit flow and reduce systemic cardiac demand.

Many of the earliest studies evaluating the cardiac effects of AVF creation were performed prior to the institution of routine erythropoietin supplementation when significant anaemia may have contributed to the haemodynamic effects witnessed. Despite this, the increased cardiac demand associated with AVF creation is undisputed, and is believed to be a significant contributor to the excess burden of CV morbidity and mortality of ESRF. Specifically, AVF creation has been discouraged in ESRF patients with significantly depressed cardiac function (LVEF<30%), due to fears of reduced physiological reserve precipitating fulminant cardiac decompensation. Patients with pre-existent coronary stenoses may suffer an unmanageable increase in cardiac work, with an
associated deterioration in sub-endocardial perfusion and worsened anginal symptoms.\textsuperscript{421} Furthermore, patients with prior CABG utilising internal thoracic artery grafts may develop coronary steal phenomenon from ipsilateral AVF creation/haemodialysis.\textsuperscript{422,423} Given the prognostic advantage of internal thoracic artery grafting in CABG\textsuperscript{424-426}, such findings may have deleterious implications for the management of CKD patients requiring coronary revascularisation. Despite these issues, AVF may still be created in elderly ESRF patients, following appropriate selection to avoid cardiovascular complications.\textsuperscript{427} Furthermore, despite the numerous disadvantageous cardiovascular adaptations, HDx via an AVF is associated with lesser cardiovascular mortality than catheter-based haemodialysis.\textsuperscript{428}

We sought to evaluate the effect of AVF creation on cardiovascular structure and function in the modern era of ESRF management utilizing recent advances in non-invasive CMR imaging.
Methods

Study Methods have been detailed previously (p90).

In brief, subjects with stable, progressive CKD were enrolled prior to clinically-driven, elective surgical AVF formation in preparation of anticipated haemodialysis commencement in the subsequent 6-12-months, as clinically indicated. Standard exclusion criteria were applied.

Subjects were then evaluated utilizing combined Cardiac and Vascular magnetic resonance protocols (protocol detailed previously, p94) at baseline, ideally 2-weeks prior to the planned surgical date. An identical follow-up CMR protocol was then performed at 6-months post-AVF creation to evaluate alterations in CV parameters in response to AVF creation.

Statistical Methods

As previously described (Statistical Methods, p111).

Power Calculation

The proposed study is multi-factorial in nature and has not previously been performed in the manner described. For the purposes of the power calculation for this Pilot Study, in line with previously published data in echocardiographic studies and our experience with CMR in other subject subsets, we set increase in left ventricular end-diastolic volume as our primary end-point. We estimated that we would need to complete baseline and follow-up studies in 18 subjects to provide 80% power to detect a 20% difference in the primary end-point assuming a standard deviation of 25% in the measured parameter, correlation
between baseline and follow-up measures of 0.5 (equivalent to an effect size [dz] of 0.63) and alpha error of 0.05 (G*Power v3.0.10).
Results

Twenty-seven subjects (median age 60-years, 59.3% male) were enrolled in the Study with initial CMR imaging undertaken approximately 2-weeks prior to planned AVF surgery. Due to the elective nature of the surgery, surgical dates were often cancelled by the Hospital in favour of more urgent cases, such that the interval from scan to surgery often exceeded the 2-week time-frame (time from scan to surgery 17±22 days). One subject was unable to undergo AVF creation at the scheduled surgical date due to surgically unattractive anatomy not apparent at pre-surgical assessment. One subject died following development of acute CVD progression between the CMR scan and scheduled surgical date, with deferral of AVF surgery and subsequent patient death. No clinical predictors of impending CV decompensation were apparent at the initial Study CMR scan. These two subjects were excluded from the Study analyses.

A complete CV protocol was possible in twenty-five (25) subjects, with two subjects unable to undergo FMD protocol due to SBP>200mmHg at time of FMD acquisition. A third subject was unable to complete the endothelium-independent component of the FMD protocol due to back pain related to lying supine in the CMR scanner.

Of the twenty-five (25) subjects who underwent successful AVF surgery (age 60±13 years, 60% male), one subject developed an occluded AVF within one month of surgery, and following commencement of peritoneal dialysis, elected not to have the AVF repaired. This subject had no follow-up CMR data and hence was also excluded from the subsequent analysis. Twenty-four patients were thus included in the follow-up study to evaluate alterations in cardiac and vascular
structure over time following successful AVF creation (age 59±12 years, 58% male). Baseline characteristics are detailed in Table 3. All baseline and follow-up scan parameters were normally distributed. The average time from surgery to follow-up scan was 198±32 days. At follow-up, all subjects completed the cardiac protocol, aortic distensibility and brachial artery blood flow protocols. One subject was unable to undertake the FMD protocol due to back pain. A further two subjects only undertook the endothelium-dependent component of the FMD protocol, unable to tolerate the endothelium-independent component of the FMD protocol due to back pain.

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>Age (years)</td>
<td>59±12</td>
</tr>
<tr>
<td>Gender (male), % (n)</td>
<td>58 (14)</td>
</tr>
<tr>
<td>Hypertension, % (n)</td>
<td>88 (21)</td>
</tr>
<tr>
<td>Hyperlipidaemia, % (n)</td>
<td>54 (13)</td>
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<tr>
<td>Type II Diabetes Mellitus, % (n)</td>
<td>38 (9)</td>
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<tr>
<td>Smoking, % (n)</td>
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<tr>
<td>- current</td>
<td>12 (3)</td>
</tr>
<tr>
<td>- former</td>
<td>17 (4)</td>
</tr>
<tr>
<td>- non-smoker</td>
<td>71 (17)</td>
</tr>
<tr>
<td>Prior Coronary Artery Disease, % (n)</td>
<td>21 (5)</td>
</tr>
<tr>
<td>Cerebrovascular Disease, % (n)</td>
<td>8 (2)</td>
</tr>
<tr>
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<td>21 (5)</td>
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<tr>
<td>Baseline Creatinine (μmol/L)</td>
<td>552±130</td>
</tr>
</tbody>
</table>

*Table 3: Subject Baseline Characteristics – Study 1*
Clinical Results

Clinical findings were predominantly unchanged following AVF-creation (weight: 80±19kg vs. 78±17kg, p=0.07; SBP: 146±19mmHg vs. 146±17mmHg, p=0.96; Hb: 115±12g/dL vs. 119±15g/dL, p=0.26). All subjects were undergoing treatment with EPO-analogues at baseline and at follow-up. Notably, resting heart rate was significantly increased at follow-up compared to baseline (HR: 71±12bpm vs. 76±11bpm, p=0.008). Furthermore, across the entire cohort, a small, non-significant increase in serum creatinine was noted (serum creatinine: 552±127μmol/L vs. 620±163μmol/L, p=0.11). When the three subjects who had commenced dialysis prior to follow-up imaging were excluded from the creatinine analysis, the serum creatinine for the remaining subjects was noted to have increased significantly 6-months following study enrolment, as would be expected clinically in this condition (529±91μmol/L vs. 652±123μmol/L, p=0.0006).

Cardiac Results

In the presence of advanced CKD / ESRF, baseline imaging revealed preserved left ventricular systolic function (LVEF 72±7%; Normal range 58-76%) with normal left ventricular size (LVEDV 149±31mL; Reference range: Males 115-198mL; Females 88-168mL) and CO (7.5±1.5L/min; Reference range: Males 2.82-8.82L/min, Females 2.65-5.98L/min). Despite the high prevalence of HT, LVM was within normal limits at baseline (132±33g; Reference ranges: Males 108-184g, Females 72-144g). LA area was mildly increased across the cohort,
with RA area at the upper limits of the normal range (LA Area 27±6cm², RA Area 23±6cm²; Normal range <24cm²).551

At follow-up, significant alterations in cardiac structure and function were identified. LVEF and RVEF were unchanged at follow-up however, confirming the limited utility of this index for the evaluation of alterations in cardiac structure and function despite its universal use in echocardiographic surveillance (LVEF 72±7% vs. 71±9%, p=0.60; RVEF 66±5% vs. 67±6%, p=0.45). Significant increases were seen for the majority of measured or derived parameters for atrial and ventricular structure and function however. Mean LVEDV increased by 17.2% (149±31mL vs. 175±43mL, p<0.0001), mean LVESV by 20.7% (43±16mL vs. 52±24mL, p=0.014), mean LVSV by 15.9% (107±24mL vs. 124±29mL, p<0.0001) and LV CO by 25.0% (7.5±1.5L/min vs. 9.4±2.2L/min, p<0.0001). Significantly, mean LVM was increased by 12.7% (132±32g vs. 149±35g, p<0.0001) 6-months following AVF-creation.

Similar results were seen for the increases in mean RVEDV (17.9% increase; 142±32mL vs. 167±42mL p<0.0001), RVESV (15.1% increase; 49±14mL vs. 56±18mL, p=0.0014) and RVSV (19.6% increase; 93±24mL vs. 112±27mL, p<0.0001). Mean LA area was increased by 11.3% (27±6cm² vs. 30±6cm², p=0.0003) and mean RA area was increased by 8.9% (23±6cm² vs. 25±6cm², p=0.0046).

Notably, 62.5% of subjects had CMR measures for LVEF, LVEDV, LVESV, LVSV and LVMI within normal limits prior to AVF creation, however this had reduced to 41.7% of subjects 6-months following AVF creation (p=0.24). The results of changes in cardiac structure and function are summarised graphically in figures 14-16.
Figure 14: Alterations in Left Ventricular Structure and Function following successful AVF creation in ESRF.
Figure 15: Alterations in Right Ventricular Structure and Function following successful AVF creation in ESRF.
Figure 16: Alterations in Left and Right Atrial Area following successful AVF creation in ESRF.

Vascular Results:

Aortic Distensibility

Aortic distensibility was not significantly altered by the creation of a surgical AVF at the 6-month follow-up scan (2.3±1.2mmHg⁻¹ vs. 2.2±1.2mmHg⁻¹, p=0.59). Ascending aorta (AA), proximal descending aorta (PDA), distal descending aorta (DDA) and average aortic distensibility ([AA+PDA+DDA]/3) were all numerically, but not statistically significantly, lower following AVF creation (Figure 17).
Prior to AVF creation, baseline aortic distensibility decreased progressively from DDA to AA, consistent with an increase in aortic stiffness (reduced aortic compliance) in the more proximal aortic segments (AA 1.41±1.0mmHg⁻¹ vs. PDA 2.20±1.3mmHg⁻¹, p=0.023; AA 1.41±1.0mmHg⁻¹ vs. DDA 3.20±2.0mmHg⁻¹, p=0.0003; PDA 2.20±1.3mmHg⁻¹ vs. DDA 3.20±2.0mmHg⁻¹, p=0.046). These finding remained similar following AVF creation, although the numerical difference between aortic distensibility at PDA and DDA did not quite reach
statistical significance (AA 1.27±1.2mmHg\(^{-1}\) vs. PDA 2.11±1.3mmHg\(^{-1}\), p=0.024; AA 1.27±1.2mmHg\(^{-1}\) vs. DDA 3.10±2.0mmHg\(^{-1}\), p=0.0004; PDA 2.11±1.3mmHg\(^{-1}\) vs. DDA 3.10±2.0mmHg\(^{-1}\), p=0.052) (Figure 18).

![Figure 18: Aortic Distensibility by Aortic Level Prior to (Pre-), and Following (Post-) AVF creation in ESRF.](image)

**Brachial Artery Flow-Mediated Dilatation**

Twenty-one subjects completed the FMD protocol at both pre-AVF and follow-up scanning. In these subjects, brachial artery endothelium-dependent vasodilatation, as measured by flow-mediated dilatation was markedly reduced at 6-month follow-up imaging following AVF-creation (9.0±9.2% vs. 3.0±5.7%, p=0.012; Figure 19). Endothelium-independent vasodilatation was unaffected by AVF-creation in the subjects evaluated (13.3±9.1% vs. 12.2±10.0%, p=0.74). Notably, in a sub-population of 17-subjects where this sequence was performed, there was no significant change in baseline brachial artery blood flow in the arm.
contralateral to the AVF – a factor with the potential to contribute to any measured alterations in endothelial function (0.054±0.030L/minute vs. 0.055±0.03L/minute, p=0.97) (Figure 20). Baseline brachial artery area was unchanged across the study cohort between baseline and follow-up scans (26.5±8mm² vs. 27.7±7mm², p=0.31). There was no significant correlation between % change in FMD and % change in AVF flow (r=0.17, p=0.52), % change in LV CO (r=-0.29, p=0.24) or % change in LVSV (r=-0.27, p=0.28).

Figure 19: Alterations in Peripheral Endothelial Function following successful AVF creation in ESRF.

Brachial Artery Blood Flow – Arterio-Venous Fistula Arm

Brachial artery blood flow ipsilateral to the AVF increased substantially following AVF creation (0.057±0.04L/minute vs. 1.158±0.44L/minute, p<0.0001)(Figure 20). This represents a more than 20-fold increase in mean ipsilateral brachial artery blood-flow 6-months following AVF-creation. Notably, there was no
significant association between increases in AVF-arm brachial artery blood flow and adaptive change in LV CO (r=0.09, p=0.71) or LVSV (r=0.13, p=0.59), potentially implicating a more complex systemic circulatory adaptation to AVF creation.

Figure 20: Alteration in CMR-assessed brachial artery blood-flow, ipsilateral and contralateral to the newly created arterio-venous fistula.
Discussion

Elective AVF creation is associated with substantial alterations in cardiac structure and function, necessary to accommodate the increase in cardiac output sufficient to service the fistula whilst maintaining homeostatic systemic supply. These changes include bi-atrial and bi-ventricular dilatation, increased ventricular stroke volume and cardiac output, as well as a clinically significant increase in LVM – a measure closely associated with adverse outcomes in ESRF. This adaptive cardiac remodelling, coupled with a >2000% increase in brachial artery blood flow ipsilateral to the newly created AVF, was also associated with a marked reduction in remote endothelial function and a consistent numerical, but not statistically significant, increase in aortic stiffness. Although necessary for the provision of HDx, it is striking that a planned medical intervention could cause such widespread maladaptation within the CV system of already high-risk individuals. That said, it is acknowledged that HDx performed via such conduits results in a substantially lower burden of overall morbidity and mortality than HDx via implanted catheters which may not contribute so significantly to CV maladaptation.428

Previous TTE studies have demonstrated the potential for increased LVM and LV dilatation following AVF creation.411,414 Since the first publication of efficacy for a synthetic EPO in the treatment of ESRF anaemia in the New England Journal of Medicine in January 1987604, substantial inroads have been made in the management of anaemia, hypertension and salt/fluid imbalance in ESRF. Downward revision of blood pressure targets, increased therapeutic options and an increased recognition of the horrifying CV prognosis of patients with ESRF.
have all contributed to improvements in care for affected patients. Despite such advances, however, our Study demonstrates comprehensively the enduring CV impact of AVF creation in these high-risk individuals.

Parfrey et al have previously demonstrated the negative prognostic implications of concentric LVH, LV dilatation and LV dysfunction as measured by TTE in ESRF patients commencing dialysis.\textsuperscript{145} Furthermore, London et al have previously demonstrated an extraordinary prognostic advantage to therapeutic interventions in ESRF that successfully reduce LVM by at least 10\%.\textsuperscript{160} Such measures were associated with a relative risk of 0.78 (0.63-0.92, p=0.0012) for all-cause mortality, and 0.72 (0.51-0.90, p=0.0016) for CV-event-free survival across the cohort. This relative risk reduction was particularly notable amongst subjects with no prior history of CVD (RR 0.69 [0.52-0.83, p=0.0182] for all cause mortality, and 0.52 [0.23-0.75, p=0.0204] for CV event-free survival).\textsuperscript{160}

In the present Study, we demonstrated a 17.2\% increase in mean LVEDV and a 12.7\% increase in mean LVM 6-months following AVF-creation (p<0.0001 for both). Although beyond the scope of this observational study, such cardiac adaptations may thus be expected to be associated with a deleterious impact on CV morbidity and mortality. Furthermore, recognition of the impact of a 25.0\% increase in mean CO 6-months following AVF creation (p<0.0001) may be an important factor in the clinical management of pre-dialysis patients – particularly as such measures are rarely reported using conventional cardiac imaging modalities, where LVEF (unchanged in this Study despite the myriad other structural and functional cardiac alterations) routinely forms the basis of a clinician’s determination of significant functional cardiac impact.
RV structure and function have historically been neglected in the evaluation of cardiac structure and function. TTE provides only a limited evaluation of RV size and systolic function, although tissue Doppler indices of RV function have recently been evaluated – particularly in the context of known pulmonary hypertension. Substantial recent research has focussed on the importance of RV structure and function in the maintenance of exercise capacity – both in healthy athletes and diverse disease states. The role of RV remodelling in the reduced exercise capacity common in ESRF remains to be determined, but it is certain that CMR will be integral to future research in this area. Unlike previous studies, CMR enabled more comprehensive RV evaluation in the present Study. Accordingly, we demonstrated substantial alterations in RV structure and function 6-months following AVF creation, with >15% increases seen in mean RVEDV, RVESV and RSVV (p≤0.0003 for all). Although intuitive, such findings are important to appreciate given the known association of AVF creation and the development of clinically significant PHT in ESRF patients. The role of sub-clinical RV remodelling and dysfunction in the exercise intolerance of ESRF remains to be determined but the results from this Study provide an important link in this line of enquiry.

LA size is more commonly increased in ESRF, in comparison to the general community. Such atrial dilatation is known to be associated with the development of patchy interstitial fibrosis within the LA myocardium, delaying local atrial conduction velocities and providing rich substrate for the perpetuation of multiple re-entrant circuits in the perpetuation of AF. In fact, LA dilatation has previously been identified as an independent risk factor for the development of AF in ESRF and may contribute to the poor CV prognosis of
patients with CKD. Furthermore, whether through the predisposition to AF or as a marker of chronic LV diastolic impairment, recent evidence confirms an association between LA dilatation and increased mortality in ESRF patients with LVH.

In the present Study, at 6-month follow-up imaging, AVF-creation was associated with an 11.3% increase in mean LA area (p=0.0003) and an 8.9% increase in RA area (p=0.0046). As previously mentioned, such alterations occurred in the context of increases in LVEDV/RVEDV, LVSV/RVSV, and LVM. Thus, increased LA and RA area may be expected to occur following AVF creation as a result of increases in both ventricular volumes and filling pressures in the context of increased myocardial mass and stretch. As such, the induced increase in atrial stretch (LA in particular) provides a more conducive substrate for the development of intra-atrial re-entrant arrhythmia, and AF more specifically.

Atrial fibrillation (AF) is the most common sustained arrhythmia in the general population and recent studies have confirmed AF as a significant negative prognostic factor in ESRF. AF is more highly prevalent in ESRF than the general community, and contributes disproportionately to morbidity and mortality in affected patients. Although haemodialysis in particular is a known risk factor for the development of AF, it remains unclear whether declining renal function contributes independently to this risk, or whether the high prevalence of AF risk factors (e.g. advancing age, hypertension, CCF, LVH, coronary artery disease, valvular heart disease) amongst CKD patients is responsible for the high prevalence of this condition in CKD. It would appear that AVF creation might play a significant role in the promotion of atrial dilatation within the ESRF milieu.
Thus, in the modern era of anaemia and uraemia management, this Study demonstrates the significant role AVF creation has in the genesis and promotion of atrial and ventricular structural and functional abnormalities. Although beyond the scope of this observational study, the potential for disadvantageous prognostic outcomes directly related to such alterations remains to be determined. Of course, the absence of a feasible, lower risk alternative to the provision of HDx remains a major stumbling block.

_Aortic Distensibility_

Arterial stiffness provides a clinically relevant measure of longitudinal vascular injury and is predictive of future cardiovascular events independent of conventional CV risk factors.\(^3\)\(^7\)\(^2\)\(^3\)\(^7\)\(^5\)\(^3\)\(^7\)\(^9\)\(^3\)\(^8\)\(^0\)\(^3\)\(^8\)\(^2\)\(^3\)\(^8\)\(^3\)\(^4\)\(^0\)\(^6\) Although risk factors such as hypertension and hyperlipidaemia may be effectively reduced by pharmacologic intervention, the vascular injury induced by such risk factors over time is not immediately negated by such therapies. Thus arterial stiffness has been postulated to provide a more relevant surrogate for future CV risk than conventional risk factors.\(^3\)\(^7\)\(^0\)

This theory was tested by the *Conduit Artery Function Evaluation* (CAFÉ)\(^4\)\(^0\)\(^4\) sub-study which drew on 2,073 patients from the larger *Anglo-Scandinavian Cardiac Outcomes Trial* (ASCOT)\(^4\)\(^0\)\(^7\) to assess this issue. CAFÉ demonstrated that despite similar reductions in brachial systolic blood pressure (SBP) and PP, the combination of amlodopine + perindopril was more efficacious in reducing central SBP and PP than the atenolol + thiazide combination\(^4\)\(^0\)\(^4\), corresponding to the demonstration of lesser cardiovascular events in the amlodopine + perindopril cohort.\(^4\)\(^0\)\(^7\) Similarly, losartan was shown to reduce arterial stiffness
and central PP to a greater degree than the comparator (atenolol) with an associated reduction in stroke in the *Losartan Intervention for Endpoint Reduction in Hypertension* (LIFE) study.\textsuperscript{408} The differing impact of alternative anti-hypertensive agents on central vascular stiffness has thus been proposed as a potential explanation for the differential effects of these agents on CV events.\textsuperscript{404,409}

Of particular relevance was the finding that the CV benefits of blood pressure lowering in ESRF were attenuated in patients where PWV was not improved by anti-hypertensive therapies.\textsuperscript{371} Thus, though the conventional risk factor was moderated by standard therapies, the modifiability of the associated arterial alterations was a more useful predictor of future CV prognosis.\textsuperscript{612}

Aortic distensibility provides a novel measure of central vascular compliance in response to systemic pulse pressure. MRI has recently been utilised to evaluate non-superficial arteries (such as the aorta) in a variety of physiological and disease states\textsuperscript{259,386-393}, and has been shown to correlate well with traditional PWV.\textsuperscript{394} Specifically, CMR-derived aortic distensibility and volumetric arterial strain have been found to be predictive of cardiovascular clinical end-points and overall survival in ESRF on multivariate analysis.\textsuperscript{395}

We evaluated aortic distensibility at baseline and 6-months following AVF-creation utilising a validated CMR methodology. Notably, aortic distensibility was not significantly altered at 6-month follow-up although a consistent numerical fall in distensibility was noted at each of the aortic levels assessed. Such a finding may of course be a chance observation, but provides substrate for the hypothesis that 6-months is too short a follow-up period to identify chronic alterations in central vascular stiffness in response to AVF-creation in the context of chronic
uraemia. Notably, the CAFÉ study evaluated vascular stiffness on repeated visits for up to 4-years in their demonstration of significant differences between the large treatment arms.

Of perhaps more significance is the finding that aortic stiffness increases progressively from distal aorta to proximal. Our research team has previously identified a significant difference between aortic distensibility at the distal descending aorta as compared to the ascending aorta in healthy elderly subjects, with a numerical, but not statistically significant trend for stepwise increasing stiffness from distal to proximal segments. This finding was not replicated in young healthy subjects in that study and had not previously been demonstrated for measures of aortic distensibility. We proposed that this finding might relate to regional differences in the aortic elastin:collagen ration, with the proportion of elastin known to be greater in the proximal segments of the aorta compared to the distal segments (almost three-fold relative difference). Elastin is thought to be particularly susceptible to fragmentation and destruction in response to the cyclical fatigue associated with repetitive systolic/diastolic pressure waves over time. Hence the proximal aortic segments would be expected to be particularly prone to deterioration in compliance with diminution of medial elastic content. By contrast, the distal aorta may behave relatively more like muscular conduit arteries that have been shown to have less elastin content and exhibit little or no age-related alteration in distensibility or compliance compared to more proximal arteries which have a higher elastin content. Such findings have previously been inferred using PWV methodologies. Such an observation is particularly significant as an increase in proximal aortic stiffness may be expected to play a prominent role in the generation of CVD. More
specifically, we hypothesised that increased proximal segmental stiffness may be associated with an increase in ventricular afterload and loss of the usual cushioning of the transmission of cyclical ventricular stroke volume to the peripheries. Furthermore, we proposed that this derangement of ventriculo-vascular coupling might play a significant role in the promotion of LVH and, potentially, deterioration in diastolic coronary perfusion resulting from reduced proximal aortic diastolic elastic recoil. Such a finding is particularly relevant in the context of CKD, as deterioration in vascular elasticity has been proposed as a metabolic consequence of chronic uraemia, with the addition of accelerated vascular calcification (common within the proximal aorta) further contributing to increased vascular stiffness in CKD.

The combination of increased arterial stiffening and reduced ventricular compliance in the context of ventricular hypertrophy and dilatation in ESRF contributes to a progressive decline in cardiac reserve, as the reduced arterial compliance leads to disadvantageous physiological responses to increased circulatory demand. Exertion is generally associated with increases in HR and LVSV (unless diastolic dysfunction is severe, where LVSV may fall with rising HR). In CKD, the increased arterial stiffness is associated with magnified blood pressure and exaggerated LV afterload in response to any increase in LVSV, due to the reduced arterial capacitance reserve. Significantly, coronary perfusion may be markedly altered during these conditions, even in the absence of obstructive coronary atherosclerosis. Such a combination may provide a potent substrate for functional myocardial ischaemia during exertion and periods of increased circulatory demand (e.g. infection, haemodialysis) and contribute further to myocardial dysfunction and CV morbidity.
**Endothelial Function**

Endothelial dysfunction is known to be a critical precondition to the development of atherosclerosis and subsequent CV sequelae.\(^{228}\) Significantly, the presence of peripheral endothelial dysfunction has previously been correlated with impairment in coronary endothelial function\(^{270}\), and hence has been used to provide a non-invasive method for predicting the presence of coronary artery atherosclerosis, and future coronary events in at-risk patients.\(^{262-266}\) Additionally, improvements in peripheral endothelial function have been associated with therapeutic interventions that improve cardiovascular risk.\(^{263,264}\)

CKD provides a particularly complex precipitant for endothelial dysfunction, resulting from multiple circulating factors injurious to the endothelium and promotional of the necessary pro-inflammatory setting for endothelial dysfunction and accelerated atherosclerosis.\(^{256}\) Notably, impairment in forearm endothelial function has been shown to be associated with an increase in all-cause mortality in ESRF.\(^{265}\)

In this Study, we found a clinically significant reduction in endothelial function (as measured by brachial artery FMD) 6-months following elective AVF creation. Our results demonstrated a reduction in mean FMD response from 9.02±9.2% to 3.05±5.7% at 6-month follow-up (p=0.012). There was no deterioration in endothelium-independent (GTN-mediated) brachial artery dilatation over the follow-up period however, indicating a primarily functional rather than structural contribution to this dysfunction. Such a finding strongly implicates a mechanistic trigger to remote endothelial dysfunction from the increased blood flow and vascular shear stress in the AVF arm and that induced by the compensatory increase in systemic cardiac output required to service the fistula.
Furthermore, such findings occurred in the absence of detectable change in baseline brachial artery blood flow in the FMD arm. Such findings implicate AVF creation in the promotion of the complex pro-atherogenic milieu of CKD and ESRF and such conduits may contribute significantly to the development of accelerated CVD associated with this condition.

**Limitations**

Although similarities exist between our Study design and that of other published studies evaluating the cardiac effects of AVF creation⁴¹,⁴¹⁴, the most significant limitation of the current study is the absence of a comparable control group. In this regard, due to clinical imperatives, there was no available cohort of stable advanced CKD subjects with functioning AVF, but not yet receiving dialysis therapy, at our institution. Comparison with a pre-dialysis, pre-AVF cohort may have provided a beneficial comparison, despite the lesser severity of CKD such a cohort would necessarily have experienced. Despite the absence of a control group in this study, the results were highly consistent across the cohort, with a high degree of biological plausibility.

Additionally, although values for the majority of clinically relevant parameters evaluated remained stable between the Study investigations, the progressive decline in renal function witnessed cannot be excluded as a potential contributor to the CV alterations demonstrated. The magnitude of change detected across the majority of evaluated parameters in this Study, however, are demonstrably out of proportion to the decline in renal function seen, implicating an alternative mechanism capable of relatively acute adaptive change. AVF creation provides such a potential mechanism.
Furthermore, re-evaluation of the cohort at an additional, earlier time-point may have provided valuable information regarding the time-course of these CV adaptations. Whether many of these CV adaptations occur acutely, or whether such alterations (other than LVM) occur progressively over months, remains to be determined.

**Conclusion**

Elective AVF creation in pre-dialysis CKD is associated with significant increases in bi-atrial and bi-ventricular chamber volumes and LVM commensurate with the requirement for a 25\% mean increase in cardiac output. Furthermore, AVF creation is associated with deterioration in systemic endothelial function, and a consistent small, but statistically non-significant trend towards increased aortic stiffening at all levels. Such alterations in CV structure and function have been strongly linked to poorer CV and all-cause mortality in previous epidemiological and observational studies. The role of therapeutic strategies in ameliorating such disadvantageous sequelae following creation of these necessary iatrogenic conduits in high-risk ESRF patients requires further study.
Study 2: Evaluation of Alterations in Cardiovascular Structure and Function following Elective Arterio-Venous Fistula Ligation following Successful Stable Renal Transplantation

Introduction

CVD is the leading cause of death in patients with ESRF and remains a leading cause of morbidity and mortality amongst successful RTx recipients. Despite substantial improvements in all-cause and CV mortality following RTx, transplant patients endure a significantly higher burden of CV and all-cause mortality than similarly-aged members of the general population. Such persistently elevated risk is multi-factorial and is influenced by the burden of maladaptive CV alterations accumulated during the period of impaired renal function, in addition to the contribution of post-transplant immunosuppressive therapies to CV risk. As such, efforts to promote the reversal of ESRF-induced maladaptive CV alteration may enhance the prognostic advantage of RTx for ESRF patients.

Previous studies have demonstrated conflicting results with regards the beneficial impact of RTx on CV structure and function. LVH is common in ESRF and is an independent predictor of adverse outcomes in ESRF and post-RTx. Furthermore, increased LA size has recently been linked with elevated mortality in association with LVH in ESRF patients referred for renal transplantation. Similarly, altered vascular function has been linked with elevated CV mortality in ESRF, however results of recent studies evaluating
improvements in vascular structure and function following RTx have been mixed.630-633

Significantly, many successful RTx recipients have persistently functioning AVF, despite the absence of ongoing clinical need. Although some AVF may spontaneously occlude following RTx, such AVF place a persistently elevated demand on cardiac function, and may contribute to the promotion of vascular structural and functional derangement that precedes clinically significant CVD.

Previous small echocardiographic studies have demonstrated improvements in LV size and LVM following elective surgical ligation of clinically large AVF post-RTx.439,634 It remains unclear whether such improvements in cardiac structure and function are limited to ligation of clinically large fistulae, or whether routine AVF ligation may be associated with beneficial cardiac and vascular effects. We sought to evaluate the effect of AVF ligation on cardiovascular structure and function in the modern era of post-RTx management utilizing recent advances in non-invasive CMR imaging.
Methods

Study Methods have been detailed previously (p92).

In brief, subjects with were enrolled following stable, successful RTx in the context of persistent AVF prior to clinically-driven, elective surgical AVF ligation. Standard exclusion criteria were applied.

Subjects were then evaluated utilizing combined Cardiac and Vascular magnetic resonance protocols (protocols detailed previously, p94) at baseline, ideally 2-weeks prior to the planned surgical date. An identical follow-up CMR protocol was then performed at 6-months post-surgery to evaluate alterations in CV parameters in response to AVF ligation.

Statistical Methods

As previously described (Statistical Methods, p111).

Power Calculation

This Pilot study was multi-factorial in nature and had not previously been performed in the manner described. For the purposes of the power calculation, in line with previously published data in echocardiographic studies and our experience with CMR in other subject subsets, we set a decrease in LVEDV as our primary end-point. We estimated that we would need to complete baseline and follow-up studies in 18 subjects to provide 80% power to detect a 20% difference in the primary end-point assuming a standard deviation of 25% in the measured parameter, correlation between baseline and follow-up measures of 0.5
(equivalent to an effect size [dz] of 0.63) and alpha error of 0.05 (G*Power v3.0.10).
Results

Eighteen (18) subjects (median age 59-years [IQR 47-65 years], 77.8% male) were enrolled in the study with initial CMR imaging undertaken approximately 2-weeks prior to planned elective AVF surgery. Due to the elective nature of the surgery, surgical dates were often cancelled by the Hospital in favour of more urgent cases, such that the interval from scan to surgery often exceeded the 2-week time-frame (time from scan to surgery 20.3±18 days). Baseline characteristics are detailed in Table 4.

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<td>Time from Transplant to Initial Scan (days)</td>
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<td>Hypertension, % (n)</td>
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<td>- former</td>
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<td>Cerebrovascular Disease, % (n)</td>
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</table>

*Table 4: Subject Baseline Characteristics – Study 2*

A complete CV protocol was possible in sixteen (16) subjects, with two subjects unable to undergo FMD protocol due to SBP>200mmHg at time of FMD
acquisition. A third subject was unable to complete the endothelium-independent component of the FMD protocol due to back pain related to lying supine for the long CMR protocol.

The median time from surgery to follow-up scan was 201 days (IQR 187-220 days). All subjects completed the cardiac protocol, aortic distensibility and brachial artery blood flow protocols. One subject was unable to undertake the FMD protocol due to back pain. A further one subject only undertook the endothelium-dependent component of the FMD protocol, unable to tolerate the additional duration in the scanner necessary to complete the endothelium-independent component of the FMD protocol due to back pain.

Clinical Results

No significant changes were detected in clinical parameters between baseline and follow-up imaging (weight: 81±16kg vs. 81±16kg, p=0.69; SBP: 144±17mmHg vs. 138±18mmHg, p=0.13; HR: 72±8bpm vs. 71±11bpm, p=0.73; Hb: 131±18g/dL vs. 134±15g/dL, p=0.26; serum creatinine: 125±63μmol/L vs. 130±65μmol/L, p=0.60). No subjects required blood transfusion or dialysis during the study period.

Cardiac Results

Eighteen subjects were included in the follow-up study and underwent initial cardiac protocol. In the presence of successful, stable RTx, baseline imaging revealed preserved mean left ventricular systolic function (LVEF 73% [67-80%]; Normal range 58%-76%) with mean LVEDV at the upper range of normal
Cardiac output was increased in this cohort at baseline (9.6±2.9L/min; Reference range: Males 2.82-8.82L/min, Females 2.65-5.98L/min). In the context of a high prevalence of prior HT, LVM was at the upper limit of normal limits at baseline (166±56g; Reference ranges: Males 108-184g, Females 72-144g). Left and right atrial area were mildly increased across the cohort at baseline (LA Area 29±5cm², RA Area 27±4cm²; Normal range <24cm²).

At follow-up, significant alterations in cardiac structure and function were identified. LVEF and RVEF were unchanged however, confirming the limited utility of this index for the evaluation of alterations in cardiac structure and function, despite its universal use in echocardiographic surveillance (mean LVEF 73% [67-80%] vs. 72% [68-78%], p=0.89; RVEF 65% [60-69%] vs. 64% [56-68%], p=0.34). Significant alterations were seen for a number of other measured or derived parameters for atrial and ventricular structure and function.

Mean LVEDV decreased by 13.1% (192±52mL vs. 167±52mL, p=0.013), mean LVSV by 14.4% (135±43mL vs. 115±33mL, p=0.008) and mean LV CO by 15.6% (9.6±2.9L/min vs. 8.1±2.3L/min, p=0.004). Significantly, mean LVM decreased by 9.7% (166±56g vs. 149±51g, p=0.0001) 6-months following AVF-ligation. Change in LVESV (10.1% decrease; 57±31mL vs. 52±32mL, p=0.16) was not statistically significant (Figures 21-23).

Similar results were seen for the decreases in mean RVEDV (12.1% decrease; 175±45mL vs. 153±43mL, p=0.006) and RVSV (14.8% decrease, 112±34mL vs. 95±29mL, p=0.003). Mean LA area was reduced by 10.1% (29±5cm² vs. 26±5cm², p=0.016) and mean RA area decreased by 8.4% (27±4cm² vs. 25±3cm², p=0.016) (Figures 21-23).
Figure 21: Alterations in Left Ventricular Structure and Function following AVF-ligation in the context of successful, stable renal transplantation.
Figure 22: Alterations in Right Ventricular Structure and Function following AVF-ligation in the context of successful, stable renal transplantation.
Figure 23: Alterations in Left and Right Atrial Area following AVF-ligation in the context of successful, stable renal transplantation.

Vascular Results:

Aortic Distensibility

Aortic distensibility was not significantly altered by the surgical ligation the AVF at the 6-month follow-up scan (2.6±1.4mmHg⁻¹ vs. 2.6±1.2mmHg⁻¹, p=0.84). Ascending aorta (AA), proximal descending aorta (PDA), distal descending aorta (DDA) and average aortic distensibility ([AA+PDA+DDA]/3) were all numerically similar following AVF ligation (Figure 24).
Prior to AVF creation, baseline aortic distensibility decreased progressively from DDA to AA, consistent with an increase in aortic stiffness in the more proximal aortic segments, though only the comparison of AA with DDA distensibility demonstrated a statistically significant difference (AA 1.74±1.5mmHg⁻¹ vs. PDA 2.51±1.2mmHg⁻¹, p=0.11; AA 1.74±1.5mmHg⁻¹ vs. DDA 3.51±2.2mmHg⁻¹, p=0.010; PDA 2.51±1.2mmHg⁻¹ vs. DDA 3.51±2.2mmHg⁻¹, p=0.11). These finding remained
similar following AVF ligation (AA 1.78±1.3mmHg⁻¹ vs. PDA 2.60±1.4mmHg⁻¹, p=0.08; AA 1.78±1.3mmHg⁻¹ vs. DDA 3.49±1.8mmHg⁻¹, p=0.002; PDA 2.60±1.4mmHg⁻¹ vs. DDA 3.49±1.8mmHg⁻¹, p=0.10) (Figure 25).

![Figure 25: Aortic Distensibility by Aortic Level Prior to (Pre-) and Following (Post-) AVF ligation in the context of stable, successful renal transplantation.](image)

Although representative of significantly different clinical cohorts, there was no significant difference detected in average aortic distensibility between Study 1 subjects with ESRF 6-months following AVF-creation, and stable post-RTx patients 6-months following AVF ligation in this Study (2.16±1.3mmHg⁻¹ vs. 2.62±1.2mmHg⁻¹, p=0.23; Figure 26).
Figure 26: Average Aortic Distensibility in ESRF subjects following AVF-creation compared to successful RTx recipients following AVF-ligation.

Flow-Mediated Brachial Artery Dilatation

Sixteen (16) subjects completed the FMD protocol at both pre-AVF and follow-up scanning. In these subjects, brachial artery endothelium-dependent vasodilatation, as measured by flow-mediated dilatation was significantly improved at 6-month follow-up imaging following AVF-ligation (2.5±6.5% vs. 8.0±5.9%, p=0.0426; Figure 27). Endothelium-independent vasodilatation was unaffected by AVF-ligation in the subjects evaluated (11.9±4.4% vs. 11.6±7.6%, p=0.87). Importantly, no significant change was detected in baseline brachial artery blood flow between initial and follow-up scans (0.08±0.04L/min vs. 0.09±0.05L/min, p=0.24) in a sub-population of 8-subjects where this sequence was performed (Figure 28). Similarly, there was no significant change in baseline brachial artery area detected across the study cohort between baseline and follow-up scans (28.4±10mm² vs. 27.4±10mm², p=0.76). There was no
correlation between % change in FMD and % change in LV CO (r=0.24, p=0.56) or LVSV (r=-0.08, p=0.84).

Figure 27: Evaluation of Alterations in Endothelial Function following AVF ligation in the context of stable, successful renal transplantation.

Brachial Artery Blood Flow – Arterio-Venous Fistula Arm

Brachial artery blood flow ipsilateral to the AVF decreased substantially following AVF creation (1.59±0.69L/minute vs. 0.083±0.05L/minute, p<0.0001)(Figure 28). This represents an almost 95% reduction in mean ipsilateral brachial artery blood-flow 6-months following AVF-ligation. Notably, there was no significant association between % change in brachial artery blood flow and % change in LV CO (r=0.22, p=0.41) or % change in LVSV (r=0.09, p=0.74).
Figure 28: Alteration in CMR-assessed brachial artery blood-flow, ipsilateral and contralateral to the ligated arterio-venous fistula in the context of stable, successful renal transplantation.
Discussion

Following successful, stable RTx, elective AVF ligation is associated with myriad desirable alterations in cardiac structure and function. Most notably, AVF ligation was associated with substantial regression of LV cavity dilatation and a clinically significant reduction in LVM – increased at baseline in this post-RTx cohort. Additionally, in the context of unchanged LVEF and RVEF, there were significant reductions in measures of RV and bi-atrial dilatation. Such alterations were noted in the context of a >15% reduction in mean CO 6-months following AVF ligation and a 95% reduction in brachial artery blood-flow ipsilateral to the ligated AVF. Notably, the improvement in contralateral brachial FMD closely mirrored the deterioration in this parameter following AVF creation in Study 1. Such alterations in cardiac structure and function were not mirrored by significant alterations in vascular structure, as measured by CMR-derived aortic distensibility however. Most importantly, the majority of the subjects studied had undergone successful renal transplantation >3 years previously, with indefinite persistence of clinically unnecessary AVF potentially providing a strong source of maladaptive CV stress in affected patients.

AVF may persist indefinitely following successful RTX. Although some RTx patients note spontaneous thrombosis of their AVF, routine post-RTx surgical AVF-ligation following a meaningful period of clinically stability remains a focus of ongoing research rather than routine clinical practice worldwide. The longer-term cardiac impact of AVF persistence following successful RTx is not known, however there is mounting evidence that AVF contribute significantly to persistence of LVH (and potentially, associated diastolic left ventricular dysfunction) in post-transplant patients. The published data in this field is
contradictory however\textsuperscript{438-440}, with some evidence that only larger AVF are responsible for disadvantageous cardiac remodelling.\textsuperscript{441} A recent study further suggests that the AVF, rather than the necessity for haemodialysis, is the propagating factor in LVH, demonstrating a marked reduction in adverse cardiac indices following failure of AVF, but ongoing intermittent haemodialysis via a central venous catheter.\textsuperscript{442} Moreover, the impact of AVF-ligation on right ventricular and atrial structure and function as well as central and peripheral vascular structure and function has not previously been determined. Our Study demonstrates regression of bi-ventricular and bi-atrial dilatation, improvement in LVM and a statistically significant improvement in remote endothelial function as measured by FMD – all factors associated with lesser morbidity and mortality. It is well appreciated that successful RTx is associated with a substantial improvement in morbidity and mortality for ESRF patients.\textsuperscript{443-449} Despite this, RTx recipients endure a persistently elevated risk of CV morbidity and mortality compared to the general population, despite substantial improvements in post-RTx care in the last two decades.\textsuperscript{451} Successful RTx has previously been shown to be associated with significant reductions in LVM and LV volumes following transplantation, though cardiac structure and function do not universally normalise.\textsuperscript{454-463} Thus, the role for novel therapies to improve post-RTx care, and CV morbidity and mortality in particular, deserves particular attention.

Endothelial dysfunction is a common finding in ESRF. Although generally improved, endothelial dysfunction commonly persists following RTx.\textsuperscript{470-472} Many of the immunosuppressive agents utilised following RTx contribute to this persistent endothelial dysfunction, and may contribute further to overall CVD risk through unfavourable effects on lipid profiles, blood pressure and glucose
intolerance.\textsuperscript{473} Thus, endothelial dysfunction post-RTx remains a potential trigger to the development of clinically significant CVD, and hence remains a potential target for therapeutic interventions, whether by improved blood pressure and lipid control, smoking cessation or by other novel means.\textsuperscript{472} In our Study, endothelium-dependent vasodilatation (as measured by FMD) demonstrated a significant improvement in mean brachial artery dilatation 6-months following AVF-ligation. Notably, endothelial function at baseline was particularly poor, despite generally preserved RTx function. The finding of improved endothelium-dependent vasodilatation is significant, as it infers a widespread benefit to systemic endothelial function, related potentially to a combination of reduced systemic blood flow and/or alteration of circulating triggers to endothelial dysfunction. Importantly, our Study did not find any significant change in baseline brachial artery blood flow in the FMD arm that may have contributed to these findings. Moreover, that a peripheral arterial bed remote from the AVF demonstrated improved endothelial function raises the possibility of improvements in clinically more important vascular beds such as the coronary and cerebral circulations. Previous studies in non-CKD cohorts have demonstrated reduction in future CV events associated with improvements in peripheral endothelial function.\textsuperscript{635} Such an hypothesis remains to be determined in post-RTx, however the potential for the relatively minimally-invasive AVF ligation procedure to induce a beneficial impact on clinical CV events through improved systemic endothelial function deserves further investigation. Anecdotally, the male subjects evaluated commented upon noticeable improvements in erectile function following AVF-ligation. Although this factor was not evaluated prospectively, it provides further basis for the hypothesis of
potential improvement in systemic endothelial function with AVF-ligation. Regardless, it remains to be determined whether the FMD finding in this Study would be replicated in other vascular beds, and whether such improvements in systemic endothelial function may abrogate this potent trigger to atherosclerosis and future CV events this high-risk cohort.

Large artery structure and compliance is also adversely affected by ESRF and long-term dialysis\textsuperscript{474-476}, with improvement but persistence of increased arterial stiffness following transplantation.\textsuperscript{477-480} The calcineurin inhibitors have been implicated in the promotion of arterial stiffness following RTx, although the process is clearly multi-factorial.\textsuperscript{481-484} Persistence of LVH and diastolic LV impairment are also believed to play a significant role in the continuation of large artery dysfunction, with disadvantageous CV sequelae.\textsuperscript{348} In this Study, we have demonstrated persistence of aortic stiffness despite marked reductions in CO and LVM following elective AVF-ligation. Such a finding may relate to the relatively less modifiable nature of aortic structure, or perhaps an insufficient duration between surgical AVF ligation and the follow-up evaluation. Interestingly however, this Study further demonstrates a stepwise increase in aortic stiffness from distal to proximal aorta. As discussed in the previous Chapter, such a finding may relate to intrinsic differences in the elastin:collagen ratio in proximal vs. distal aortic segments. Regardless, we propose that an increase in proximal aortic stiffening may contribute significantly to LV afterload, and have a particularly deleterious impact on coronary artery perfusion resulting from reduced proximal aortic diastolic elastic recoil.\textsuperscript{394}

Despite persistence of aortic stiffness in this RTx cohort, there was no statistically significant difference in aortic distensibility between ESRF patients 6-months
following AVF-creation and the successful RTx recipients evaluated 6-months following AVF-ligation. This is despite the post-RTx subjects being generally younger than the ESRF subjects evaluated in Study 1 (54.6±12 years vs. 59.2±12 years, p=0.22). Such a finding would suggest that previously published improvements in reported exercise capacity following RTx may stem more from improvements in cardiac, rather than vascular, structure enabling improved cardiac reserve despite persistently elevated ventricular afterload. Such an hypothesis remains to be investigated.

We have demonstrated significant improvements in CV structure and function following elective AVF-ligation, utilising recent advances in cardiac and vascular imaging through CMR. CMR provides for substantially greater accuracy and reproducibility in the detection of cardiac abnormalities as compared to traditional TTE or nuclear technologies. In fact, TTE has less than 50% of the precision of CMR in evaluation of LVM – a strong independent predictor of CV events in ESRF and post-RTx patients. As such, studies utilising CMR require a substantially reduced sample size to detect significant differences in CV structure and function following therapeutic intervention. In this regard, our Study is unique, in that we have been able to address alterations in CV structure and function following AVF-ligation more accurately and comprehensively than previously possible, with our results highlighting this capacity.

Limitations

This Study represented a Pilot examination of the potential for advantageous CV remodelling in response to elective AVF ligation in the context of successful, stable RTx. Although prospectively conducted and similar in design to previously
published work evaluating alterations in cardiac structure and function as assessed by TTE\textsuperscript{440}, the absence of a control group provides a notable limitation to the generalizability of the Study results. Ideally, a control group consisting of successful RTx recipients with persistently functioning AVF, matched for time from AVF creation, time from RTx and baseline characteristics such as age and gender would have served to optimise the Study design. Using this Pilot study for power calculations, such a study is currently being undertaken at our centre. Despite this, the cohort evaluated in the present Study represented patients with long-standing successful RTx, with stable renal function and best-practice co-morbidity management. Clinical stability was an overriding clinical imperative prior to referral for AVF ligation, reducing the chance of RTx management or renal function to contribute to the alterations seen. Furthermore, it remains to be determined whether a larger sample size may have enabled the detection of significant alterations in aortic distensibility, or whether such changes require a longer follow-up interval, given the more structural nature of this parameter. Given the recent recognition of the prognostic significance of this measure, further work in this area is certainly warranted.

**Conclusion**

Cardiac structure and function is significantly improved by elective AVF-ligation in the context of stable successful RTx. Furthermore, AVF-ligation was associated with significant improvement in systemic endothelial function, as evaluated by FMD. Aortic distensibility was unaltered by AVF-ligation in this Study. Thus despite a >15% reduction in overall CO and a ~95% reduction in brachial artery
blood flow ipsilateral to the ligated AVF, disadvantageous alterations in vascular structure may be more entrenched than derangements in vascular function or disadvantageous cardiac remodelling. Efforts to minimise the duration of maladaptive triggers to vascular dysfunction require further attention in the prevention of potentially irreversible deleterious vascular remodelling in ESRF and following successful RTx. We propose elective AVF ligation be considered in all successful RTx recipients following a suitable period of clinical stability.
Study 3: Evaluation of the Diagnostic Accuracy of Dobutamine-Stress Cardiac MRI in the Detection of Angiographically-Significant Coronary Artery Disease prior to Renal Transplantation

Introduction

RTx is associated with significant improvements in QOL, morbidity and mortality for successful recipients. In comparison to ESRF patients accepted for transplantation but remaining on the waiting list, cadaveric RTx has been shown to more than triple the life-expectancy of ESRF patients on dialysis. A substantial proportion of this benefit relates to a reduction in CV death. Despite this, CVD remains a leading cause of death amongst RTx recipients and RTx recipients experience an incidence of CV mortality that is many times that of the age-matched controls from the general population. Thus RTx is associated with substantial CV benefits, but a significant burden of CVD persists in this vulnerable, high-risk patient cohort.

Current pre-RTx screening processes are somewhat heterogeneous, but are relatively successful in identifying patients at lower risk of ischaemic CV events. Despite this, RTx is associated with an early increase in all-cause and cardiovascular mortality for recipients. Notably, the survival advantage of RTx appears not to be apparent until beyond 250-days post-transplantation, making survival to this point a necessary pre-condition to RTx benefit. Furthermore, according to a large Scandinavian study, between the second and
third years post-RTx, 41.4% of failed grafts are lost due to chronic rejection, but 42% are lost due to patient death with a functioning graft.\textsuperscript{450} A substantial burden of this early mortality is due to CVD.\textsuperscript{468} To reduce peri-operative CV morbidity and mortality, or early graft loss due to CVD, potential RTx recipients undergo rigorous screening processes. These pre-RTx screening investigations must consider not only peri-operative risk, but should also be designed to provide information regarding CV risk into the early years post-RTx to ensure recipients are most likely to enjoy the mortality advantages associated with the RTx process.\textsuperscript{487} Although there is widespread support for CV evaluation prior to RTx\textsuperscript{485,488-492}, there is no universally accepted screening process and hence each RTx centre may have different routines depending on local experience and expertise. Much of this debate also centres around the appropriateness of coronary revascularisation in often asymptomatic subjects in whom ‘significant’ coronary artery disease has been detected.

There is substantial debate regarding the necessity to perform ICA to exclude significant CAD prior to RTx. Numerous studies have been published demonstrating the advantages and limitations of non-invasive investigations in the ESRF context.\textsuperscript{499,501,505,636} In particular, in ESRF it appears that vasodilator stress is inferior to tachycardia-stress in the non-invasive detection of significant CAD.\textsuperscript{513} Exercise-capacity is often limited in ESRF however, limiting the capacity for exercise-based stress imaging to provide a universally applicable non-invasive methodology for the exclusion of significant coronary artery disease. Nuclear-based technologies have been widely utilised for this purpose, though suffer from technical limitations and a not-insignificant radiation dose, particularly for younger patients requiring recurrent studies. The incidence of
malignancy is known to be increased following RTx, hence it would appear reasonable to avoid unnecessary medical radiation in the pre-RTx evaluation. As a result, many authors have favoured stress echocardiography for the evaluation of pre-RTx subjects.\textsuperscript{493,505,512,637} This technique, however, is highly dependent on imaging adequacy and interpreter expertise, and is confined to a limited range of imaging windows.

DS-CMR is a relatively more recent addition to the non-invasive imaging pantheon, but provides a high level of diagnostic accuracy in studies evaluating non-CKD patients at intermediate-high clinical risk.\textsuperscript{638,639} Following a standard dobutamine-stress protocol (as for dobutamine-stress echocardiography), DS-CMR provides superior image quality and unlimited imaging planes, without the administration of exogenous contrast agents or ionising radiation. The diagnostic accuracy of this methodology is yet to be determined in the ESRF / pre-RTx context however. Hence, we sought to evaluate the diagnostic accuracy of DS-CMR in comparison to ICA, and to compare these results to the current institutional standard – myocardial perfusion scintigraphy.
Methods

Study Methods have been detailed previously (p103).

In brief, Subjects were enrolled following referral for exclusion of significant CAD, prior to listing for RTx for the treatment of advanced CKD / ESRF by the South Australia RTx Team. Standard exclusion criteria were applied. All subjects underwent DS-CMR as per validated protocol. Offline analysis was subsequently performed to exclude inducible wall motion abnormalities indicative of regional ischaemia. On a separate day, all subjects then underwent ICA to determine the presence and severity of epicardial coronary stenoses. Offline analysis of angiographic stenosis severity was determined by two experienced observers, with clinical significance determined for coronary stenoses of ≥70% in luminal diameter by visual estimation.

Comparison of DS-CMR and invasive coronary angiographic findings was determined following independent reporting of each investigation by observers blinded to the results of the alternative investigation.

Statistical Analysis

As previously described (Statistical Methods, p111)
Results

Twenty-two subjects were evaluated with DS-CMR prior to ICA, as per the clinical protocol of the Royal Adelaide Hospital Renal Medicine Department and South Australian Renal Transplant Unit (Age 56.1±8.5 years, 68.2% male). Baseline characteristics are detailed in Table 5. Twenty subjects (90.9%) also underwent SPECT perfusion imaging, as a component of the standard current clinical work-up for higher-risk pre-transplant candidates at our institution. All subjects were asymptomatic with regards myocardial ischaemia.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Proportion (n=22)</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>56.1±8.5</td>
</tr>
<tr>
<td>Gender (male), % (n)</td>
<td>68 (15)</td>
</tr>
<tr>
<td>Hypertension, % (n)</td>
<td>91 (20)</td>
</tr>
<tr>
<td>Hyperlipidaemia, % (n)</td>
<td>27 (6)</td>
</tr>
<tr>
<td>Type II Diabetes Mellitus, % (n)</td>
<td>41 (9)</td>
</tr>
<tr>
<td>Smoking, % (n)</td>
<td></td>
</tr>
<tr>
<td>- current</td>
<td>5 (1)</td>
</tr>
<tr>
<td>- former</td>
<td>18 (4)</td>
</tr>
<tr>
<td>- non-smoker</td>
<td>77 (17)</td>
</tr>
<tr>
<td>Prior Coronary Artery Disease, % (n)</td>
<td>36 (8)</td>
</tr>
<tr>
<td>Peripheral Vascular Disease, % (n)</td>
<td>9 (2)</td>
</tr>
<tr>
<td>Cerebrovascular Disease, % (n)</td>
<td>9 (2)</td>
</tr>
<tr>
<td>Receiving Dialysis, % (n)</td>
<td>95 (21)</td>
</tr>
</tbody>
</table>

*Table 5: Subject Baseline Characteristics – Study 3*
The median time-period between DS-CMR and ICA was 13 days (IQR 8-31 days). At DS-CMR, target HR was achieved in 19 subjects (86.4%), with one subject reaching 93% of predicted target heart rate at a Dobutamine dose of 40mcg/kg/minute and supplemental Atropine (1.2mg). One subject's DS-CMR protocol was ceased prematurely due to the development of clinically significant regional myocardial dysfunction, indicative of a large territory of inducible myocardial ischaemia at sub-maximal dobutamine stress. One subject failed to reach target HR at maximal dobutamine dose, with evidence of widespread, non-viable myocardial infarction noted (deemed a positive study for CAD, hence atropine not administered). There were no other haemodynamic or clinically relevant complications related to Dobutamine-infusion in the subjects evaluated. Mean LVEF was preserved overall (61±15%) with six subjects having LVEF <55%. LVM was significantly increased within this cohort, indexed to body-surface area (113g/m²; Reference range: 45-81g/m² for men, 31-79g/m² for women).

The median time-period between SPECT and ICA was 108 days (IQR 49-231 days). This is substantially longer than that for the DS-CMR, as the SPECT imaging was performed as a clinically-referred test, with subsequent referral for coronary angiography proceeding after assessment of the results within the clinical practice of the SA Renal Transplant and RAH Cardiology Units. Of the SPECT studies, 11 (55%) utilised Dobutamine as the stressor, 7 (35%) utilised exercise stress and two studies (10%) utilised dipyridamole – a vasodilator stress previously associated with reduced diagnostic accuracy in the context of ESRF.\(^{513}\) Notably, one dipyridamole-SPECT study result was a true negative for ischaemia and the other a false negative for ischaemia.
Diagnostic Accuracy:

At ICA, seven subjects (31.8%) had coronary artery stenoses of ≥70% severity within the major epicardial coronary arteries. DS-CMR correctly identified the presence of significant coronary artery disease in all seven subjects (Sensitivity = 100%; 95% CI 54-100%). Of the fifteen subjects without significant coronary artery disease, DS-CMR was negative in 14 (Specificity = 93%, 95% CI 68-100%). A single subject was deemed to have a positive DS-CMR study with no haemodynamically significant coronary artery disease identified at ICA. Positive predictive value (PPV) of DS-CMR was 88% (95% CI 47-100%) and negative predictive value (NPV) was 100% (95% CI 77-100%). (Table 6)

SPECT imaging correctly identified the presence of significant coronary stenoses in four of the seven subjects with stenoses ≥70% severity (Sensitivity = 57%, 95% CI 18-90%). SPECT falsely identified the presence of myocardial ischaemia related to epicardial coronary artery disease in eight of thirteen subjects without significant stenoses at ICA (Specificity = 38%, 95% CI 14-68%). PPV of SPECT in this cohort was 33% (95% CI 10-65%) and NPV was 63% (95% CI 25-91%). (Table 6)
\[
\begin{array}{|c|c|c|}
\hline
 & \text{DS-CMR} & \text{SPECT} \\
\hline
\text{Sensitivity} & 100\% & 57\% \\
\text{Specificity} & 93\% & 38\% \\
\text{Positive Predictive Value} & 88\% & 33\% \\
\text{Negative Predictive Value} & 100\% & 63\% \\
\text{Accuracy} & 95\% & 42\% \\
\text{Likelihood Ratio} & 15.00 & 0.93 \\
\hline
\end{array}
\]

Table 6: Diagnostic performance of DS-CMR and Stress-Perfusion SPECT in the detection of angiographically significant coronary stenoses.

Utilising an identity binomial generalised estimating equation, the diagnostic performance of DS-CMR was significantly superior to SPECT imaging for the detection of significant coronary stenoses ≥70% at ICA (p<0.001).

Assessment of Left Ventricular Ejection Fraction

Assessment of cardiac structure and function was performed for all DS-CMR studies. SPECT-based assessment was reported quantitatively for 18 of the 19 SPECT studies. No other structural or functional information was available from the SPECT studies. In those subjects undergoing both studies, mean LVEF by CMR was 60.6±15%, but mean LVEF by SPECT was only 50.2±13%. This represents a statistically and clinically significant 10.4±4% mean underestimation by SPECT compared to the internationally-recognised gold-standard assessment of LVEF by CMR (p<0.001) (Figure 29).
Figure 29: Comparison of CMR-derived vs. SPECT-derived evaluation of LVEF.
Discussion

To our knowledge, the diagnostic performance of DS-CMR has not previously been evaluated in the detection of significant coronary artery disease in ESRF patients prior to RTx. In this pilot study, we have demonstrated a very high level of diagnostic accuracy for this technique, with highly reassuring NPV and strong PPV. Furthermore, DS-CMR clearly outperformed the institutional standard (SPECT) on all fronts, with SPECT concerningly misdiagnosing three patients as having no significant CAD despite stenoses ≥70% at ICA. The procedure was safe and well tolerated by all subjects and CMR is additionally able to provide gold-standard evaluation of left and right ventricular structure and function, LVM, as well as measures of atrial volumes, valvular function and thoracic aorta dimensions and compliance. Other than LVEF, SPECT is unable to reliably evaluate any of these cardiac parameters, with SPECT-based evaluation of gated LVEF proving systematically unreliable in this Study. Given the importance of this measure in prognostic evaluation of ESRF patients, such a clinically significant discrepancy is highly concerning, and illustrates the unreliability of SPECT imaging in correctly excluding significant coronary artery disease or accurately quantifying LVEF.

Death with a functioning transplant remains a leading cause of long-term graft failure and CVD remains a leading cause of post-transplant mortality. As such, CV evaluation prior to RTx remains an important component of determining eligibility for RTx. Although resting abnormalities of LV structure and function have been shown to regress following successful RTx, in the absence of myocardial ischaemia, the presence of myocardial ischaemia on myocardial perfusion scintigraphy or stress echocardiography has been shown to be
associated with a markedly poorer prognosis.\textsuperscript{51,63,642} Previous authors have suggested that all potential RTx recipients undergo ICA for the exclusion of significant CAD, with some studies demonstrating ICA to provide superior prognostic value over non-invasive investigations.\textsuperscript{500} The optimal form of pre-RTx evaluation remains to be determined definitively however.

DS-CMR is a relatively new addition to the non-invasive armoury for the exclusion of significant coronary artery disease in patients at intermediate-high risk. Despite this, the technique has been evaluated in a variety of non-ESRF cohorts, demonstrating a high level of diagnostic accuracy with sensitivity and specificity generally \( \geq 80\%-85\% \) for each.\textsuperscript{638,639} This compares very favourably to studies evaluating other non-invasive functional cardiac imaging tests. In the context of this high level of diagnostic accuracy, DS-CMR has also recently demonstrated strong prognostic value in predicting future CV events.\textsuperscript{540,541,643,644} Reassuringly, a negative DS-CMR has been shown to predict a low rate of cardiac events in subsequent years with previous large studies in this field having consistently demonstrated a cardiac event rate of \(~1\%\) per year in the 2-4 years following a negative DS-CMR study.\textsuperscript{540,541,645} This is similar to the widely published event rates for CV event-free survival following negative myocardial perfusion imaging\textsuperscript{646} and stress echocardiography.\textsuperscript{647} Additionally, DS-CMR benefits from a high spatial resolution and hence a low rate of inter-observer variability with regards reporting of inducible ischaemia.\textsuperscript{648} Such attributes are highly desirable in a non-invasive investigation, particularly in the screening evaluation of a generally asymptomatic cohort at high risk of future CV events. Although previously demonstrated to be of prognostic value in the pre-operative assessment of patients undergoing non-cardiac surgery, but unsuitable for stress
echocardiography\textsuperscript{643}, to our knowledge, DS-CMR has not previously been assessed in the evaluation of ESRF patients prior to RTx. This population provides unique challenges in the use of DS-CMR, potentially adversely influencing diagnostic accuracy. Non-ischaemic resting wall motion abnormalities related to uraemic cardiomyopathy have the potential to influence the detection of myocardial ischaemia through the false attribution of inducible ischaemia to such wall motion defects, or the concealment of true adjacent ischaemia. Furthermore, the high prevalence of LVH in the ESRF cohort may influence the prognostic value of DS-CMR, even in the presence of a negative scan.\textsuperscript{649,650} Despite these limitations, all non-invasive imaging modalities suffer from limitations in respect of their accuracy in various cohorts. More specifically, SPECT is known to suffer from attenuation artefacts related to the diaphragm and body habitus and stress echocardiography suffers from impaired image quality in the presence of obesity, cachexia and obstructive pulmonary disease. Though CMR is able to overcome most limitations related to body habitus, the detection of inducible regional wall motion defects at very high heart rates provides some technical challenges for CMR. The temporal resolution of CMR is generally lower than that for echocardiography, and SSFP imaging generally requires 8-12 cardiac cycles to generate a composite cine image. This may be problematic in the context of irregular cardiac rhythms such as AF or frequent ventricular ectopy, causing the endocardial margins on the composite cine to become indistinct. Such issues can usually be overcome by converting to a prospectively-gated acquisition and altering the triggering delay time to avoid incorporation of cardiac cycles with shorter R-R intervals. During dobutamine-stress imaging, tachycardia is the prime objective of the study protocol as a surrogate marker of the achievement of
peak pharmacological stress. The rate of cardiac contraction and relaxation during peak tachycardia may be associated with loss of image quality due to insufficient temporal resolution. Such an issue sometimes necessitates the combination of standard ECG-triggered, retrospectively-gated SSFP imaging with real-time, non-triggered True-FISP imaging – a sequence that provides a marginally lower level of spatial resolution in favour of increased temporal resolution during peak stress imaging. We have found the combination to be advantageous in the clinical reporting of DS-CMR scans.

Despite its high-level of diagnostic accuracy, DS-CMR is not the most commonly utilised stress-CMR modality at our institution or internationally. Adenosine-stress perfusion CMR has received significantly greater attention for the detection of significant CAD in at-risk individuals. This technology utilises a gradient-echo sequence, imaging during first-pass myocardial perfusion following intravenous administration of a gadolinium-based contrast agent. The spatial resolution of this technique is marginally lower than that used for SSFP wall-motion imaging (~2.3-2.8mm in-plane resolution for perfusion imaging sequences vs. 1.8-2.0mm in-plane resolution for SSFP), however this level of resolution allows for the assessment of 5-8 radial myocardial image pixels in the presence of normal myocardial thickness. This compares to 1-2 pixels for conventional SPECT imaging. Thus, the detection of sub-endocardial myocardial ischaemia during vasodilator stress is greatly enhanced over standard nuclear imaging techniques.\textsuperscript{651-654} Furthermore, the gadolinium-based contrast agent used is biologically inert in its chelate form, excreted efficiently via renal clearance within 24-48 hours in the presence of preserved renal function and is
not associated with the not-insignificant burden of iatrogenic ionising radiation associated with SPECT imaging.

Adenosine-stress perfusion CMR additionally provides the capacity to evaluate the myocardium for evidence of fibrosis indicative of prior myocardial infarction or non-ischaemic fibrotic replacement. At our institution, a conventional adenosine-stress cardiac perfusion scan with standard evaluation of cardiac structure and function, stress and rest perfusion imaging and delayed contrast-enhancement imaging is generally completed within 40-45 minutes of scan commencement. Thus, a wealth of information was available from a relatively short scan time, elegantly combining many of the technically superior aspects of CMR imaging into a single study. Unfortunately, however, the administration of gadolinium-based contrast agents in the presence of CKD has been associated with the development of a potentially fatal condition termed Nephrogenic Systemic Fibrosis (NSF). Such a condition is believed to develop following the dissociation of gadolinium from its chelate in the presence of reduced/absent renal excretion and hence more prolonged circulation time. As such, the disassociated gadolinium may deposit in the dermis and solid organs such as the liver, causing an inflammatory reaction that may lead to local cell death and fibrosis. As such, regulatory agencies have advised against the use of gadolinium-based contrast agents in the absence of extreme clinical need, largely preventing the use of this imaging technique in patients with ESRF, particularly within the confines of a research study. CMR clinicians therefore continue to await a safe, approved contrast agent capable of providing comparable diagnostic accuracy without the associated risk of gadolinium-based chelates in individuals.
with CKD. The capacity for higher Tesla MRI scanners to detect alterations in regional myocardial oxygen consumption also offers future promise.

Despite these issues, a recent head-to-head study evaluating the diagnostic performance of adenosine-stress perfusion and dobutamine-stress CMR in a non-ESRF population demonstrated almost identical results on a per patient and per segment basis.\textsuperscript{659} Previous studies have suggested a marginal advantage for DS-CMR in terms of specificity however, when compared to anatomical assessment of coronary patency at ICA, leading to the recommendation of DS-CMR as the “method of choice for current state-of-the-art treatment regimens to detect ischemia in patients with suspected or known coronary artery disease but no history of prior myocardial infarction”.\textsuperscript{539} In this regard, although small, our Study has demonstrated that DS-CMR is highly accurate in the detection of angiographically significant coronary artery disease in ESRF, despite a high prevalence of LVH and conventional CV risk factors in the Study cohort at baseline.

DS-CMR has previously been demonstrated to be a safe Study for the detection of CAD.\textsuperscript{660} During our Study, there were no clinically-significant arrhythmias or haemodynamic complications. A single subject experienced a significant reduction in blood pressure during dobutamine stress, related to the development of a large burden of segmental myocardial dysfunction due to inducible myocardial ischaemia, and the test was promptly terminated. Such a result represents a strongly positive study (confirmed at ICA) rather than an adverse outcome \textit{per se}, with no other clinical sequelae noted during prolonged post-scan monitoring. Previous studies of dobutamine stress echocardiography have demonstrated a high level of safety with this technique, though serious
complications may occur.\textsuperscript{661-663} This risk profile does not appear exaggerated in ESRF and although small, this Study confirms the safety of Dobutamine stress imaging in ESRF prior to RTx.

At ICA, we prospectively determined coronary stenosis severity ≥70\% to be haemodynamically significant. This cut-off was determined based on usual clinical practices, both at our institution and more generally amongst the international cardiology community. Such a cut-off is routinely used in determining, angiographically, suitability for coronary revascularisation in the absence of alternative justifications (e.g. classical exertional symptoms in the presence of convincing non-invasive evidence of inducible ischaemia in the territory supplied by a 50-69\% stenosis). All anatomical cut-offs for haemodynamic significance are based on the false presumption of the infallibility of ICA, and the neglect of the numerous other lesion characteristics important in the induction of downstream myocardial ischaemia. The \textit{Fractional Flow Reserve versus Angiography for Multivessel Evaluation (FAME)} Study recently demonstrated the superiority of functional over purely anatomical evaluation of coronary ischaemia in improving outcomes in patients with significant multivessel CAD.\textsuperscript{531} Furthermore, the evaluation of fractional flow reserve (FFR) revealed striking findings regarding the diagnostic accuracy of ICA in determining lesion significance. In this landmark study, 80\% of lesions of 70-89\% stenotic severity were haemodynamically significant as determined by FFR, and 96\% of lesions ≥90\% stenosis. Of stenoses 50-69\% severity, 35\% were deemed functionally significant, despite conventional practices deeming such lesions angiographically “moderate” rather than “severe”, and generally undeserving of coronary revascularisation.\textsuperscript{532} Thus, although a cut-off of 70\% stenosis provides a
high level of diagnostic accuracy, comparison of a functional study (such as DS-CMR) evaluating the development of stress-induced regional ischaemia with an anatomical but ultimately imperfect “gold-standard” such as ICA, may result in anatomical “false positive” and/or “false negative” results that may, in fact, be correctly identifying the presence/absence of myocardial ischaemia in the context of a wide range of non-critical epicardial coronary lesions. Arguably, the FAME study provides the strongest available evidence for the angiographic determination of the functional significance of coronary stenotic severity and the prognostic relevance of revascularisation in the presence of multivessel coronary disease. Furthermore, although inferior to FFR, this study utilised subjective evaluation of coronary stenosis severity, rather than QCA to determine lesion severity (in keeping with universal clinical practice), we elected to utilise a visually-determined cut-off of 70% stenosis for our Study. No such data exists comparing QCA favourably to gold-standard coronary FFR to refute such an approach.

As an aside, adenosine-stress perfusion CMR has been validated against invasive FFR in a number of studies, with sensitivity and specificity approaching or exceeding 90% in most studies – values that are generally superior to that reported for diagnostic accuracy based on anatomical coronary stenotic severity alone.\textsuperscript{664-668} SPECT, however, has demonstrated poor concordance with invasive FFR determination of myocardial ischaemia due to epicardial coronary disease.\textsuperscript{533} At this stage, DS-CMR has not been evaluated against the invasive gold-standard, though this work is proceeding currently.

It is important to note also, that there may be a significant difference between the determination of haemodynamic significance of single or multiple coronary
stenoses, and the clinical significance the presence of even mild-moderate epicardial coronary atherosclerotic disease may signify. In this regard, the 2-year FAME study follow-up demonstrated a risk of myocardial infarction of only 0.2% for lesions where revascularisation was deferred based on an FFR>0.80 (haemodynamically non-significant). 534 Furthermore, such deferred lesions were associated with disease progression necessitating revascularisation in 3.2% at 2-years. Whether such results may be applied to the dissimilar pathobiology of coronary atherosclerosis in the ESRF and post-RTX cohorts remains to be determined however.

**Limitations**

Our Study suffers from a relatively small sample size, potentially leading to overestimation of the diagnostic accuracy of DS-CMR. Alternative manuscripts evaluating stress-echocardiography and stress-SPECT in similar patient cohorts were based on the evaluation of 40-50 subjects each, necessitating (as planned) continued recruitment prior to publication. 505,513,669 Despite this, the current study provides the first opportunity to assess the utility of DS-CMR in this high-risk patient subset, with results demonstrating a high level of accuracy and no safety concerns. The results for the institutional standard (SPECT) are highly disappointing, regardless of the Study sample size, and raise significant concerns regarding the appropriateness of continued use of this modality at our institution. A further limitation of our Study is the absence of an invasive functional gold-standard such as FFR to compare the results of the non-invasive functional study. As highlighted, the comparison of anatomical coronary stenotic severity with the
results of a functional study evaluating the development of myocardial ischaemia in response to dobutamine stress may be compromised by the imperfect nature of ICA lesion characterisation. Studies evaluating DS-CMR against per-vessel FFR are ongoing.

Ideally, to allow for optimal comparison of the stress methodologies studied, all subjects would have undergone both DS-CMR and SPECT prior to ICA. In our Study, two subjects did not undergo SPECT imaging, but were referred directly to ICA as a component of the clinical pathway, due to the perception of very-high pre-test risk. Interestingly, neither of these subjects had ischaemia detected at DS-CMR, nor significant CAD detected at ICA. It is uncertain whether performance of SPECT in these subjects would have led to an improved measure of specificity for SPECT, in light of the poor specificity demonstrated in the subjects evaluated. Although relatively small, DS-CMR demonstrated an unequivocal statistical and clinical superiority in the subjects evaluated in this Study, though further research is required to ensure our initial results remain robust in a larger, less highly selected ESRF patient cohort.

Conclusion

In this pilot study, we have demonstrated a high level of diagnostic accuracy for DS-CMR in the detection of angiographically significant CAD in ESRF subjects undergoing CV risk assessment prior to RTx. Furthermore, DS-CMR significantly out-performed the institutional standard – SPECT myocardial perfusion imaging. In this high-risk cohort, DS-CMR was safe, highly accurate and provided a wealth of prognostically significant structural and functional cardiac data to the referring
clinician. Although further work in this area is necessary, we propose DS-CMR as a new standard of care in ESRF patients undergoing evaluation prior to RTx.
Study 4: Evaluation of Dynamic Coronary Endothelial Function in End-Stage Renal Failure

Introduction

CVD is a leading cause of morbidity and mortality in ESRF and following successful RTx. A significant component of this burden relates to the end-organ injury resulting from impaired oxygen delivery due to atherosclerotic stenotic disease in subtending vessels. Atherosclerosis represents a final common pathway resulting from myriad causes of endothelial injury and subsequent endothelial dysfunction. Systemic endothelial dysfunction is highly prevalent in the presence of CKD, with numerous studies demonstrating a strong link between systemic endothelial dysfunction and future CV events. In fact, a substantial body of literature has focused on the evaluation of systemic endothelial function in ESRF, with numerous methodologies claiming superiority in predicting future coronary and cerebrovascular events. Remarkably, little (if any) work has focused on the evaluation of coronary endothelial function in this condition however.

Coronary endothelial dysfunction has previously been associated with a significantly increased burden of future cardiac events.\textsuperscript{262,670} Research evaluating the link between coronary and peripheral endothelial function has revealed varying results however, suggesting that coronary endothelial function may not be directly predicted by evaluating peripheral artery function.\textsuperscript{270,671-674} We sought to invasively evaluate coronary endothelial function in the presence of advanced CKD in subjects undergoing clinically-indicated ICA prior to potential RTx.
Furthermore, we sought to compare indices of coronary macrovascular and microvascular function in RTx candidates with values for coronary vascular function for subjects with normal renal function and minimal CAD.
Methods

Study Methods have been detailed previously (p107).

In brief, CKD subjects were enrolled following referral for ICA prior to listing for RTx for the treatment of advanced CKD / ESRF by the South Australia RTx Team. Non-CKD subjects were enrolled from patients with eGFR>60mL/min/1.73m² undergoing clinically-driven ICA for exclusion of significant CAD. Subjects from both cohorts were excluded if significant CAD was identified within the epicardial coronary arteries at ICA. Standard exclusion criteria for the performance of coronary endothelial function assessment were applied.

At ICA, if coronary anatomy was suitable, coronary endothelial function was evaluated utilizing a 0.014-inch coronary Doppler Flo-wire (Volcano Therapeutics, CA, USA) advanced into the Study vessel via an infusion catheter. Following achievement of stable Doppler Flow velocity signals, resting coronary flow velocities were determined, with determination of epicardial coronary diameter by standardized quantitative angiography with offline analysis.

Coronary endothelial function was then evaluated by validated protocol⁶⁰₂,⁶⁰³, consisting of the following interventions:

- 5% dextrose control infusion for 2-minutes (Control 1)
- Acetylcholine (3-minute infusions of Ach 10⁻⁷M [1.6mcg/min], then 10⁻⁶M [16mcg/min]
- 5% dextrose control infusion for 5-minutes (Control 2)
- bolus dose of GTN (50mcg)
- bolus dose of Adenosine (48mcg)
At the completion of each infusion, a coronary angiogram was performed using 9mL of non-ionic contrast (Omnipaque, GE Healthcare, Waukesha, USA) injected through a Medrad infusion pump at 5mL/s, with images saved for offline analysis. Doppler indices, haemodynamic data (systemic blood pressure and heart rate) and ECG rhythm strip were recorded continuously throughout the study for offline analysis.

**Quantitative Coronary Angiography**

Following acquisition of standardised coronary angiography during the Study protocol, offline analysis was performed using proprietary edge-detection software (QCA-CMS™, Medis Medical Imaging Systems, Leiden, Netherlands). Mean coronary diameter was assessed 5mm distal to the tip of the Doppler guidewire over a 5mm segment.

**Coronary Blood Flow**

Coronary velocity measurements were analysed by a technician blinded to the results of the QCA analysis. Coronary blood flow (CBF) was then calculated by validated formula.\(^275\)

\[
[\pi \times 0.125 \times (\text{Coronary Diameter})^2 \times (\text{Coronary Blood Flow APV})] \times 60
\]

Coronary flow velocity reserve (CFR) was then determined as a ratio of:

\[
\text{APV (post-adenosine) / APV (baseline)}
\]

A normal response is considered to have occurred if endothelial-dependent CBF increases by more than 50% in response to Ach, and the coronary flow reserve (CFR) - the ratio of maximal to baseline CBF - is greater than 2.5 following adenosine.\(^275\)
Statistical Analysis

As previously described (Statistical Methods, p111)

Power Calculation

The outlined Study was a Pilot Study performed to evaluate the scope of coronary endothelial dysfunction in the presence of advanced CKD, as compared to a non-CKD cohort. As such, a formal Power calculation was not performed.
Results

Following determination of coronary artery suitability at ICA, coronary endothelial function was invasively evaluated in eight (8) pre-RTx subjects and thirteen (13) non-CKD subjects. Baseline characteristics are described in Table 7.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CKD (n=8)</th>
<th>Controls (n=13)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.5±10</td>
<td>53.9±14</td>
<td>0.72</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>63%</td>
<td>62%</td>
<td>p=0.69</td>
</tr>
<tr>
<td>Hypertension</td>
<td>100%</td>
<td>45%</td>
<td>p=0.006</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>63%</td>
<td>36%</td>
<td>p=0.17</td>
</tr>
<tr>
<td>Type II Diabetes Mellitus</td>
<td>63%</td>
<td>18%</td>
<td>p=0.04</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- current</td>
<td>13%</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>- former</td>
<td>25%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>- non-smoker</td>
<td>62%</td>
<td>60%</td>
<td>p=0.86</td>
</tr>
<tr>
<td>Prior Coronary Artery Disease</td>
<td>13%</td>
<td>54%</td>
<td>p=0.07</td>
</tr>
<tr>
<td>Receiving Dialysis</td>
<td>88%</td>
<td>0%</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Baseline Creatinine (μmol/L)</td>
<td>709±140</td>
<td>68±21</td>
<td>p=0.0002</td>
</tr>
<tr>
<td>Baseline Haemoglobin (g/dL)</td>
<td>108±10</td>
<td>138±13</td>
<td>p=0.0004</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Aspirin</td>
<td>25%</td>
<td>45%</td>
<td>p=0.87</td>
</tr>
<tr>
<td>- Statin</td>
<td>63%</td>
<td>36%</td>
<td>p=0.17</td>
</tr>
<tr>
<td>- Beta-blocker</td>
<td>75%</td>
<td>36%</td>
<td>p=0.06</td>
</tr>
<tr>
<td>- ACEI / ARB</td>
<td>88%</td>
<td>27%</td>
<td>p=0.006</td>
</tr>
<tr>
<td>- Diuretic</td>
<td>0%</td>
<td>18%</td>
<td>p=0.37</td>
</tr>
<tr>
<td>- Calcium Channel Blocker</td>
<td>63%</td>
<td>18%</td>
<td>p=0.04</td>
</tr>
<tr>
<td>- Insulin</td>
<td>13%</td>
<td>9%</td>
<td>p=0.50</td>
</tr>
</tbody>
</table>

Table 7:  Subject Baseline Characteristics – Study 4

ACEI = Angiotensin Converting Enzyme Inhibitor; ARB = Angiotensin Receptor Blocker
There was no difference in the mean age of the subjects within the two cohorts (52.5±10 vs. 53.9±14, p=0.72). All subjects had only minor coronary artery disease within the study vessel (≤20% epicardial coronary stenoses)[LAD=100%] and subjects with diffuse atherosclerosis were specifically excluded. Constant haemodynamic monitoring was performed throughout the Study. Baseline SBP, DBP and HR were not statistically dissimilar between the cohorts (SBP: 142±24mmHg vs. 125±24mmHg, p=0.13; DBP: 82±14mmHg vs. 80±10mmHg, p=0.76; HR: 79±23bpm vs. 67±14bpm, p=0.26). There was no statistically significant alteration in HR or blood pressure detected during endothelial function assessment within the cohorts.

*Endothelium-Dependent Coronary Function*

There was no difference between the CKD and normal cohorts with respect to the maximum change in CBF following Ach 10⁻⁶M administration (81.0±68% vs. 89.8±71%, p=0.79). Notably, study vessel diameter at the site of assessment was greater in the non-CKD cohort at baseline (2.7±0.5mm vs. 3.6±1.0mm, p=0.02), with this difference persisting during high-dose Ach infusion (2.8±0.7mm vs. 3.6±1.0mm, p=0.048). Despite this, there was no statistically significant difference in either baseline (48.4±20mL/min vs. 64.7±23mL/min, p=0.12), or peak (88.2±46mL/min vs. 113.8±35mL/min, p=0.17) CBF between the cohorts, although the CKD cohorts CBF was numerically lower at both time-points (in the presence of significantly smaller Study vessels). Change in CBF following Ach was also not significantly different between the cohorts (81.0±68% vs. 89.8±71%, p=0.97). There was no significant difference between the cohorts in the change in vessel diameter in response to high-dose Ach (2.2±10.3% vs. 1.2±5.7%, p=0.80).
No subject in either cohort had a significant epicardial coronary constriction response to Ach $10^{-6}$M (defined as a $>50\%$ reduction in cross-sectional area as derived from QCA).\textsuperscript{276}

*Endothelium-Independent Macrovascular Coronary Function*

There was no statistically significant difference between the CKD and non-CKD cohorts with respect to endothelium-independent conduit vessel vasodilatation in response to intracoronary GTN (Vessel QCA: $10.9\pm12\%$ vs. $9.7\pm21\%$, $p=0.73$).

*Endothelium-Independent Microvascular Function*

There was a highly significant difference in microvascular function between the CKD and non-CKD cohorts, as measured by CFR. CFR was significantly reduced in the CKD cohort, but preserved in the non-CKD cohort ($1.9\pm0.4$ vs. $3.0\pm1.1$, $p=0.01$). Despite the potentially greater prevalence of anaemia and LVH in the CKD cohort, there was no significant difference in baseline APV between the cohorts ($27.0\pm5.4\text{ cm/s}$ vs. $24.1\pm11.5\text{ cm/s}$, $p=0.44$) to explain the differences in CFR, as has been proposed by other authors.\textsuperscript{277}
Discussion

We have found a significant difference in coronary flow reserve (CFR) following adenosine administration between subjects with advanced CKD and those with preserved renal function, in the presence of only minor epicardial coronary disease at ICA. This finding is striking, in that adenosine is an endothelium-independent vasodilator\(^\text{675}\), acting directly via activation of the A2 receptors on vascular smooth muscle of resistance vessels \(\leq 100\mu\text{m}\) in diameter.\(^\text{676}\)

Preservation of microvascular function has previously been defined as a CFR>2.5 in response to adenosine administration.\(^\text{276}\) The results of our Study demonstrate impaired augmentation of coronary microvascular dilatation in response to a potent microvascular dilatory stimulus in the CKD cohort, strongly implicating coronary microvascular dysfunction in the presence of CKD.

Previous studies in this area have demonstrated conflicting results. In 2004, Ragosta et al published a Study evaluating invasive CFR amongst subjects with TII DM evaluating differences in the presence and absence of associated ESRF. In their Study, the presence of advanced CKD was associated with a reduction in CFR, predominantly mediated via an increase in basal coronary average peak velocity (APV), rather than a failure of APV augmentation in response to intravenous adenosine.\(^\text{277}\) The authors hypothesised this impairment in CFR in CKD to potentially result from the greater prevalence of LVH in that cohort and the possible presence of otherwise unidentified circulating vasodilatory humoral factors specific to renal failure directly mediating the elevated baseline APV. Furthermore, they refute the potential contribution of anaemia to the elevated baseline APV in their CKD cohort due to the absence of statistically significant
difference in haematocrit between subjects with normal vs. abnormal CFR. Of course, the absence of a statistically significant difference in this sub-analysis may simply reflect Type II statistical error, rather than the true absence of an association.

This finding of elevated basal CBF has recently been affirmed non-invasively utilising PET. Published in *Circulation* in 2009, the Study by Koivuviiita *et al* evaluated basal and hyperaemic (post-dipyridamole) myocardial perfusion in twenty-two patients with moderate to severe CKD (divided into three cohorts according to eGFR), and compared these results to ten healthy control subjects with preserved renal function. Although this Study found preserved CFR amongst the CKD patients (as distinct from the previously discussed invasive study and our own results), basal myocardial blood flow was significantly elevated amongst the CKD patients vs. the non-CKD controls (p<0.001), with a negative correlation between eGFR and myocardial blood flow seen (Spearman correlation coefficient=0.63, p=0.0001). An ultrasound-based FMD protocol was also undertaken in this Study, with FMD (% change in brachial artery diameter) significantly reduced amongst the CKD cohorts compared to controls (p<0.03).

The authors conclude that “coronary and peripheral vascular function are disturbed by different mechanisms in patients with CKD”, although direct comparison of the two methodologies utilised in this Study is questionable. The evaluation of large-vessel endothelial-dependent function in the brachial artery is distinct from the evaluation of coronary endothelium-independent, microvascular function evaluated by the administration of intravenous dipyridamole in this study. Although previous studies have demonstrated a link between peripheral microvascular function (as assessed by ‘gold-standard’
venous occlusion plethysmography) and peripheral arterial FMD responses, the two are not equivalent and comparison of coronary microvascular responses to dipyridamole with FMD as a surrogate marker of peripheral microvascular function is somewhat disingenuous.

A further Study by Chade et al published in 2006 evaluated invasive CFR amongst 124 patients with milder renal dysfunction (GFR<60mL/min/1.73m$^2$), compared to 482 control subjects with preserved renal function (GFR>60mL/min/1.73m$^2$), finding reduced GFR to be predictive of impaired CFR by univariate, but not multivariate, analysis. Although the mean CFR was reduced in subjects with mildly impaired renal function (compared to controls), only increasing age, female gender, rising BMI and co-existent hypertension were significantly associated with impairment of CFR by multivariate analysis. No comment was made on differences in basal APV values between the two cohorts in this Study.

In our Study, the baseline APV was not significantly different between the cohorts and coronary angiography failed to demonstrate the presence of diffuse atherosclerosis as a contributor to a falsely impaired CFR. The difference between cohorts derived almost entirely from the failure of vasodilatory augmentation of coronary flow velocity, strongly implicating microvascular dysfunction as the primary aetiology. This finding is significant, as no previous Study has demonstrated such a finding in ESRF, although the phenomenon has been strongly suspected by clinicians. Importantly, the demonstration of microvascular dysfunction in advanced CKD provides a sound biological basis for published work demonstrating angina to occur in the absence of obstructive epicardial coronary disease in up to 50% of ESRF patients undergoing ICA.

In this regard, a recent publication by Sicari et al in a non-CKD cohort
demonstrated a significant prognostic disadvantage for patients presenting with chest pain, but in whom ICA revealed normal or near-normal coronary arteries. In this Study, patients with a CFR<3.0 (as assessed by echocardiography) had a markedly lower infarct-free survival out to 4-years follow-up (55% vs. 96%, p<0.0001). In our Study, the finding of reduced CFR may be impacted by the presence of factors such as anaemia and supply-demand mismatch in the context of LVH, the presence of microvascular disease must certainly be considered a significant component of the milieu in CKD, and may contribute to the adverse CV outcomes seen in CKD patients.

The documentation of impaired CFR in CKD is particularly significant in considering the non-invasive evaluation of possible myocardial ischaemia affecting CKD and ESRF patients in clinical practice. Vasodilator stress imaging (particularly SPECT-based imaging) provides a cornerstone for the non-invasive evaluation of possible myocardial ischaemia in most developed nations. Whether in the evaluation of CV risk prior to possible RTx, or in the assessment of clinically suggestive symptoms in CKD/ESRF patients, vasodilator stress imaging predominantly depends upon the demonstration of differential regional blood flow induced by maximal hyperaemia following vasodilator (e.g. adenosine) administration. Myocardial territories subtended by epicardial coronary vessels with haemodynamically significant stenoses already have maximal or near-maximal microvascular vasodilatation as a component of the intrinsic autoregulatory mechanisms within the coronary microcirculation. Thus, at rest, in the absence of active ischaemia, microvascular auto-regulation maintains sufficient (relatively homogenous) blood flow to the myocardium, even within territories supplied by epicardial coronary stenoses. During vasodilator
administration, the microvasculature subtended by normal or minimally diseased epicardial arteries undergoes substantial vasodilatation leading to a 3-5-fold increase in regional blood flow. Due to the near-maximal microvascular dilatation at rest in stenosed territories, further microvascular dilatation may not be possible, leading to the development of a relative hypoperfusion of the “ischaemic” territory. In the presence of global coronary microvascular dysfunction, as may be seen in CKD/ESRF, the augmentation of blood flow into “non-ischaemic” territories appears to be blunted, potentially reducing the capacity for relative hyper-perfusion to these myocardial segments. Thus, the capacity to detect perfusion inhomogeneity during vasodilator administration in the presence of significant epicardial coronary disease is impaired, potentially reducing the sensitivity of the non-invasive imaging study. Furthermore, the capacity of the CKD/ESRF microvasculature subtended by stenosed coronary arteries to maximally vasodilate may also be blunted, potentially leading to myocardial ischaemia in the presence of less severe epicardial disease.

This theory concurs with the available body of literature evaluating vasodilator stress imaging against ICA in ESRF. Marwick et al reported an exceedingly low sensitivity for dipyridamole SPECT in their prospective Study – 29% for the detection of coronary stenoses ≥70% at ICA. Although numerically superior to that published by Marwick et al, the results from Vandenberg's retrospective Study in Transplantation revealed a sensitivity of only 62% - a result that fails to inspire confidence in the technique for the accurate exclusion of significant CAD in this high-risk condition. Boudreau et al published more reassuring results in 1990, however concerns remain regarding the use of this technique in clinical practice. In light of the biological theory regarding the prevalence of
microvascular dysfunction in CKD, previous authors have proposed tachycardia stress as a more appropriate stress-modality in this condition, whether utilising echocardiographic or SPECT imaging.\textsuperscript{505,513} Published results for tachycardia stress in this condition appear to provide a greater level of diagnostic accuracy, though head-to-head studies are lacking.\textsuperscript{513} The inherent limitations of the imaging technology may also play a significant role in determining diagnostic accuracy, as demonstrated by the poor results achieved for tachycardia-stress SPECT reported in the previous Chapter of this Thesis.

\textit{Endothelium-dependent Coronary Function}

Perhaps surprisingly, our Study failed to demonstrate a significant difference in endothelium-dependent epicardial coronary vasodilatation between the cohorts in response to intra-coronary Ach infusion. While the standard deviations around the cohort means were wide, the numerical difference between the cohorts was small, suggesting the absence of a clinically significant difference that was simply underestimated due to the small sample sizes. Both cohorts demonstrated a >50\% increase in CBF in response to Ach, indicating preservation of endothelium-dependent coronary vascular function, even in the presence of advanced CKD in this Study.\textsuperscript{276} Despite this, it is also notable that the non-CKD cohort failed to demonstrate a significant increase in epicardial coronary diameter in response to the endothelium-dependent vasodilator Ach and the resultant increase in CBF detected. One subject in the non-CKD cohort and two subjects in the CKD cohort demonstrated a >5\% reduction in vessel diameter in response to Ach $10^{-6}$M, however there was no other evidence of significant endothelial dysfunction detected during Ach administration.
Such findings run counter to the commonly held belief that CKD must be associated with significant epicardial coronary endothelial dysfunction, as an extension of the numerous studies demonstrating impairment of endothelial function in the peripheral circulation. The absence of significant endothelial dysfunction in this pre-RTx cohort is also notable as the increased burden of peripheral endothelial dysfunction has commonly been used to infer a similar pattern of dysfunction in the coronary beds, predisposing to the accelerated coronary atherosclerosis commonly encountered in this condition. The explanation for our findings may lay in the highly selective nature of the population studied, rather than truly reflecting the absence of coronary endothelial dysfunction as a component of CVD in ESRF and/or a small sample size. In this regard, the CKD subjects studied represented a group, albeit with coronary risk factors, at lower risk than standard dialysis patients who are not considered ‘potential’ renal transplant candidates. ESRF patients on dialysis who are not being considered for RTx generally represent the CKD cohort at greatest risk of CV morbidity and mortality.\textsuperscript{451} Mortality for this group is exceedingly high, relating often to the presence of prior CVD and/or advanced age. Such patients were not considered for this Study, and the study population was further sub-selected by excluding pre-RTx patients with anything more than minor angiographically apparent coronary disease. It might therefore be hypothesised that the subjects enrolled and evaluated in this Study were specifically enrolled on the basis of an absence of indirect markers of coronary endothelial dysfunction, by virtue of the absence of significant resultant end-organ vascular disease. Whether such coronary endothelial findings are truly representative of the wider ESRF population remains to be determined.
Endothelium-Independent Macrovascular Coronary Function

There was no significant difference between the cohorts in vessel diameter by QCA following administration of intracoronary GTN in this Study. Such a finding confirms the presence of intact epicardial endothelium-independent vasodilatation in the two cohorts, consistent with the absence of fixed epicardial coronary disease preventing vascular smooth muscle dilatation in response to GTN. Although ESRF may be associated with a significant burden of both intimal and medial vascular calcification, the CKD cohort in this Study was specifically selected based on the absence of significant angiographically-apparent coronary disease. Hence, given the less severely diseased ESRF cohort studied, it is not surprising that the response to GTN was similar between the cohorts.

Limitations

There are a number of important limitations of the current Study. The sample size of the two cohorts was small, potentially affecting our statistical power in the detection of a significant difference between the groups studied for endothelium-dependent function. In this regard, the Study was powered to detect a statistically significant difference with 80% power for an effect size (d) of 1.22. The effect size of the current study was only 0.14, potentially necessitating a substantially greater sample size to detect any statistically significant difference. Despite this, our Study failed to demonstrate a numerical trend towards endothelial dysfunction (%change in CBF <50%) in the CKD cohort as would indicate the presence of a clinically significant difference in coronary endothelial function between the CKD and non-CKD cohorts.
Subjects were specifically selected based on the absence of angiographically significant coronary atherosclerosis within the epicardial coronary vessels, however in the absence of IVUS, diffuse epicardial atherosclerosis cannot be entirely excluded. Moreover the presence of diffuse microvascular atherosclerosis was unable to be excluded by the Study protocol. The presence of such disease could certainly have led to diminution of both basal and peak APV in response to adenosine, resulting in the findings described. Every effort was made to exclude this potential confounder, and all of the CKD subjects studied were asymptomatic with regards clinical features of haemodynamically significant CAD. Furthermore, each of the CKD subjects studied had participated in the previously reported Study of DS-CMR, with all those included in this Study having no inducible ischaemia detected using this highly sensitive and accurate modality. With regards the accuracy of our results, microvascular function was assessed in this Study utilising CFR as assessed by the Volcano Flow-wire system (Volcano Therapeutics, CA, USA). It is recognised that CFR may be influenced by the concurrent haemodynamic state, with elevation of HR and increased contractile state potentially influencing CFR measurements. More recently, the index of microvascular resistance (IMR) has been proposed as a more specific and reproducible measure of microvascular function. IMR is derived by determination of the distal coronary pressure (derived from the pressure wire) divided by coronary blood flow during maximal hyperaemia. CBF in this instance is derived by a thermodilution method for evaluating coronary transit time utilising a thermistor at the distal end of an alternative commercially available pressure wire system. Importantly, IMR has been demonstrated to be less affected by epicardial coronary stenosis or haemodynamic state of the patient.
In this regard, IMR may provide a more specific evaluation of microvascular function than CFR, although to date the Doppler flow-wire derived CBF assessment remains the ‘gold-standard’ and most widely published invasive evaluation of coronary blood flow.

Selection bias may have played a role in the results of this Study, in light of the markedly different indications for ICA between the two cohorts. Although the CKD cohort was evaluated to exclude asymptomatic CAD prior to RTx, the non-CKD cohort were referred for ICA following suggestive symptoms or demonstration of myocardial ischaemia by non-invasive evaluation. Previous studies have demonstrated reduced endothelial function in patients presenting with chest-pain syndromes, even in the absence of significant epicardial coronary disease at ICA. Despite such differences in clinical indication for ICA, the CFR of the non-CKD cohort was within the normal range, largely excluding diffuse microvascular disease as a consistent finding in this cohort.

Finally, we administered adenosine via an intracoronary route, in line with recommended protocols for invasive assessment of coronary vascular function.²⁵⁴ It remains to be determined whether 48mcg of adenosine is sub-maximal in the context of advanced CKD, but regardless, such findings indicate the presence of objective microvascular dysfunction. Moreover, whether alternative findings would have been obtained with intravenous adenosine administration, or the use of other vasodilatory agents (e.g. substance P), remains to be determined.
Conclusion

In this Study, we have documented the presence of significant coronary microvascular dysfunction in the presence of advanced CKD, as compared to subjects with minimal coronary artery disease and preserved renal function. This finding related to the blunting of microvascular dilatation in response to intracoronary adenosine, rather than elevation of basal APV as had previously been reported. We found no significant difference in endothelium-dependent or – independent epicardial coronary function in response to Ach and GTN respectively. Although surprising, such a finding occurred in the context of a highly selected CKD population, with minimal epicardial coronary atherosclerosis. Our findings are particularly significant in regards the potential implications for the investigation and management of CKD patients where microvascular dilatation in expected following administration of adenosine analogues. Vasodilator-stress SPECT may be particularly inappropriate for the exclusion of significant CAD in this cohort, and alternative stress modalities should be considered in the presence of significant renal dysfunction to avoid an unacceptable prevalence of false negative studies that may result from the reduced sensitivity implied by our results.
CONCLUSION
Thesis Summary

The primary aims of this Thesis were directed to the evaluation of alterations in CV structure and function in ESRF, and more specifically to the evaluation of: the CV impact of AVF creation prior to dialysis commencement; the CV impact of elective AVF ligation following successful, stable RTx; the diagnostic performance of DS-CMR in excluding significant CAD prior to RTx, and; coronary endothelial and microvascular function in advanced CKD/ESRF.

As detailed in the Background section of this Thesis, the prevalence of CKD is rising progressively in developed countries, in tandem with the rising incidence of obesity and TIIDM. The CV sequelae of advancing CKD are significant, and ultimately lead to a markedly elevated incidence of CV morbidity and mortality, with associated increases in personal and societal health care costs. Despite substantial advances in the care of CV disease in the general community over the last three decades, CKD and ESRF continue to confer a markedly shortened lifespan on affected individuals, regardless of age. Significant markers for particularly elevated risk included increases in LVH, increased atrial and ventricular dimensions, the presence of peripheral endothelial dysfunction and increased vascular stiffness. In light of the appallingly high mortality rates of ESRF, greater efforts are clearly required to better identify and direct specific care to the prevention and management of CVD in this condition.

In Study 1, we demonstrated the utility of CMR in the evaluation of CV structure and function in ESRF, and further demonstrated the significant cardiac and vascular alterations associated with the elective formation of an iatrogenic AVF prior to the commencement of dialysis. Although prognostically superior to large-
bore central venous catheters, the creation of AVF was associated with substantial alterations in CV structure and function including substantial increases in CO, atrial and ventricular chamber dimensions, and LVM at 6-month follow-up imaging. Furthermore, we identified a significant reduction in endothelium-dependent brachial artery vasodilatation contralateral to the AVF at follow-up, occurring in the absence of any alteration in baseline brachial artery blood-flow. Notably, there was no detectable change in aortic distensibility during the Study period, potentially indicating this parameter to be a less acutely adaptable component of the vascular system. This Study demonstrated the medium-term impact on prognostically significant structural and functional CV parameters, and the need to consider therapeutic options that may help to offset such disadvantageous CV adaptation to AVF formation.

In Study 2, we demonstrated the utility of CMR in the evaluation of CV structure and function following successful RTx. CMR again demonstrated a high level of spatial and temporal resolution, in the absence of ionizing radiation or exogenous contrast agents, and provided for the detection of significant alterations in CV structure and function with a high level of statistical significance despite modest sample sizes. This Study demonstrated the potential for dramatic alterations in prognostically-sensitive CV structural and functional parameters associated with the elective ligation of clinically unnecessary, but persistent AVF following RTx. Remarkably, despite the disparate clinical characteristics of the cohorts, the response to AVF ligation closely mirrored those findings demonstrated in Study 1, with Study 2 demonstrating a “reversal” of many of the disadvantageous alterations identified in the subjects evaluated in Study 1.
Study 3 focused on the evaluation of DS-CMR in the detection of haemodynamically significant CAD in a cohort of advanced CKD / ESRF subjects referred for coronary angiography prior to consideration of RTx. Many of these subjects had also been investigated utilising the institutional standard, SPECT-imaging – as a component of the routine clinical pathway evaluating potential RTx recipients. This Study demonstrated a dramatic improvement in diagnostic accuracy associated with DS-CMR imaging, as compared to SPECT, with 100% sensitivity and diagnostic accuracy approaching 100% for the CMR technique. Furthermore, it was demonstrated that SPECT imaging performed poorly in the accurate quantification of LVEF in this condition, as compared to the “gold standard” evaluation provided by CMR. Such results demonstrated the diagnostic superiority of DS-CMR in the routine evaluation of ESRF patients with suspected CAD, potentially opening an avenue for prognostic assessment of high-risk ESRF patients in alternative clinical scenarios.

In Study 4, we undertook to invasively assess coronary endothelial function in advanced CKD/ESRF and compare these findings with findings of coronary endothelial function within a non-CKD cohort. The most striking finding of this Study was the failure of microcirculatory vasodilatation in response to adenosine in the CKD cohort, and the inference of significant microvascular dysfunction in this condition. This finding was particularly notable in the absence of any detectable dysfunction of coronary endothelium-dependent function as might have been expected to be present in an ESRF cohort. Such findings of microvascular dysfunction support a physiological basis for previous research demonstrating impaired diagnostic accuracy associated with vasodilator-dependent stress-imaging techniques (notably SPECT). The absence of significant
endothelial dysfunction in this ESRF cohort was somewhat surprising, but may be more reflective of the highly selected Study population, than of the true absence of significant endothelial dysfunction in ESRF more generally. Further work is clearly necessary in this area.

**Future Directions**

The demonstration of diagnostic utility provided by CMR in the Studies outlined opens a plethora of potential research avenues. The high diagnostic reproducibility of CMR has previously been shown to allow for the detection of highly significant alterations in cardiac and vascular structure without the requirement for large sample populations. Hence CMR provides a potentially superior imaging modality for future studies evaluating the CV impact of alternative therapeutic interventions in this condition. More specifically, CMR provides an outstanding opportunity to accurately detect potential CV benefits associated with lifestyle (e.g. exercise) and pharmaco-therapeutic interventions (e.g. alternative phosphate binders) in CKD, ESRF and following RTx.

Following the results of the AVF-ligation Study, we are progressing with a larger randomised Study of systematic AVF ligation following successful RTx. Such a Study would focus on the evaluation of the prognostic benefits that might be expected to arise from the advantageous structural and functional alterations seen in this initial Study.

The documentation of superior diagnostic accuracy associated with the use of DS-CMR in the exclusion of significant CAD in a relatively healthy ESRF cohort being assessed for suitability prior to RTx provides the basis for extending the use of
this imaging modality further in ESRF. Specifically, in light of the markedly elevated risk of MI in ESRF, and the abysmal prognosis of affected patients, prevention of such events is of paramount importance. It remains to be determined whether a diagnostic technique of high accuracy may be able to provide prognostic information regarding the future likelihood of coronary events in this condition. By direct extension, such a modality may then allow for the identification of ESRF patients at particularly high-risk who may benefit from “prophylactic” revascularisation prior to a fatal cardiac event. Such a premise remains to be proven however, despite the therapeutic attractiveness of such a concept.

Finally, the demonstration of intact coronary endothelial function in a small group of pre-RTx patients, where endothelial dysfunction might reasonably have been expected, raises the potential for research aimed at the identification of specific clinical / genetic characteristics that may provide relative protection to the endothelium despite the presence a uraemic milieu. We are progressing with this study to increase our numbers, and hence certainty that endothelial dependent vasodilation is truly unaffected in our pre-renal transplantation cohort. Further studies however evaluating coronary endothelial function in a variety of higher-risk ESRF subjects may better illustrate the burden of coronary endothelial function and dysfunction in this condition, although the presence of significant CAD will remain a technical limitation to the use of the invasive techniques described herein.

Renal dysfunction is associated with myriad alterations to CV structure and function, many of which may not be recognised utilising standard clinical investigations. CMR provides a novel avenue for the more comprehensive clinical
and investigational assessment of ESRF patients in the detection of sub-clinical cardiac and vascular disease and the impact of therapeutic interventions in this condition. Although limited by cost and availability, the increased penetration of CMR into routine clinical practice more generally, and in ESRF more specifically, provides an exciting opportunity to advance our understanding of the CV sequelae of this condition, and to progress towards more effective preventive CV care for these high-risk patients.
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