EFFECTS OF A REDUCTION IN GFR ON CARDIOVASCULAR STRUCTURE AND FUNCTION: A FIVE YEAR FOLLOW UP STUDY

ANNA MARIE PRICE

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Institute of Cardiovascular Sciences

School of Clinical and Experimental Medicine

College of Medical and Dental Sciences

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ABSTRACT

The inverse association between glomerular filtration rate (GFR) and cardiovascular risk is well recognised but not fully explained. Furthermore, chronic kidney disease is associated with atherosclerosis, arteriosclerosis, left ventricular hypertrophy and myocardial fibrosis. Kidney donation reduces renal function by approximately 30% and allows the study of the cardiovascular effects of a reduced GFR in healthy subjects without confounding comorbidities.

This thesis aims to examine the isolated effects of a reduction in GFR as a result of nephrectomy on haemodynamics, blood pressure, cardiovascular structure and function, myocardial tissue characterisation and blood biomarkers.

In a multi-centre prospective controlled study, there were no significant changes in peripheral blood pressure or pulse wave velocity in donors compared to controls at 12 months. In a prospective 5 year longitudinal study of donors and controls, no significant differences were observed in left ventricular volumes or mass, strain parameters or ejection fraction at 5 years after nephrectomy. In a cross-sectional comparison of 1:1 age and sex matched donors and controls there were no significant differences in T1 or T2 times, extracellular volume or late gadolinium enhancement.

The reduction in GFR after donation does not lead to deleterious changes in cardiovascular structure and function at 5 years.

DEDICATION

To my remarkable fiancée, Richard, and my wonderful family.

ACKNOWLEDGMENTS

This study was undertaken with the supervision of Professor Jonathan Townend (Consultant Cardiologist) and Professor Charles Ferro (Consultant Nephrologist) at the University of Birmingham and University Hospitals Birmingham National Health Service Foundation Trust. I will be forever grateful to my lead supervisor Professor Townend, for his exceptional mentorship throughout. His intellectual input, enthusiasm and ongoing support has been pivotal in enabling me to achieve my goals and ensure that this research study was a success. His passion for research and endless motivation confirmed my desire to pursue an academic career. I am also indebted to my co-supervisor Professor Ferro for his mentorship, intellectual insights and for being an inspirational role model to an aspiring Nephrologist.

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Finally I would like to thank my fiancée, Richard for his infinite understanding during this period and for encouraging me to pursue my own dreams. On a personal note, the final year, 2020, presented us with some immense challenges. Without his backing, love and stability none of this would have been possible.

EXTENT OF PERSONAL CONTRIBUTION

The work in this thesis was carried out by myself with the following exceptions. The original hypothesis and concept for the Chronic Renal Impairment in Birmingham (CRIB)-DONOR II study was developed by Professor Jonathan Townend, Professor Charles Ferro and Dr William Moody. Grant funding was obtained by myself and Professor Townend as part of a British Heart Foundation Clinical Training Fellowship (FS/16/73/32314). Ethical approval from the Research Ethics Committee/Health Research Authority and study sponsor was obtained by myself. I obtained all study amendments, ensured that regulatory documents were up to date and completed all sponsor-led audits. The recruitment and consent of all CRIB-DONOR II participants was carried out by myself along with arranging and performing the 5-year follow up study visits. Participants' baseline and 12-month data from the original CRIB-DONOR study was provided by Dr Moody.

Cardiac magnetic resonance (CMR) imaging safety checklist, scanning and administration of gadolinium to participants was carried out by myself with the assistance of radiographers (Mrs Jan Kennedy, Mrs Elizabeth Squire and Miss Eleanor Derrick) and Dr Victoria Stoll. I am grateful to Dr Roman Wesolowski for providing the phantom and carrying out regular scanning of the phantom throughout the study. I carried out all arterial stiffness measures (pulse wave velocity/pulse wave analysis), ultrasound for carotid intima thickness and blood pressure measurements including analysis of ambulatory blood pressure monitoring results. I also performed all blood sampling and delivered them to the Clinical Biochemistry unit at University Hospitals Birmingham for immediate analysis and prepared serum and plasma samples for storage with assistance from staff at the Wellcome Trust Birmingham Clinical Research Facility. At the end of the study, frozen serum samples were analysed for N-terminal-pro B type natriuretic peptide and highly sensitive Troponin T by laboratories within University Hospitals Coventry and Warwickshire and laboratories within Birmingham

Heartlands Hospitals for the analysis of High-sensitivity C-reactive protein (CRP). The analysis of Fibroblast growth factor-23 (FGF23), α-klotho and multiplex magnetic immunoassays were carried out by myself under the supervision of Dr Kirsty McGee. The administration of radioactive chromium for isotopic glomerular filtration rate assessment was carried out within the Nuclear Medicine Department by specialist technologists and analysed by Clinical Scientists with support from Medical Physics Expert Dr Peter Anderson.

All CMR volumetric, feature tracking, T1/T2 mapping and late gadolinium enhancement assessment was carried out myself for the baseline and 5 year visit scans. Dr Ravi Vijapurapu and Dr William Moody carried out reproducibility assessment for T1, extracellular volume and volumetric analysis. Statistical analysis for the CRIB-DONOR II study was carried out by myself with the advice and guidance of Dr Peter Nightingale.

In addition to the CRIB-DONOR study, I was responsible for the Birmingham cohort of the Effect of a reduction in glomerular filtration rate after nephrectomy on arterial stiffness and central haemodynamics (EARNEST) study. This study was a collaborative multi-centre study involving 7 United Kingdom (UK) centres. The original hypothesis for the EARNEST study was developed by Professor Jonathan Townend, Professor Charles Ferro and Dr William Moody. Grant funding was obtained by Professor Townend as part of a British Heart Foundation Clinical Project Grant (PG/12/35/29403). I was responsible for arranging and performing the follow up study visits for the Birmingham cohort. I conducted the study visits with the assistance of research nurse Ms Carole Green. I was also responsible for the site file, all regulatory documents and any audits conducted by the sponsor for the Birmingham site. All centres data was collated electronically by Cambridge Clinical Trials Unit and provided by study coordinator Dr Katan Patel. Data cleaning was carried out by Dr George Greenhall. Statistical analysis was carried out with the assistance of Dr George Greenhall and advice from Dr Laurie Tomlinson.

My supervisors Professor Townend and Professor Ferro have provided advice and guidance with preparation of manuscripts throughout the study.

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95% CI	confidence interval
2D/3D	two-dimensional/three-dimensional
ACR	albumin creatinine ratio
ADA	Aortic Distensibility Analysis
AHA	American Heart Association
AIx	augmentation index
AI_{75}	augmentation index corrected to 75 beats per minute
ANOVA	one way analysis of variance
APKD	Adult polycystic kidney disease
ARIC	Atherosclerosis Risk in Communities
ARSAC	Administration of Radioactive Substances Advisory Committee
BMI	body mass index
CIMT	carotid intima-media thickness
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMR	cardiac magnetic resonance
CRIB-DONOR	Chronic Renal Impairment in Birmingham-DONOR
^{51}Cr-EDTA	^{51}Cr-ethylenediamine tetraacetic acid
CRP	C - reactive protein
CT	computerised tomography
DCM	dilated cardiomyopathy
DICOM	Digital Imaging and Communications in Medicine
DNA	deoxyribonucleic acid
DOCA	deoxycorticosterone acetate
EARNEST	Effect of a Reduction in Glomerular Filtration Rate after Nephrectomy on Arterial Stiffness and Central Haemodynamics
ECG	electrocardiogram
ECM	extracellular matrix
ECV	extracellular volume
EDTA	ethylenediamine tetraacetic acid
eGFR	estimated glomerular filtration rate

eGFR cys	CKD-EPI equation with cystatin alone
eGFR creat	CKD-EPI equation with creatinine alone
eGFR creat-cys	CKD-EPI equation with cystatin and creatinine
ELISA	enzyme linked immunosorbent assay
ESRD	end stage renal disease
FA	flip angle
FGF23	fibroblast growth factor-23
FOV	field of view
GBCA	gadolinium based contrast agents
GCS	global circumferential strain
GFR	glomerular filtration rate
GLS	global longitudinal strain
GN	glomerulonephritis
GRS	global radial strain
HLA	horizontal long axis
HR	heart rate
hr	hour
HRP	horseradish peroxidase
Hs Trop T	high sensitivity troponin T
iGFR	isotopic glomerular filtration rate
IL	interleukin
IRMER	ionising radiation medical exposure regulations
KDIGO	Kidney Disease Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
LA	left atria
LGE	late gadolinium enhancement
LV	left ventricle
MACS	magnetic assisted cell sorting buffer
MAP	mean arterial pressure
MCP-1	monocyte chemoattractant protein-1
MDRD	Modification of Diet in Renal Disease
MFI	mean fluorescence intensity

mGFR	measured glomerular filtration rate
MMP-9	matrix metallopeptidase
MOLLI	modified look-locker inversion recovery
MRI	magnetic resonance imaging
NHANES	National Health and Nutrition Examination Survey
NHS	National Health Service
NSF	nephrogenic systemic fibrosis
NT-pro BNP	n-terminal pro B type natriuretic peptide
PBMC	peripheral blood mononuclear cells
PTH	parathyroid hormone
PWA	pulse wave analysis
PWV	pulse wave velocity
RAAS	renin angiotensin aldosterone system
RV	right ventricle
SAX	short axis stack
SCMR	Society of Cardiovascular Magnetic Resonance
SSFP	steady state free precession
TE	echo time
TI	inversion time
TNF	tumour necrosis factor
TR	repetition time
TSE	turbo spin echo
UK	United Kingdom
US	United States
VLA	vertical long axis

CHAPTER 1

INTRODUCTION

1.1 Extent of personal contribution

This chapter is based on the following published first author articles.[1-3] The first drafts of the manuscript were written by myself and I was responsible for all edits and revisions.

Price AM, Ferro CJ, Hayer MK, Steeds RP, Edwards NC, Townend JN. Premature coronary artery disease and early stage chronic kidney disease. *QJM*. 2017; 11 (10):683-686. http://doi.org/10.1093/qjmed/hcx179.[1]

Price AM, Edwards NC, Hayer MK, Moody WE, Steeds RP, Ferro CJ and Townend JN. Chronic kidney disease as a cardiovascular risk factor: lessons from kidney donors. *Journal of the American Society of Hypertension*. 2018; 12:497-505. http://doi.org/10.1016/j.jash.2018.04.010.[2]

Price AM, Hayer MK, Vijapurapu R, Fyyaz SA, Moody WE, Ferro CJ, Townend JN, Steeds RP, Edwards NC. Myocardial Characterization in pre-dialysis chronic kidney disease: a study of prevalence, patterns and outcomes. *BMC Cardiovascular disorders.* 2019; 19 (295). https://doi.org/10.1186/s12872-019-1256-3.[3]

1.2 The kidneys

The kidneys are two retroperitoneal organs derived from the mesoderm and are approximately 10-12cm in length.[4] They are composed of an inner medulla and a renal cortex comprising individual functioning nephrons.[5]

They have a complex and multifaceted physiological role including: filtration of excretory products, regulation of bone mineralisation, blood pressure, fluid status and production of erythropoietin.[5]

1.3 Chronic kidney disease

1.3.1 Definition and classification of CKD

Chronic kidney disease (CKD) is a term used to describe a wide variety of diseases that persistently affect the structural and/or the functional composition and integrity of the kidney.[6] Historically, the definition of CKD has been indistinct and the terminology used has been inconsistent.[7]

In an attempt to standardise this, the United States (US) National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) developed a definition in 2002, with later recognition in 2004 from the Kidney Disease Improving Global Outcomes (KDIGO).[8, 9] This finally allowed recognition of a global uniform definition. The definition centred on a few fundamental principles; there must be evidence of 'kidney damage' (anatomical component) or the glomerular filtration rate (GFR) must be $<60ml/min/1.73m^2$ (functional component) and abnormalities must persist for greater than three months (temporal component) in order to be defined as chronic.[7, 9] Classification was then determined by sub dividing CKD into 5 categories based on the severity of the GFR.[8] It was the view of KDOQI and KDIGO that any CKD classifications should be based on patient outcomes and prognostic indicators.[10]

In 2009, a KDIGO controversies conference was held to address issues with the current system and a collaborative meta-analysis was undertaken to establish composite relative risks.[7] Following the conference and analysis of data from 1,555,332 CKD participants the definition was upheld with the addition of adding in a proteinuria classification and subdividing stage 3 CKD into two components of severity.[10]

The 2012 KDIGO guidelines are currently in use.[11] The addition of proteinuria was due to the strong association between proteinuria and adverse outcomes even at the earliest stages of CKD.[12] The 2012 KDIGO guidelines classify CKD by abnormalities in structure (e.g. cysts on imaging) or function for at least three months.[11] Evidence of structural damage includes histological or imaging evidence, electrolyte disturbance suggestive of tubular disorders, urinary sediment or an albumin creatinine ratio (ACR) of >3mg/mmol.[11] Further category classifications centre on risk, using a 2 dimensional (2D) matrix comprised of proteinuria (A1-A3) and glomerular filtration rate (G1-G5), both well-established independent predictors of long term outcome in CKD, see **Figure 1.1**.[12-15]

GFR ml/min/1.73m^2			A1 Normal/high-normal <3mg/mmol	A2 High 3-30mg/mmol	A3 Very high/nephrotic >30mg/mmol
G1	Normal or high	>90			
G2	Mild	60-89			
G3a	Mild to moderate	45-59			
G3b	Moderate to severe	30-44			
G4	Severe	15-29			
G5	Kidney failure	<15			

Figure 1.1. Heat map illustrating the 2012 KDIGO CKD classification.

Adapted from Romagnani[14] et al and Levey[10] et al The heat map represents composite ranking for adjusted relative risk for five outcomes: all-cause mortality, cardiovascular mortality (death from stroke, myocardial infarction, heart failure or sudden cardiac death), kidney failure (treated by dialysis or transplant), acute kidney injury and progressive CKD.

Green indicates low risk, yellow moderate risk, orange high risk and red very high risk.

1.3.2 Prevalence of CKD

CKD is rapidly becoming a global health epidemic with severe economic consequences.[16, 17] The burden is particularly high in the elderly, where early stage CKD affects approximately 1 in 3 of those over the age of 70.[18] Healthcare costs can be up to four fold of those without CKD as a result of both increased hospital admissions and outpatient appointments.[19]

Globally, the prevalence is reported to be between 5-10% of the population but varies considerably depending on geographical location.[18] In a meta-analysis global prevalence for stages 3–5 was calculated as a mean of 10.6% (95% confidence interval [CI] 9·2–12·2%) and a mean of 13.4% (95% CI 11.7-15.1%) for all stages of CKD.[20] In a review of random samples of 13,896 participants over the age of 16 from the Health Survey for England (HSE) between 2003 and 2010 Aitken et al. reported overall prevalence of CKD between 5 and 6%.[21] More recent estimates from the same survey in 2016 has suggested the prevalence in the whole population is 7.3%.[22] Prevalence is higher in women and increases with advancing age.[22] Estimates from a UK population cohort study of primary care data of those over the age of 60 suggest that prevalence is higher at around 18% with 8% undiagnosed prior to the study.[23]

1.3.3 The cardo-renal relationship

The heart and the kidney have a complex co-dependent relationship due to common biochemical pathways and haemodynamic relationships.[24] This relationship is so strong that the risk of those with CKD progressing to end stage renal disease (ESRD) is substantially lower than the risk of death from cardiovascular disese.[25] The first published reference of this link was a description by Bright in 1836 where a series of cases of patients with “albuminous urine” were described as dying from neurological complications secondary to uraemia.[26] Their presentation, clinical appearance and, on occasion, autopsy results were detailed.[26] The patients died from the consequences of advanced renal failure in an era without dialysis.[26] It

was noted that their hearts were enlarged, "flabby" and had significant ventricular hypertrophy.[26] This was the first known report of left ventricular disease in CKD.[26]

In many circumstances this connection means that dysfunction (acute or chronic) in one organ inevitably leads to some degree of dysfunction in the other organ system.[24] The cardio-renal types are a classification system encompassing the clinical syndromes seen as a result of the interaction between the cardiovascular and renal system.[24] In 2004, the National Heart, Lung and Blood institute initially attempted to categorise the syndromes.[24] This was later adapted by the Acute Dialysis Quality Initiative in 2008 following a consensus conference segregating cardio-renal syndromes into 5 distinct types, **see Table 1.1**.[24] [27] This thesis and the aim of these studies focuses on type 4 chronic reno-cardiac syndrome in which CKD leads to chronic changes within the heart.[27]

Table 1.1. Nomenclature of cardio-renal syndromes developed by the Consensus Conference of the Acute Dialysis Quality Initiative. Adapted from Rangaswami et al and Ronco et al.[24, 27]

Phenotype	Syndromes	First organ to fail	Description	Typical scenario
Type 1	Acute cardio-renal		Acute heart failure→Acute kidney injury	Acute coronary syndrome and circulatory collapse causing renal injury
Type 2	Chronic cardio-renal		Chronic heart failure→Chronic kidney disease	Long standing heart failure
Type 3	Acute reno-cardiac		Acute kidney injury→Acute heart failure	Volume overload as a result of acute kidney injury
Type 4	**Chronic reno-cardiac**		**Chronic kidney disease→Chronic heart failure**	**Uraemic/CKD associated cardiomyopathy as a result a long term chronic kidney disease**
Type 5	Secondary cardio-renal		Systemic process	E.g. Amyloidosis or sepsis etc.

1.4 Cardiovascular mortality in CKD

1.4.1 Epidemiology of all-cause and cardiovascular mortality

Life expectancy is a useful measure of overall health and disease burden at a population based level.[28] In large scale epidemiological studies CKD is associated with a significantly reduced life expectancy.[28, 29] In a study of participants aged 35-80yrs, Turin et al. demonstrated that life expectancy for both men and women with CKD declined in steps with each progressive CKD stage, with the most dramatic reductions in the most advanced stages of disease.[29] For example, based on the abridged life table methods used by the authors, a male aged 40 years would have an estimated life expectancy of 20 years less with a GFR of 15-29ml/min/1.73m^2 as opposed to an aged matched male with a GFR of >60 ml/min/1.73m^2.[29]

The reasons for this reduced life expectancy are numerous and are not limited to the development of ESRD.[25] In 2004 the landmark study of Go et al. sought to determine whether there was an independent relationship between poor outcomes (defined as hospitalisation, mortality and cardiovascular events) and GFR in non-dialysis subjects within the community.[13] Using health care records from 1996 to 2000, in over 1 million subjects, there was a stepwise increase in the risk of death and major cardiovascular events from a GFR of <60 ml/min/1.73m^2 and below even after adjustment for confounding variables.[13] Although the median follow up period was very short (2.8 years) and their cohort was restricted to insured participants in North California, it was the first study demonstrating an independent inverse relationship between GFR, mortality and cardiovascular events.[13]

Since then, further large scale population studies have established strong mortality links to both proteinuria and GFR over longer time periods of follow up.[15, 30] In Taiwan, Wen et al studied 462,293 subjects undertaking routine medical screening, followed participants up for a median of 7.5 years and recorded 14,436 deaths.[30] In this study subjects identified with

CKD were at a 83% higher risk of mortality than the general population [hazards ratio (HR) 1.83 (95% CI 1.73-19.30].[30] This further increased to a 100% increased risk of cardiovascular mortality [HR 2.0 (95% CI 1.78-2.25)] compared to the general population.[30] The most compelling data, however, comes from two large scale systematic reviews and meta-analysis from the Chronic Kidney Disease Progress Consortium which was formed in 2009 in an effort to investigate the prognostic implications of GFR and proteinuria.[15, 31] These studies both demonstrated strong and profound relationships between GFR/proteinuria and mortality. In 2010, the Chronic Kidney Disease Progress Consortium included 105,872 subjects from 14 studies with proteinuria data from urine ACRs and 1128,310 subjects from a total of 7 studies with urine dipstick measures from the general population.[15] Pooled adjusted hazard ratios began to increase from <75ml/min/1.73m^2 and proteinuria had an additive risk.[15] All-cause and cardiovascular mortality increased with increasing proteinuria and declining GFR with effects most profound in those over the age of 75 years.[15] In a second systemic review and meta-analysis using data from subjects considered at high risk of CKD (hypertension, cardiovascular disease or diabetes) which included 10 cohorts of 266,975, the hazard ratio increased progressively from 1.03 in those with a GFR of 60 ml/min/1.73m^2 to 3.11 in those with a GFR of 15 ml/min/1.73m^2 for all-cause mortality with similar results for cardiovascular mortality.[32] Proteinuria had a multiplicative effect for prediction and was a major independent risk factor for cardiovascular mortality.[32]

Much of the disproportionate increased risk of mortality and reduced life expectancy is due to cardiovascular disease. In a study of 27,998 participants with a GFR <90 ml/min/1.73m^2 the risk of requiring renal replacement therapy was 1.1%, 1.3% and 19.9% for stage 2, 3 and 4 CKD respectively.[25] In contrast, the risk of death was 19.5% for stage 2, 24.3% for stage 3 and 45.7% for stage 4.[25] Those who died also had higher rates of heart failure and coronary artery disease.[25] This suggests that the risk of cardiovascular disease in CKD is so substantial

that those at the earliest stages of CKD are more likely to die of cardiovascular disease than progress to ESRD.[25] Thompson et al. also reviewed the deaths of 81,064 participants from Alberta, Canada.[33] Death from cardiovascular disease increased with each progressive stage of CKD, 20.7% in stage 2 (with proteinuria), 36.8% in stage 3a, 41.2% in stage 3b, and 43.7% in stage 4.[33] An increase in heart failure, valvular disease and arrhythmia deaths were also observed with advancing CKD, see **Figure 1.2**.[33] In addition, left ventricular hypertrophy (LVH) is widespread in those commencing haemodialysis and both arrhythmia and sudden cardiac arrest account for over a third of deaths according to US Renal Data System data. [34-36] This suggests that not only does cardiovascular disease increase with advancing CKD but that the underlying mechanisms may change as GFR deteriorates.

A

B

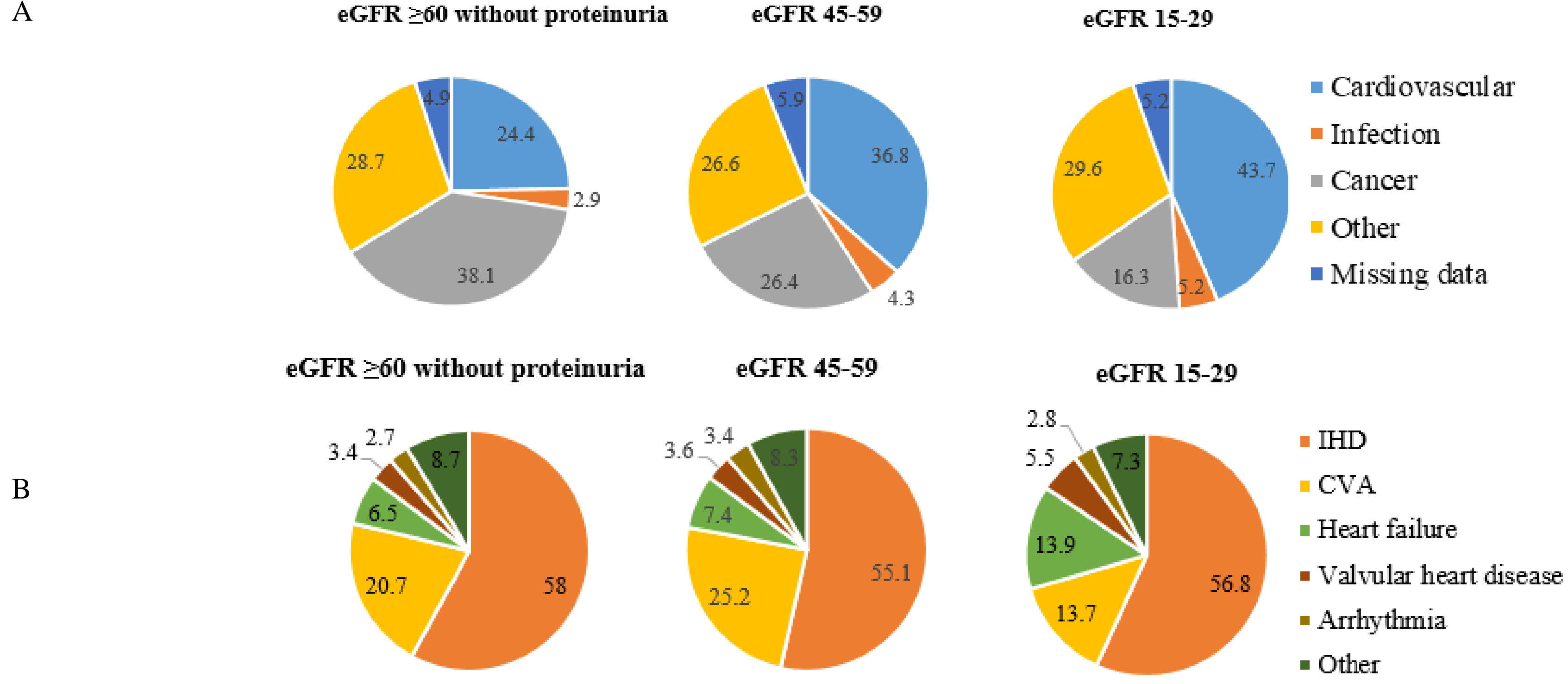


Figure 1.2. Unadjusted relative percentages for cause of death according to estimated glomerular filtration rate (ml/min/1.73m^2). Adapted from Thompson et al.[33]

A. The top panel illustrates the four main causes of death for each CKD category. For those with a GFR >60ml/min/1.73m^2 the commonest cause of death was cancer. For those with a GFR less than 60ml/min/1.73m^2 the leading cause of death was cardiovascular disease.
B. The bottom panel segregates the causes of cardiovascular death. For those with a GFR >60ml/min/1.73m2 ischaemic heart disease was the greatest cause of death. All other causes of cardiovascular disease such as heart failure and valvular heart disease increased with advancing stages of CKD.

IHD; ischaemic heart disease. CKD; chronic kidney disease. CVA: cerebrovascular accident.

1.4.2 Cardiovascular risk prediction in CKD

Hypertension is extremely common in patients with CKD with a prevalence approaching 70% and is recognised as an important contributor to cardiovascular disease.[37] Similarly, diabetes is a leading cause of ESRD and a known strong predictor of cardiovascular disease.[38, 39] Despite this, the use of Framingham equations (using 'traditional' atherosclerotic risk factors) which are used to predict morbidity and mortality from coronary artery disease in the general population perform very poorly in the CKD population.[40] Using data pooled from the Atherosclerosis Risk In Communities (ARIC) and Cardiovascular Health Study Weiner et al. examined the accuracy of the model in participants with a GFR of 60-15ml/min/1.73m^2.[40] The Framingham equations tended to underestimate cardiovascular events in the CKD population.[40] The models accurately predicted events at 5 years in CKD participants in only 62% vs. 72% in controls without CKD and at 10 years the values were 60% and 69% respectively.[40] The problem of using traditional risk factors in CKD for predicting cardiovascular disease was further illustrated in two meta-analysis of data from the Chronic Kidney Disease Prognosis Consortium.[15, 41, 42] Mahmoodi et al. conducted a large scale meta-analysis of 45 cohorts including cohorts from those with CKD, the general population and high risk cohorts to assess whether the presence of hypertension changes the relationship between a declining GFR and all-cause and cardiovascular mortality.[41] From a GFR of >55ml/min/1.73m^2 for all-cause mortality and >45 ml/min/1.73m^2 for cardiovascular mortality those with hypertension had a higher adjusted HR.[41] At a GFR of 45ml/min/1.73m^2, however, all-cause mortality was similar in those with and without hypertension [HR 1.24 (95% CI 1.11-1.39)] vs. [HR 1.77 [95% CI 1.57-1.990)] with similar patterns seen in cardiovascular mortality.[41] The relationship between GFR and mortality was graded irrespective of hypertension and surprisingly the relationship was steeper and stronger for

those without hypertension.[41] Similar results were observed for proteinuria but the relationship was linear.[41]

Using data from over a million participants from the same Chronic Kidney Disease Prognosis Consortium cohorts, a further meta-analysis was conducted in an accompanying manuscript to investigate the impact of diabetes.[42] Mortality was higher in those with diabetes across all stages of GFR.[42] However, when the reference points were segregated for each group there was no significant difference in mortality between groups.[42] The hazard ratio for all-cause mortality at an GFR 45ml/min/1.73m^2 was similar in those with diabetes [HR 1.35 (95% CI 1.18-155) and without diabetes [HR 1.33 (95% CI1.19-1.48)].[42] The presence of diabetes, therefore, made little difference to relative risks with similar results seen for proteinuria.[42] In an attempt to predict cardiovascular disease in CKD, Matsushita et al. also conducted a meta-analysis of 24 cohorts of over 600,000 participants from the Chronic Kidney Disease Prognosis Consortium data.[43] The meta-analysis concluded that both GFR and ACR were independently linked to coronary artery disease, stroke, heart failure and cardiovascular mortality.[43, 44] Furthermore, both markers of renal dysfunction were superior predictors of all four cardiovascular outcomes than any single traditional cardiovascular risk factor. [43]

It is clear that reliance on traditional risk factors to predict cardiovascular events in CKD lacks accuracy and underestimates risk.[40] A major reason for this is that the excess mortality in CKD is probably not due to atherosclerotic disease alone, there are other cardiovascular pathological processes that appear to change with progression of CKD.[34] Whilst death due to occlusive coronary artery disease (ST elevation and non ST elevation myocardial infarction) is prominent in the early stages of CKD (atherosclerotic causes) there are increasing rates of heart failure and valve abnormalities (non-atherosclerotic causes) in the more advanced stages, see **Figure 1.3**.[33] More recently, it has been acknowledged that those with CKD are at increased risk of arrhythmias including supraventricular tachyarrhythmia, ventricular

arrhythmias, atrial fibrillation/flutter and sudden cardiac death.[45] In a retrospective analysis of data obtained from the Multi-centre Automatic Defibrillator Implantation Trial-II it was found that for every 10ml/min//1.73m^2 reduction in GFR the risk of sudden cardiac death increased by 17%.[46] Furthermore, occlusive coronary events account for very few of overall cardiovascular deaths.[47] This suggest that cardiac muscle disease plays an important role in cardiovascular disease in those with CKD.[45] Non-atherosclerotic cardiovascular structural and functional changes may be major intermediary contributors to adverse cardiovascular outcomes in CKD. [34]

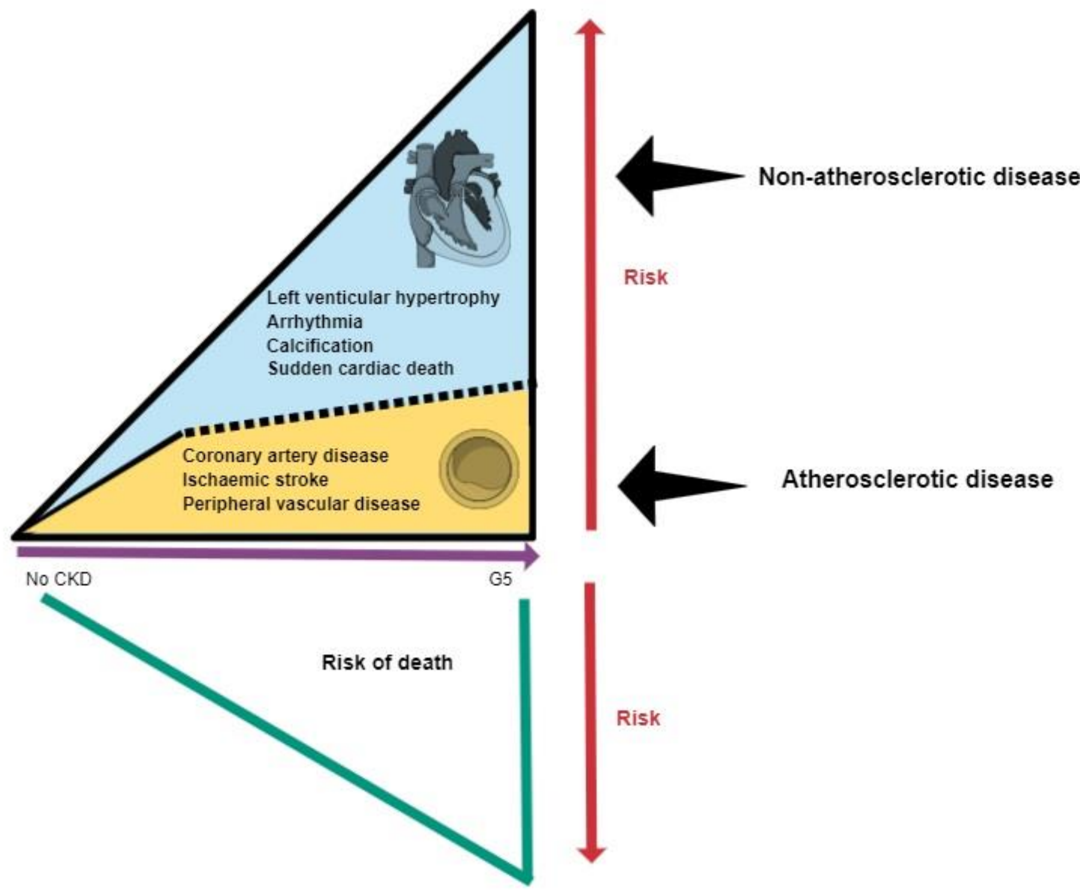


Figure 1.3. Spectrum of cardiovascular disease with CKD progression

Adapted from Sarnak et al and Wanner et al. [34, 48]

The upper triangle indicates all cardiovascular events. It is split into non-atherosclerotic causes in the upper part and atherosclerotic causes in the lower part. As CKD stages advance, (indicated by the purple arrow) the presentation of cardiovascular disease changes with progressively more non-atherosclerotic causes as CKD progresses.[34] The red line indicates increasing risks. Furthermore, the outcomes are poorer after cardiovascular events in those with the most advanced stages of CKD with greater risk of fatality indicated by the green lower triangle.[34]

1.5 Cardiovascular pathophysiology in CKD

1.5.1 Severe atherosclerosis and premature coronary artery disease

Atherosclerosis pathology

Atherosclerosis is an inflammatory disease of large and medium sized arteries which occurs in response to endothelial injury from a wide range of factors such as hypertension, hypercholesterolemia and cigarette smoking.[49-51] Although it can occur in any artery it tends to predominantly effect areas of high blood flow which are susceptible to mechanical stress and shear.[49] It is a disease of the intima, patchy in distribution and interspersed with areas of plaque.[50] It is highly prevalent in the asymptomatic general population in over 15% of subjects less than 20 years old and over 80% in those over 50 years old.[52]

In the earliest stages 'foam cells' otherwise known as cholesterol rich macrophages accumulate in the subendothelium, widely described as the fatty streak.[49] Over time with further risk factor exposure, advanced lesions develop in which both lipid and smooth muscle cells accumulate to eventually form a necrotic lipid core and a fibrosis cap.[49] It is typically the rupture of the cap which promotes thrombus and occlusion of the artery although thrombus can also occur on areas of plaque, both mechanisms can cause myocardial infarction.[48, 49] This manifests clinically as an acute disruption to blood flow but the clinical picture depends on the location of the artery involved.[49]

Prevalence of atherosclerosis in CKD

Shortly after the introduction of the world's first haemodialysis unit in 1960 in Seattle, Lindner et al. reported a disproportionate number of deaths in the 39 patients who had commenced regular dialysis treatment.[53] Mortality was high at over 50% and the mean survival was only 6.5 years.[53] Over half of the deaths observed were secondary to accelerated atherosclerosis, defined as myocardial infarction or stroke.[53] The authors concluded that the atherosclerotic process had been 'accelerated'.[53]

The accelerated process of atherosclerosis is not just limited to those on dialysis.[54] Using data sets from 15,350 subjects aged 45-54yrs in the ARIC study Manjunath et al. observed that GFR was an independent risk factor for atherosclerotic disease even after adjustment for traditional risk factors.[54] The risk of new onset atherosclerotic cardiovascular disease increased by an adjusted HR of 1.05 (95% CI 1.02, 1.09) for every 10ml/min/1.73m^2 decline in GFR thus increasing in a linear fashion with declining GFR.[54] Over a mean follow up of 6.2 years there were significantly greater rates of both new and recurrent atherosclerosis disease with declining GFR.[54]

Carotid intima-media thickness (CIMT) is considered a useful non-invasive surrogate marker of atherosclerosis by the assessment of vascular remodelling using ultrasound.[55] It is considered a general assessment of atherosclerosis.[56] It has been shown to correlate with major traditional risk factors, coronary artery disease and also enhance prediction of adverse cardiovascular events using the Framingham risk score.[57] In a study of 203 participants with stages 3 to 4 CKD, Szeto et al. found that CIMT is a robust prognosticator of future cardiovascular events.[58] Nearly 60% had atherosclerotic plaques present on ultrasonography.[58] Each higher quartile of CIMT increased the risk of subsequent cardiovascular events by 40%.[58]

Pathophysiology of atherosclerosis in CKD

The high rate of cardiac mortality and observations of accelerated atherosclerotic disease has led to several studies directly observing coronary arteries in those with CKD.[59] Whilst atherosclerosis in the general population primarily effects the intima there is a greater degree of media thickness and calcification seen in those with CKD although both do occur.[55]

In a randomly selected cohort of 126 autopsy samples Nakano et al. found the frequency of severe atherosclerosis (as determined by the American Heart Association classification system) increased with declining GFR.[59] This relationship persisted even after adjustment for

confounders, so much so that those with stage 4 CKD were at almost twice the risk of advanced atherosclerotic lesions [odds ratio 3.02 (95% CI 1.22, 7.49)] than those with stage 3a CKD [odds ratio of 1.40 (95% CI 0.76, 2.55).[59] Although this study lacked a comparator group it suggests the severity of atherosclerosis increases as renal function deteriorates.[59]

Clinical presentation of atherosclerosis in CKD

In addition to morphological differences, the clinical presentation of coronary artery disease in CKD is also atypical and their presentation has often been described as oligo-symptomatic.[48] Recognition and diagnosis requires a high level of clinical acumen and investigation of more vague symptoms such as fatigue or shortness of breath.[48] For example, in cases of acute myocardial infarction those with CKD are much less likely to describe chest, arm, shoulder or neck pain and more likely to report shortness of breath [odds ratio 1.35 (95% CI 1.13, 1.62)] in contrast to those without CKD.[60]

Furthermore, patients with CKD can have advanced coronary artery disease and be completely asymptomatic. In a study of 30 asymptomatic patients commencing haemodialysis over half had significant coronary artery disease (defined as >50% stenosis on coronary angiography) despite being completely asymptomatic. [61] Within our research group a 30 year old with known stable CKD and hypertension underwent a cardiac magnetic resonance (CMR) as part of a research study.[1] The presence of focal subendocardial late gadolinium enhancement was seen on the scan eluding to potential underlying coronary artery disease.[1] A computerised tomography (CT) coronary angiogram revealed no flow limiting lesions, however, he had a significantly elevated Agatston score for someone of his age indicating the presence of premature coronary atherosclerosis, see **Figure 1.4**.[1]

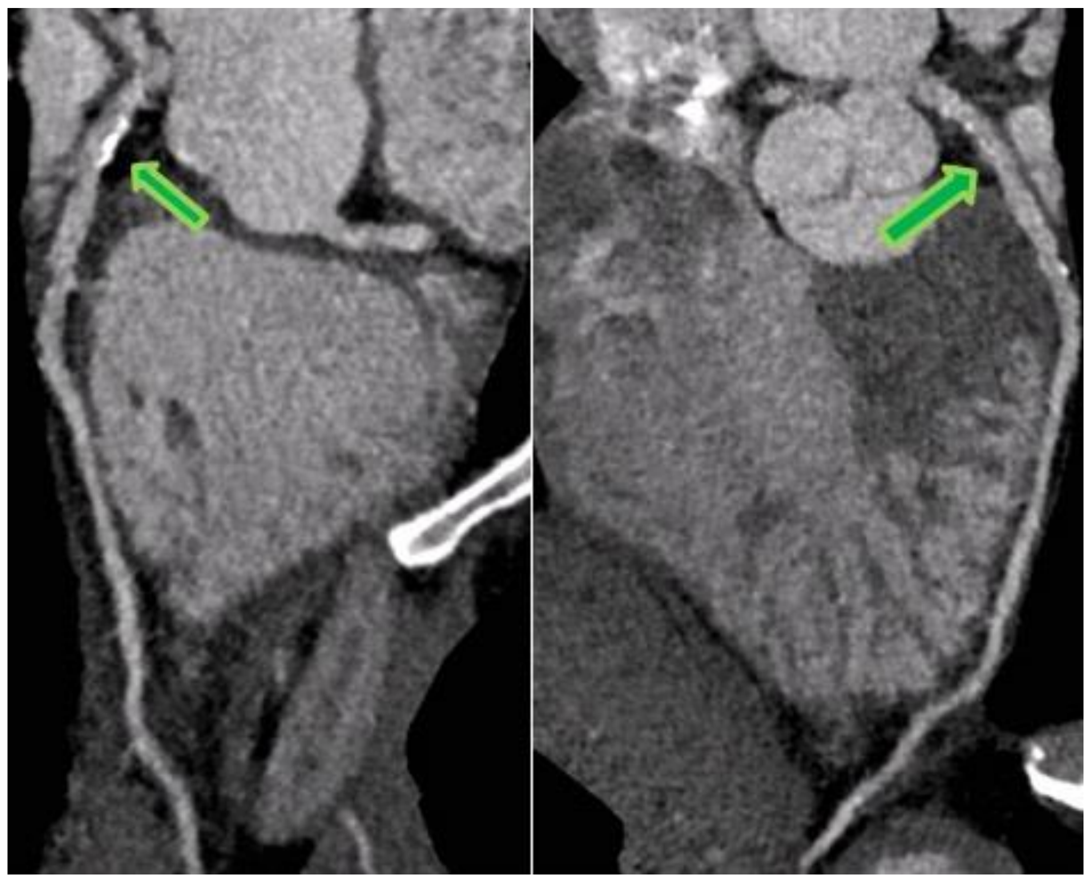


Figure 1.4. Mild diffuse coronary atheroma with moderate coronary calcification (Agatston score: 112) on CT coronary angiogram in an asymptomatic patient with stage 3 CKD.

CT coronary angiogram images with green arrows indicating areas of coronary calcification in the left anterior descending coronary artery. Although coronary CT angiogram adequately detects calcification it cannot determine whether there is intimal calcification or the medial calcification typical of CKD.[55]

CT; computerised tomography. CKD; chronic kidney disease.

1.5.2 Progressive arteriosclerosis and haemodynamic consequences of a stiff vascular system

Arteriosclerosis pathology

In addition to atherosclerotic disease, CKD is also characterised by a second structural vascular pathology, arteriosclerosis.[62] Arteriosclerosis is the result of vascular remodelling which is non-occlusive, does not encroach into the lumen, and unlike atherosclerosis, is more widespread, including many peripheral arteries typically unaffected by atheroma.[50, 63]

The arterial wall is composed of a combination of collagen fibres, elastic lamellae, connective tissue, smooth muscle and fibroblasts.[50, 64, 65] Central arteries have the highest elastin to collagen content in comparison to peripheral, smaller arterioles which have higher collagen and smooth muscle content.[50, 64, 65] Collagen is deposited and degraded in a response to environmental and mechanical stressors.[64] Vascular remodelling is an adaptive response to direct injury and haemodynamic stressors causing shear and tensile stress to the endothelium.[50] The endothelium therefore acts as a mechanical sensor which may instigate remodelling.[50] Whilst any temporary stressors may lead to a reversible change in vasomotor tone, chronic stress will cause responses within the arterial wall itself (structural stiffness).[50]

While atherosclerosis is a disease of the intima, arteriosclerosis predominately effects the medial layer of the artery.[62, 63] It is indicated by the presence of medial thickening, widespread calcification, increases in collagen content and both hyperplasia and hypertrophy of vascular smooth muscle cells.[62] The rise in calcification and collagen content of the arterial wall ultimately leads to increased vascular stiffness.[50] Early pathological studies of atherosclerotic disease in those with CKD all describe medial thickening and therefore are likely to be describing different processes, namely arteriosclerosis. For example, the histological examination of coronary arteries in 27 ESRD patients and 27 age and gender matched controls without renal dysfunction but known coronary disease demonstrated

significant morphological differences between groups.[66] The media thickness was significantly greater in ESRD compared to the control group (187±53µm vs. 135±29 µm) and there were significantly more calcified plaques.[66] In comparison, the control group had predominantly fibroatheromatous plaques.[66] Arguably the presence of medial thickening is describing arteriosclerosis here rather than atherosclerosis.

An increase in arterial stiffness is associated with a number of unfavourable functional consequences for target organs.[62] There are two main physiological explanations for this.[62] The first is the 'cushioning effect' concept or Windkessel model which suggests arteries are intended to be distensible to mediate and buffer any changes in blood pressure.[62] Elastic recoil within the aorta leads to approximately 50% of stroke volume (forward flow) and is stored fleetingly within the aorta (cushion) whilst the remaining blood flow is distributed to peripheral tissues.[67] During diastole the remaining blood within the aorta is then dissipated peripherally as a result of discharged energy from the arterial wall.[67] This is principally to ensure that there is a continual, constant blood flow being delivered to target organs and they are not subject to peaks in pressure.[62] The lack of compliance and thus 'cushion' in the vascular system therefore has two notable implications.[50] Firstly, the pressure ejected from the left ventricle (LV) is higher (leading to an augmentation in systolic blood pressure) as the rigidity within the aorta is less able to accommodate ejected blood and secondly, the diastolic pressure will be comparably lower as there is less vascular recoil and energy to dissipate during relaxation.[50, 63]

Another widely cited explanation for the effects of arterial stiffness is that of arterial wave propagation and reflection.[67] In healthy compliant arteries the forward wave, represented by ejected flow from the ventricle, propagates slowly down to peripheries.[50, 67] At bifurcations in the vascular tree, waves are reflected back toward the aorta and represent backwards flow.[62, 67] In health, the backward wave propagates slowly and returns to the aorta in diastole,

augmenting diastolic pressure, see **Figure 1.5**.[62, 67] By contrast, in a stiffer vascular system it is proposed that forward waves travel rapidly and reflected waves thus return to the aorta earlier.[67] The earlier return of the reflected wave therefore augments systolic pressure and decreases diastolic pressure.[67] The resultant combination is an increase in ventricular afterload and elevated central blood pressure. [67]

There is now an abundance of evidence indicating that the wave propagation and reflection theory is less likely.[68] Using physiological techniques to, in essence, separate reflected waves from reservoir pressure (stored blood and energy within the aorta) Wang et al. indicated the effect of reflected waves contributed very little to aortic pressure.[68] Central aortic pressure was the result of ejected flow and reservoir pressure.[68] In a large meta-analysis of over 60 studies of participants aged 4 to 9yrs Baksi et al. found that all reflection times were well within systole.[69] Younger participants did have later wave reflections on the whole but for this to return in diastole the authors estimated that the participant would need to have been 200yrs old![69] Physiologically the wave reflection theory has, therefore been subject to serious doubt although it continues to be heavily cited in recent literature.[67]

Nonetheless, irrespective of the underlying mechanisms progressive arterial stiffness limits the ability of the vascular system to regulate blood pressure downstream.[63] The largest organs, notably the brain, heart and kidneys are the most vulnerable as their anatomy is such that they are supplied by short large arterial branches from the aorta. Consequently, there is little time for cushioning and target organs are subject to oscillations in blood pressure which contribute to microvascular organ damage. [63] The resultant clinical picture is that of high central systolic blood pressure, low central diastolic blood pressure and an increase in pulse pressure, all surrogate markers of arterial stiffness.[50]

The effect on target organs is evident as small vessel disease within the brain and kidney but also leads to damage to the heart. A reduction in central diastolic blood pressure leads to a decrease in coronary perfusion pressure and is associated with myocardial ischaemia.[62, 70] As a consequence, perfusion is heavily dependent on systolic coronary perfusion, increasing sensitivity of the heart to subtle changes in systolic function and mean arterial pressure.[71] An elevation in central systolic blood pressure also increases ventricular work and is associated with LVH and fibrosis.[67]

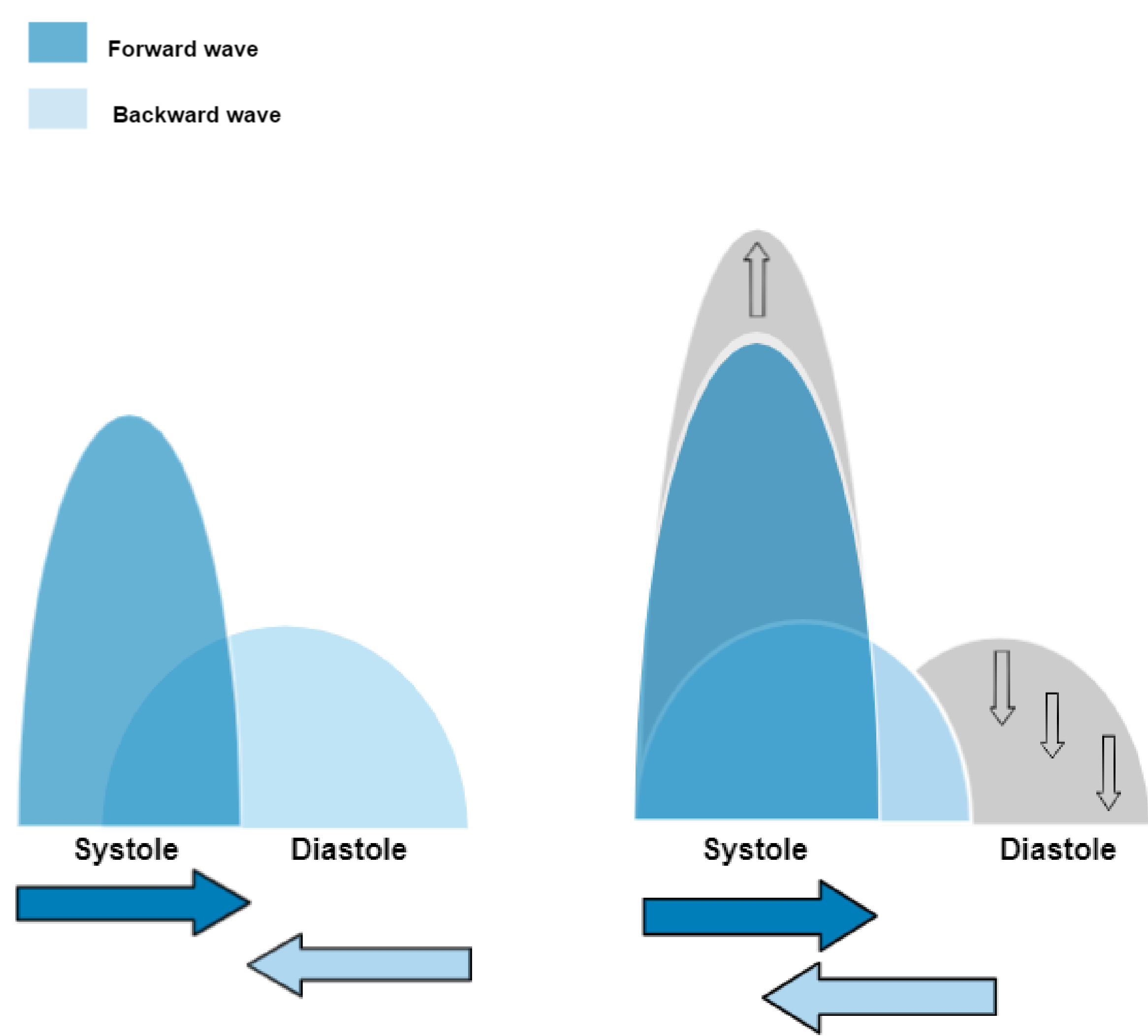


Figure 1.5. Diagram illustrating the wave reflection theory of arterial stiffness.

Adapted from Zanoli et al.[67]

The wave reflection theory is one explanation for the observation of an elevated systolic blood pressure and a subsequent decline in diastolic pressure associated with arterial stiffness. Waves in dark blue are forward waves whilst light blue waves are backwards waves. In young elastic arteries (seen on the left) the wave reflected back arrives slowly and augments diastole. As arteries stiffen (see on the right) the reflected wave returns earlier augmenting systole whilst resulting in a drop in diastole and is a pattern typically seen in older, stiffer arteries. Grey waves indicate the change to blood pressure as arteries become stiffer.

Pulse wave velocity and pulse wave velocity to determine arterial stiffness

Non-invasive measures of arterial stiffness are based on calculations of vessel distensibility and pulse wave velocity (PWV)-the speed at which blood travels along the arterial wall.[64] Both calculations are based on the 1922 Bramwell-Hill model which uses the cross sectional area of the vessel and the density of blood to determine the speed blood at which is transmitted along the artery, see below.[64]

$$D = \frac{1}{A}\frac{\partial A}{\partial P} \qquad PWV = \sqrt{\frac{A}{p}\frac{\partial P}{\partial A}} = \sqrt{\frac{1}{pD}}$$

D: Distensibility, PWV: Pulse wave velocity, P: Pressure, A: Luminal area, p: Blood density

Therefore, distensibility and PWV are linked, a decline in vessel distensibility results in a square root increase in PWV.[64] PWV also increases with a rise in pressure or a reduction in the cross-sectional area of the lumen.[64] The relationship between area and pressure is non-linear due to the mechanical response of the arterial wall (recruitment of collagen fibres) to increases in pressure which, in effect, produces a functional stiffening.[64] Whilst elastin responds to low pressures, collagen, which is less distensible, responds at the highest pressures to stiffen the arterial wall.[50] It is a protective response to prevent pressure induced damage.[50] As a consequence, PWV is pressure dependent and thus normally determined at diastole.[64] Whilst PWV is often used interchangeably with arterial stiffness the two are not the same.[64] PWV is determined using techniques in clinical practice to determine time and distance of blood flow, see below:[64]

$$PWV = \frac{\Delta\, Distance\ (between\ two\ anatomical\ sites)}{\Delta\, Time\ (of\ an\ arterial\ pulse\ to\ travel\ from\ the\ proximal\ to\ distal\ site)}$$

Distance and time can be determined using magnetic resonance techniques or using transit time methods.[64] Transit time methods measure the distance between two superficial arterial

sites (e.g. carotid-femoral). Determination of the pulse wave with an ultrasound in conjunction with electrocardiogram gating can then infer time.[64]

Arterial stiffness in CKD

Arterial stiffness has strong associations with hypertension, diabetes, advancing age and atherosclerosis, conditions which often occur alongside renal disease.[72] In 2018 the European Society of Cardiology and the European Society of Hypertension concluded that PWV could be used to risk stratify patients with levels over 10m/s due to its strong association with adverse cardiovascular outcome.[73] London and colleagues were, however, the first group to identify PWV as an independent predictor of mortality in those with ESRD.[72, 74] In a study of over 200 participants with ESRD (participants were assessed over a period of 6 years) PWV was a stronger independent predictor of mortality than either age or dialysis vintage.[72] Using a PWV of <9.4m/s for reference the odds ratio was over 5 fold for both all-cause and cardiovascular mortality even after adjustment for confounding variables for those with a PWV>12m/s.[72]

The increase in mortality is particularly compelling where PWV is not reversed by blood pressure reduction.[74] In those with ESRD with adequate control of blood pressure, cardiovascular mortality was significantly elevated in those who failed to achieve a corresponding decline in PWV [HR 2.35 (95% CI 1.51, 4.43)] despite a fall in blood pressure.[74] This suggests that a persistent raised PWV is evident of non-reversible structural changes to the cardiovascular system.[74] Whereas in contrast, an initial reversible ‘functional’ arterial stiffness is likely to reflect a nonlinear relationship between volume and pressure on the arterial wall as a result of transient changes in blood pressure.[75]

In 2010 a systematic review and meta-analysis of over 17 studies (general population, those with ESRD and those with hypertension) of more than 15,000 participants demonstrated that there was a stepwise increase in the pooled relative risk of mortality with corresponding

increases in PWV.[76] The relationship was linear and determined that for every 1m/s increase in PWV there was a 14% increase in cardiovascular events and 15% increased risk of all-cause and cardiovascular mortality.[76] In addition, in a sensitivity analysis, those with ESRD and those with hypertension had a greater pooled relative risk of total cardiovascular events compared to the general population, [RR 2.81 (95% 1.97, 4.02)] vs. [RR 2.46 (95% 1.93, 3.13)] vs [RR 1.68 (95% 1.45, 1.96)] respectively.[76]

Increases in PWV appear to begin in the earlier stages of CKD.[77] In a study of 102 patients with stage 1-5 CKD and an age matched control group there was a significant upwards trend in PWV from stage 3 onwards.[77] In multivariate analysis both a declining GFR and increasing systolic blood pressure were independently associated with a higher PWV.[77] Other evidence suggests changes can be identified as early as stage 2.[78] In a cross sectional study of 117 patients with stage 2-3 CKD Edwards et al. found that aortic distensibility was significantly higher than that in 40 healthy controls.[78] Aortic distensibility was also positively correlated with GFR.[78] Changes in arterial stiffness in those with early stage CKD have also been linked to higher mortality.[79] In a study of 134 patients with stage 2-4 CKD, PVW remained an independent predictor of mortality with a 5 fold increased risk for those with a PWV over 10m/s.[79]

1.5.3 Uraemic cardiomyopathy

In 1836 Bright was the first to publish a series of case studies detailing the severe cardiac abnormalities observed at autopsy in those with ESRD.[26] In wasn't until 1967, however, when Bailey et al. reported several case studies of severe biventricular heart failure in those with critical uraemia that the term 'cardiomyopathy' was first used in the medical literature in conjunction with ESRD.[80]

In the 1970s and 1980s several reports were published using the term 'uraemic cardiomyopathy' to describe the changes in cardiac structure and function associated with renal dysfunction.[34, 81-84] This terminology began to occur with the advent of more widespread use of dialysis and was usually used to describe structural and functional changes such as cardiomegaly, severe systolic dysfunction and pericarditis in those receiving haemodialysis.[83, 85, 86]

Abnormalities in cardiac structure in ESRD

In 1995 Foley et al. studied 433 patients commencing haemodialysis and found that over 70% had evidence of LVH on echocardiography.[87] Over a third of participants had dilated left ventricles and 15% already had evidence of systolic dysfunction measured by echocardiography and defined as fractional shortening less than 25%.[87] Both ventricular dilatation and hypertrophy were independently associated with mortality at two years.[87] A further study of the same cohort revealed that even after adjusting for confounding variables (diabetes, age and sex) a lower mass to volume ratio and larger ventricular cavity volume was associated with an increased risk of mortality after starting dialysis.[81] The high reported incidence of LVH and associated mortality in ESRD has also been corroborated in other larger echocardiography studies.[88] Using data from the Dialysis Morbidity and Mortality Study, Stack et al. reviewed the echocardiography results of 2584 participants and found the mortality risk was greatest for those with LVH within the first 6 months of commencing

dialysis [relative risk 1.61 (95% 1.17, 2.22)] suggesting that the presence of LVH was associated with shorter term survival.[88]

The adverse association between LVH and survival appears to be independent of traditional cardiovascular risk factors and tends to progress over time.[36] In a prospective study of 161 haemodialysis patients undergoing two echocardiograms (18 months apart) a significant increase in LV mass was associated with a 62% increased risk of major cardiovascular events even after accounting for traditional risk factors such as diabetes, age and smoking history.[89] Similarly, Foley et al. reviewed 596 patients starting haemodialysis who were asymptomatic of significant cardiac disease.[36] Substantial increases in LV mass were seen at follow up by a mean of 14g/m^2 over a 22 month period.[36] LVH in this cohort was concentric, progressive and associated with hypokinesis.[36]

Although the use of echocardiography is accessible, quick and reliable its use leads to an overestimation of LV mass compared to cardiovascular magnetic resonance (CMR), particularly in those with ESRD.[90] In a study of 134 patients receiving haemodialysis who underwent CMR, however, the prevalence of LVH was still over 70% and 11.2% had significant LV dilation.[91] This suggests that structural abnormalities in those with ESRD are widespread and severe.

Clinical importance of LVH

While LVH can be an adaptive physiological response to exercise training, particularly endurance training, which may have beneficial effects on cardiac output, mediate increased wall stress and increase oxygen consumption it should not be confused with pathological hypertrophy.[92-94] LVH in response to long term loading changes has detrimental consequences and is a pathological maladaptive response.[92] Firstly, LV compliance is reduced, therefore a stiffer ventricle is less likely to cope with change in LV filling pressures.[92] Any abrupt changes in LV filling may lead to pulmonary oedema or hypotension

and syncope.[92] This is frequently seen in clinical scenarios during the haemodynamic stresses of haemodialysis.[92] Secondly, even in the absence of significant coronary stenosis, patients may experience angina pectoris as a result of under perfusion of the myocardium due to an increased vascular resistance.[92] This is further exacerbated by a reduction in capillary density and increase in myocyte hypertrophy.[92] Finally, LVH is associated with arrhythmia.[92] In the Convective Transport Study, a study of echocardiography results and clinical outcomes of 327 patients with ESRD, those with the highest tertile of LV mass had a 13 fold increased risk of sudden cardiac death.[95] This risk of all-cause [HR (95% CI 1.11, 2.9)] and cardiovascular mortality [HR 3.66 95%CI 1.35-10.05)] was also increased in those with the highest mass.[95] Whilst initially cardiac function may be conserved there is eventual decompensation resulting in ventricular dysfunction (diastolic and systolic) and heart failure.[96]

Left ventricular mass as an outcome measure

LV mass is a strong independent predictor of all-cause mortality and cardiovascular events in the general population and in CKD.[89, 97] It is the strongest predictor of cardiovascular risk after age.[98] The association between LV mass and outcomes is also highly consistent.[98, 99] Furthermore, LV mass regression in the case of anti-hypertensive use and renal transplantation is associated with reduction in the risk of cardiovascular disease.[100, 101] LV mass has been widely used in randomised trials in CKD as a surrogate end point for cardiovascular mortality.[101-103]

Myocardial tissue abnormalities

In addition to the macroscopic changes in cardiovascular structure (LVH), structural changes have also been demonstrated at a microscopic level within myocardial tissue.[104, 105]

Normal myocardial tissue

Normal myocardial tissue is made up of a cellular component (cardiomyocytes, fibroblasts, vascular smooth muscles cells, macrophages) and a non-cellular component.[106] The non-

cellular component is the extracellular matrix (ECM) and is not only responsible for the physical scaffolding of cells but also maintenance of tissue through cell-ECM interactions.[107] The ECM is made up of predominantly water, proteins and polysaccharides although the exact make up varies from tissue to tissue.[107] In cardiac tissue the ECM is mainly fibrillary collagen type 1 and collagen type 2 and has important tensile properties within the heart.[106] Fibroblasts are responsible for the synthesis of collagen within the myocardium.[106] Although in many other organs the ECM can regenerate and repair, the heart has a limited ability to repair effectively. [106] Even the smallest injuries to the heart can lead to an overwhelming cascade of events leading to the activation of cardiac fibroblasts and ECM remodelling (increased accumulation of proteins within the interstitium, mainly collagen 1).[106, 108, 109]

Cardiac fibrosis

Cardiac fibrosis can be defined as a reactive pathological response to injury characterised by ECM deposition and remodelling which is detrimental to cardiac function.[106, 108] Irrespective of the underlying injury, cardiac fibrosis causes ECM stiffness which ultimately leads to cardiac dysfurnction.[110] There are three main patterns of cardiac fibrosis observed: reactive (e.g. accumulation of ECM without myocyte loss), infiltrative (e.g. glycolipid build up seen in Fabry disease) and finally replacement fibrosis (e.g. myocardial infarction as a result of loss/ damage to myocytes and replacement fibrosis).[106] The pattern of cardiac fibrosis is highly variable between cardiac pathologies but can be broadly categorised as interstitial (collagen between myocytes), compact (large areas of collagen without myocytes), diffuse (short fibres of collagen) and patchy (long fibres of collagen).[106, 108]

Cardiac fibrosis in ESRD

Animal studies have indicated that the observed increases in LV mass in those with ESRD are associated with parallel increases in cardiac fibrosis.[111] In a study of male Sprague-Dawley rats, Mall et al. induced uraemia by total nephrectomy of the left kidney and subtotal nephrectomy of the right kidney (a model of ESRD).[111] After 21 days of uraemia there was a

significant increase in total heart weight in uraemic rats compared to controls (1040 ±73 mg vs. 871± 81mg) which was associated with a corresponding increase in volume of the interstitium and deposition of collagen.[111] Although there was a significant increase in the non-cellular component, changes to the interstitial cell nuclei and cytoplasm also suggest that the cells responsible for maintaining the ECM were activated.[111] In addition the findings were not reversed by the treatment of hypertension nor reproduced in hypertensive controls and thus appear to be a consequence of renal dysfunction independent of blood pressure.[111]

In a study of human LV samples, Mall et al. attempted to repeat the findings seen in rats by specifically selecting patients with both pre-dialysis and dialysis dependent CKD.[105] Those with known coronary artery disease were excluded.[105] Over 90% of subjects had evidence of diffuse 'intermyocardiocytic' fibrosis with extensive collagen deposition after assessment by two independent observers.[105] Histological severity was scored based on myocyte hypertrophy and excessive collagen in the intersitium.[105] Fibrosis was not evident in the 50 non-hypertensive and non-diabetic 'controls' recruited for comparison.[105] Moreover, the severity of histological findings related to duration of dialysis, remained persistent even after transplantation and was independent of hypertension and diabetes on regression analysis.[105] The authors concluded that the pattern of cardiac fibrosis seen in ESRD, a diffuse interstitial pattern, differs from patterns typically seen in hypertension (perivascular fibrosis) and coronary artery disease (patchy scars).[105] In addition, the fibrosis only affected the heart, leaving the other organs largely unscathed.[105]

To further investigate the histological findings in ESRD, Amann et al. examined the hearts of 9 patients on dialysis, 9 with essential hypertension and 10 healthy controls.[112] Those on dialysis, had a significant increased density of myocardial interstitial tissue and myocyte size while the density of capillaries decreased compared to both controls and those with hypertension see **Figure 1.6.** These findings led the authors to theorise that cardiomyocytes

in those with CKD were exposed to hypoxia as capillary growth did not match that of cell hypertrophy.[112]

Cardiac fibrosis does not only have a detrimental effect at a cellular level.[113] Diffuse fibrosis and subsequent accumulation of collagen may act as a substrate for the induction of re-entry arrhythmia, heart failure, myocardial dysfunction and sudden cardiac death.[104, 113] Over a period of 8 years, Aoki et al. reviewed all those who had coronary angiography at their centre in Japan and identified 286 on haemodialysis.[104] Of these, 40 patients with a LV ejection fraction <50%, LV end diastolic volume >90ml/m^2 and without coronary artery disease were selected and compared to 50 patients with idiopathic dilated cardiomyopathy (DCM) without renal disease.[104] Those on haemodialysis demonstrated hypertrophic myocytes, myocyte disarray and irregular nuclei.[104] There was a marked difference in myocyte hypertrophy (37.6 ± 10µm in the haemodialysis group vs. 25.6 ± 7.7µm in the group with idiopathic DCM) and extensive interstitial fibrosis in those on haemodialysis.[104] Furthermore, during follow up those with the most severe fibrosis were at highest risk of cardiac death with several patients dying of ventricular arrhythmia or sudden cardiac death.[104] On multivariable analysis using cox proportional hazards, only percentage area of LV fibrosis was a predictor of mortality even after accounting for hypertension, diabetes, ejection fraction and New York Heart Association score.[104] The authors concluded that the hearts of those on haemodialysis were comparable to those with dilated phase hypertrophic cardiomyopathy.[104]

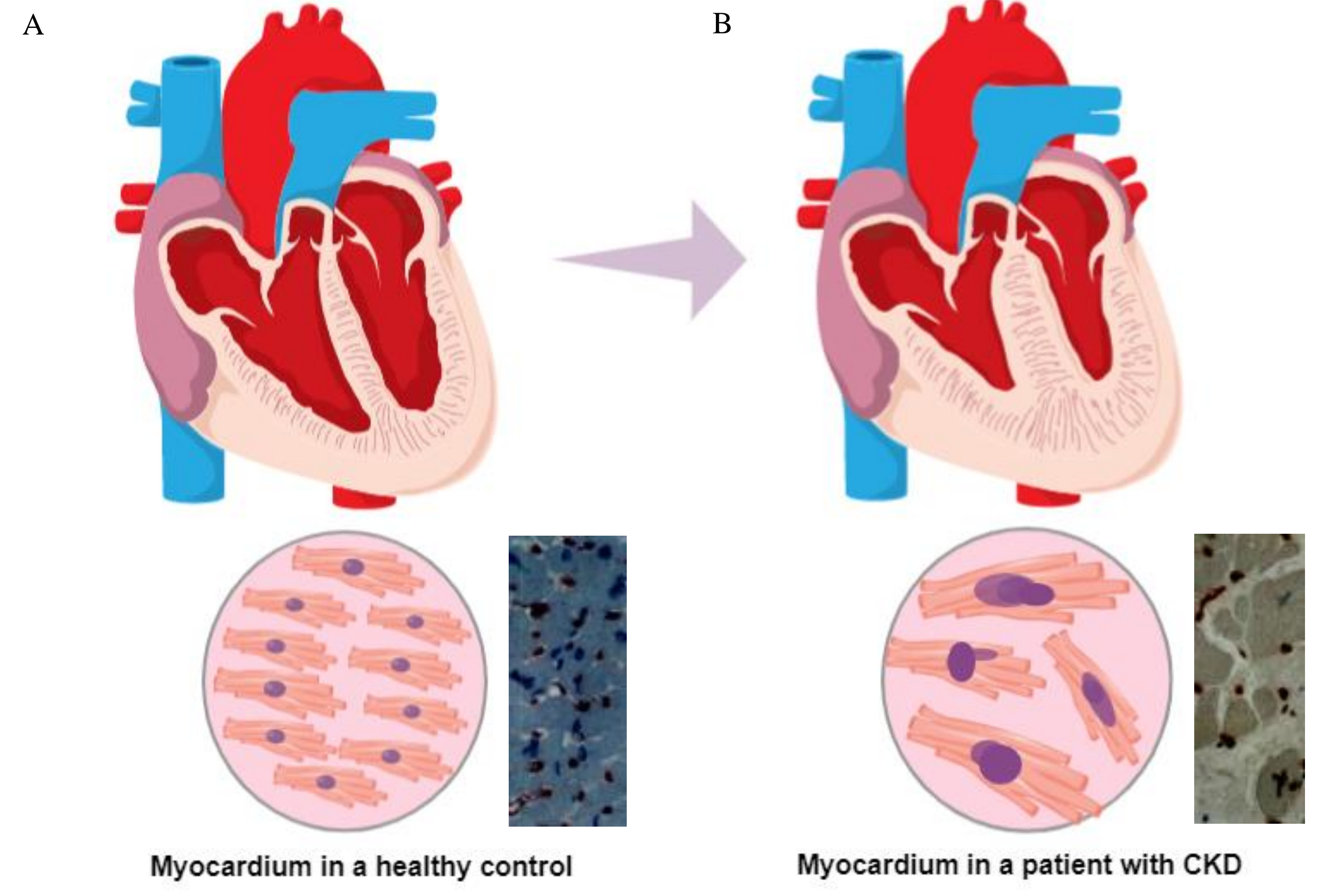


Figure 1.6. Comparison of the myocardium in a healthy control and patient with CKD
Adapted from Hinderer et al.[106] The histology examples are reproduced with permission from Amann et al.[112]

A. Example of normal myocardium.
B. In those with CKD myocytes are hypertrophied, in disarray with unusual shaped nuclei.[104, 112] In addition the density of capillaries is reduced with a notable increase in interstitial space with lack of organisation of fibres.[104, 112]

Utilisation of advanced CMR for detecting cardiac fibrosis

Although cardiac biopsy and histological assessment is not always possible in subjects with CKD novel cardiac imaging techniques have given us further insight into uraemic cardiomyopathy. One of the many benefits of magnetic resonance imaging (MRI) is soft tissue characterisation.[114] Determination of soft tissue character in MRI employs the measurement of T1 relaxation time, in other words, the time in which it takes protons within tissue to return to equilibrium following excitation by a radiofrequency pulse.[114] This is often termed longitudinal relaxation time or spine-lattice relaxation time because spin magnetisation is being measured within the same direction of the scanner.[115] The radiofrequency pulse generated by the scanner acts to invert the magnetisation.[114] T1 relaxes in an exponential manner to equilibrium and the rate of recovery is dependent on the tissue properties and surrounding molecules.[114] T1 relaxation can thus act as a biomarker of tissue pathology which can be enhanced using contrast and encoded to generate visual representations of myocardium.[114, 115]

Late gadolinium enhancement techniques

Since the introduction of gadolinium based contrasts agents in the mid-1980s, contrast based MRI has revolutionised the detection of regional abnormalities in myocardium.[116] Gadolinium is a strong paramagnetic element, toxic in the unbound form, it is always chelated with a large molecular agent to prevent entrance into the intracellular space.[117] In health, gadolinium remains in the extracellular space.[117] In pathological conditions (acute or chronic) where the extracellular space is disrupted for example due to excess collagen deposition or cell membrane rupture, gadolinium accumulates in the extracellular space.[117] It is one of the few elements magnetised at room temperature and shortens T1 time.[117] When used with an inversion recovery principle to null any signal from normal myocardium, areas with gadolinium appear bright white in contrast to the black normal myocardium where gadolinium has washed out (where T1 time is longer).[115, 117] T1 relaxation can, in effect, be

visualised by coding the times according to pixel intensity after the use of contrast.[114] This differentiation is known as T1 weighting which in principle allows the detection of regional, focal areas of fibrosis where there is clear differentiation between normal and fibrotic tissue and is termed gadolinium enhancement.[114, 117] ‘Late’ gadolinium enhancement (LGE) refers to the time in which sequences are obtained after contrast administration usually >10 minutes (as opposed to early, 1-3 minutes) and is the time in which gadolinium has usually washed out of any normal myocardium.[118]

Furthermore, in other cardiac pathology the presence of LGE has been demonstrated to be a useful risk stratification tool with strong associations with prognosis.[119] In a meta-analysis of 9 studies of patients with LGE there was an increased risk of all-cause mortality [odds ratio 3.7], hospitalisation secondary to heart failure [odds ratio 2.91] and a profound increased risk of sudden cardiac death [odds ratio 5.32].[119] The distinct patterns of LGE observed have been used for diagnostic purposes in hypertrophic cardiomyopathy, myocardial infarction, and myocarditis and is of particular use in distinguishing between ischaemic and non-ischaemic aeitologies.[115, 120] In addition, areas of LGE have been associated with corresponding areas of myocardial collagen deposition on histology.[121]

Late gadolinium enhancement in ESRD

In 2006, Mark et al. used LGE techniques to assess cardiac fibrosis patterns in ESRD.[91] In a study of over 130 patients two main patterns of LGE were observed.[91] The first was that of subendocardial LGE, observed in 14%, a pattern typical of ischaemic pathologies indicating poor perfusion of the subendocardium.[91] This pattern of LGE was also associated with traditional atherosclerotic risk factors such as diabetes and hypercholesterolemia, poorer systolic function and LV dilatation.[91] Of more interest was the second pattern observed, largely unexpected, which the authors termed ‘diffuse LGE’, seen in 14% and was associated with greater LV mass than those without LGE.[91] This cohort were patients undergoing work

up for transplantation and consequently there may have been selection bias but, nevertheless, it was the first study of its kind using MRI to suggest that cardiac fibrosis may be present in the hearts of those with CKD.[91] The authors suggested that whilst systolic dysfunction and LV dilation may be related to ischaemic patterns of LGE, diffuse LGE is associated with greater mass and could indicate a specific uraemic cardiomyopathy.[91] The association between LGE and LV mass provides support for the paradigm that the LVH observed in renal disease is pathological.[113]

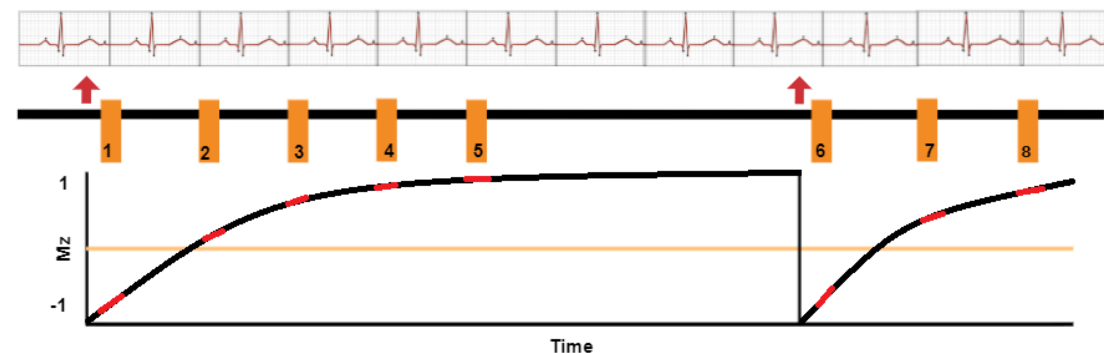
Although LGE techniques have been used widely in other cardiac pathology, shortly after the publication by Mark et al. Nephrogenic Systemic Fibrosis (NSF) in relation to gadolinium based contrast agents (GBCA) was reported in the literature.[122, 123] NSF is an incurable dermatological condition almost exclusively reported in those on dialysis or with very low renal function exposed to intravenous GBCA.[123] Even though it is extremely rare, NSF leads to aggressive skin induration, similar to scleroderma which is both disabling, incurable and associated with increased mortality due to organ involvement.[123, 124] Unsurprisingly, the use of GBCA was restricted by the Medicines and Healthcare Products Regulatory Agency and since 2012 has rarely been given to those with an GFR of $<30 ml/min/1.73 m^2$ unless the diagnostic benefits outweighs the risk.[3] Gadolinium use in this cohort would be unethical for research purposes and therefore there is very little further literature available for both patterns and prognostic implications of LGE in CKD.

T1 and T2 mapping techniques

Despite the restrictions of GBCA use in CKD, the development of novel advanced imaging techniques including T1 and T2 mapping have provided further insight into myocardial tissue characterisation.[125] T1 mapping has two distinct advantages over LGE, particularly for those with CKD.[119] Firstly, native T1 imaging does not require the use of contrast (ideal for ESRD) and secondly, whereas LGE is reliant on focal areas of fibrosis (with limited special

resolution for the detection of microscopic fibrosis) T1 mapping allows better characterisation of diffuse or interstitial fibrosis often seen in CKD.[115] In contrast to LGE techniques, T1 mapping techniques allow visualisation of each individual voxel as a pixel.[114] Each pixel is then encoded based on signal intensity to represent colour.[114, 115] This method generates a colour coded map for the entire myocardium based on absolute T1 time.[114, 115] This allows detailed microscopic pathology to be visualised.[115]

Current methods for T1 measurement require the acquisition of raw images at a variety of times following inversion, therefore images are generated at different inversion times (TI) giving different T1 weighted images.[115] The signal intensity from each T1 weighted image is subsequently fitted to the equation for T1 relaxation.[114] Techniques for image acquisition were initially proposed by Look and Locker in 1970 and modified to the Modified Look-Locker sequence (MOLLI) which is used widely although there are slight variations to the sequence.[126] MOLLI sequences generally acquire the data over successive heart beats (either 17 beats or 11 beats) in separate cardiac cycles but at the same cardiac phase during a single breath hold, see **Figure 1.7**.[127] The breath hold is critical for a good quality image to minimise respiratory motion, however, the images can be motion corrected to some extent to minimise artefact.[128]



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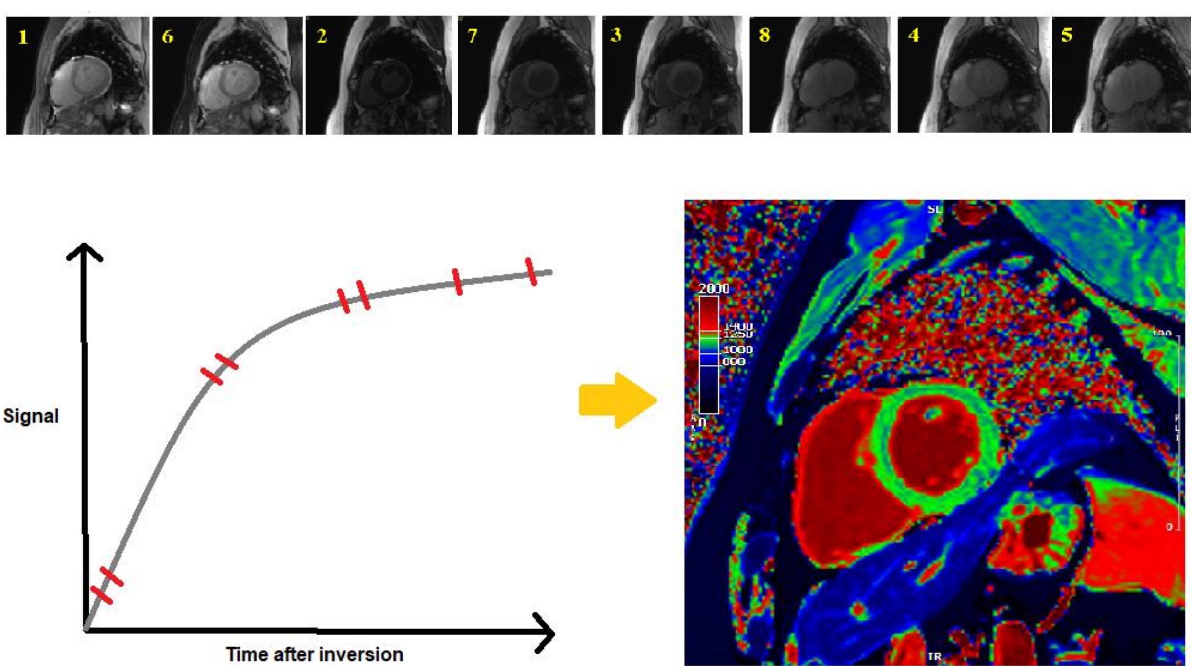

Figure 1.7. Modified Look-Locker sequence (MOLLI) T1 mapping.
Adapted from Taylor et al.[114]

A. An electrocardiogram tracing is seen at the top of the image, red arrows indicate the timing of the inversion pulses.[114] The graph below illustrates magnetisation (Mz) against inversion recovery time.[114] Magnetisation is inverted after a pulse.[114] In this sequence there is an inversion pulse and then 5 images (shown in orange squares) of the short axis stack are acquired in diastole over 5 successive heat beats at a variety of times after the inversion.[114] This is followed by a gap of three heart beats and then a second inversion pulse and a further 3 images acquired over the next 3 heart beats.[114] Therefore this is described as a 5(3)3 sampling scheme over 11 R-R intervals.[114]

B. The raw images acquired are shown in black and white with corresponding image numbers. All images are ordered according to inversion recovery time and then fitted to the T1 recovery curve (indicated by red lines on the graph on the left).[114] This is done in each pixel to generate a T1 map (shown on the right) based on the signal intensity in each pixel.[114]

Broadly, the main reasons for elevated native T1 times fall into two categorires.[115] An increase in oedema (i.e. secondary to inflammation) or changes in the interstitium due to infiltration (i.e. amyloid or collagen deposition).[115]

For further tissue characterisation the combination of using native T1 mapping and contrast allows the determination of extracellular volume (ECV).[115] The myocardium includes a cellular component, the interstitium and finally an intravascular component.[114] ECV is considered a measure of the interstitium and intravascular component in combination and changes are thought to predominantly reflect changes in the ECM. [114] Quantification of ECV requires native and post contrast T1 maps of both the myocardium and blood pool along with haematocrit taken at the time of the images which accounts for the cellular fraction of blood.[114, 115] ECV is defined as a volume fraction and calculated using the following equation.[114]

$$ECV = (1 - haematocrit) - \frac{\frac{1}{post\ contrast\ T1\ myo} - \frac{1}{native\ T1\ myo}}{\frac{1}{post\ contrast\ T1\ blood} - \frac{1}{native\ T1\ blood}}$$

The use of ECV and T1 mapping in combination provides a powerful diagnostic tool to determine myocardial tissue changes in a range of cardiac pathologies.[115] Native T1 times have been found to correlate with fibrosis quantified on biopsy in severe aortic stenosis are widely considered as a surrogate marker of myocardial fibrosis.[129, 130] ECV has also been shown to correlate with collagen volume fraction in those undergoing heart transplantation.[131] A combination of LGE, T1 and ECV is likely to give the best interpretation of fibrosis.[132]

In contrast to T1, T2 measures transverse relaxation time.[133] Unlike T1, which can be elevated due to oedema and/or deposition on the interstitium, T2 is only elevated in cases of myocardial oedema.[133] T2 may be an important differentiator for myocardial oedema in patients with CKD when used in combination with T1 mapping.[134]

T1 and ECV in ESRD

Two United Kingdom (UK) studies published in Kidney International in 2016 were the first to use native T1 mapping techniques in ESRD.[130, 135] Rutherford et al. studied 38 haemodialysis patients and 28 healthy controls matched for age and sex.[130] Participants underwent 3 tesla CMR including MOLLI T1 mapping.[130] Global and septal T1 were higher in haemodialysis patients compared to healthy controls.[130] Furthermore, T1 times correlated with LV mass without a significant difference in LV ejection fraction between the two groups.[130] T1 time in the septum was also related to both high sensitivity troponin T levels and corrected QT interval suggesting that native T1 times in ESRD may be indicative of diffuse interstitial myocardial fibrosis and have adverse arrhythmic effects proceeding any observed changes to systolic function.[130]

In a similar study design, Graham-Brown et al. studied 35 haemodialysis patients and compared them to 22 healthy controls.[135] Global T1 time was markedly higher in those on haemodialysis compared to the control group (1270ms vs. 1085ms) and in addition, septal times were significantly higher than non-septal times (1293 vs 1252ms).[135] Furthermore, global circumferential strain (GCS) and global longitudinal strain (GLS) were impaired in the haemodialysis cohort and correlated with longer T1 time.[135] This study suggested that interstitial fibrosis in ESRD may be asymmetrical, (predominantly effecting the septum) and that T1 time is related to changes in functional correlates.[135]

Despite this, although patients were scanned on interdialytic days in both of these studies, T2 times were not measured.[135] Therefore the effect of fluid status, which is highly relevant for those on haemodialysis, cannot be excluded as a component causing elevated T1 times.[135]

T1 and ECV as an outcome measure

T1 mapping and the use of ECV is emerging as a promising new outcome measure of myocardial fibrosis and a new surrogate biomarker of major cardiac pathology.[113] It has

recently been determined that in both healthy controls and CKD, T1 times are stable over time making it of particular interest for longitudinal assessment over and above the subjective visual assessment of LGE.[136] Furthermore, in those receiving haemodialysis, native T1 time has been demonstrated to have high inter-study, inter-observer and intra-observer reproducibillity.[137] Despite this, there is still a lack of histological confirmation specifically in those with CKD leaving its use subject to some criticism.[138]

Abnormalities in cardiac function in ESRD

In addition to the observed macroscopic and microscopic structural changes those with ESRD are also subject to major systolic and diastolic functional abnormalities. Recent studies have also suggested that GLS is a more sensitive predictor of cardiovascular and all-cause mortality than ejection fraction.[139] GLS is a measure of the longitudinal contractile ability of the heart. In a study of 187 participants with stage 4-5 CKD (including those on dialysis), a cox multivariate regression analysis revealed that GLS was a stronger predictor of cardiovascular mortality [HR 1.16 (95%1.04-1.30), p=0.008] compared to that of ejection fraction [HR 1.04 (0.99-1.05), p=0.08] even after adjustment for age, smoking history and LV mass.[139] For those with a preserved ejection fraction an impaired GLS carried a 5 fold risk of cardiovascular mortality [HR 5.59 (95% CI 1.23-25.30), p=0.03] indicating that early functional changes in ESRD are not benign.[139] A further study also indicated that systolic dysfunction may further progress as renal function declines. In the first longitudinal study of structural and functional change in CKD, Bansal et al. followed the Chronic Renal Insufficiency Cohort recruited between 2003 to 2008, up until 2011.[140] Participants with a GFR <20ml/min/1.73m^2 underwent serial echocardiography during their progression from advanced CKD to ESRD.[140] During follow up LV mass was high but remained relatively stable without further increases yet LV ejection fraction began to deteriorate.[140] This marks an important transition and suggests that whilst there is initial compensation, abnormalities in cardiac structure and function are likely to be an initial precursor to heart failure. [140]

As CKD progresses diastolic dysfunction also becomes evident.[141] Diastolic dysfunction is an impairment of relaxation and filling of the LV.[142] In a prospective observational study over 4 years, Farshid et al. studied 153 patients with stage 4-5 CKD who underwent echocardiography at two time points.[141] Diastolic dysfunction was reported in 85% of those studied in comparison to 22% who had an ejection fraction less than 55%.[141] Diastolic dysfunction but not ejection fraction was also found to be an independent predictor of all-cause mortality.[141]

Current definitions of heart failure are based on the 2016 European Society of Cardiology guidelines.[143] It is recognised, however, that there are no definitions of heart failure specifically for those with CKD and the pattern of heart failure may differ from other cardiac pathology.[144] As a consequence many CKD patients miss out on guideline directed care.[144]

Cardiac structural and functional changes in early stage CKD

Historically the majority of research studies have focused on the cardiovascular abnormalities of those with ESRD, however, significant LV abnormalities have also been shown to be inversely related to declining renal function demonstrated even in the earliest stages of CKD.[145]

In a cross sectional review of data from the Chronic Renal Impairment Cohort study, Park et al. assessed LVH using echocardiography in over 3000 participants with a wide range of GFRs.[145] Half of all participants had LVH and there was a progressive increase in the percentage of those with LVH and mean LV mass with each deteriorating stage of CKD, see **Figure 1.8**.[145] In effect those with a GFR of $<20 ml/min/1.73m^2$ had a twofold higher risk of LVH on echocardiography compared to those with a GFR of greater than $60 ml/min/1.73m^2$.[145] Those with a GFR of $<30 ml/min/1.73m^2$ had a 2.2 fold increased risk of abnormal ventricular geometry compared to those with a GFR $>60 ml/min/1.73m^2$ even after adjustment for confounding variables.[145]

LV mass has even been shown to rise with declining GFR in those with a preserved renal function (>60ml/min/1.73m^2).[146] In a study of over 2000 participants recruited as part of the Coronary Artery Risk Development in Young Adults cohort in comparison to those with a GFR >90ml/min/1.73m^2, participants with a GFR between 60 to 75 ml/min/1.73 m^2 had a significant increase in LV mass on echocardiogram.[146] Small declines in GFR in the 60-75ml/min/1.73m^2 range were associated with an average 6g/m^2 increase in LV mass at 10 years.[146] This suggests that cardiovascular disease and structural change begins early in the course of CKD.[146]

There is also evidence from retrospective studies that the presence of LGE is not confined to those with ESRD but also observed in much earlier stages of CKD.[147] In 2015, Dandamudi et al. assessed all comers having cardiac MRI between 2006 and 2008 at a single centre (over 900 participants) and risk stratified them according to a GFR of greater or less than 70ml/min/1.73m^2.[147] The existence of LGE was significantly associated with a lower renal function than those without LGE (73 vs. 78ml/min/1.73m^2) and remained significant after adjustment for confounding variables.[147]

Furthermore, LGE appeared to have similar adverse prognostic implications as in other cardiac pathologies.[147] In the same study Dandamudi et al. reported the presence of LGE was associated with increased risk of mortality [HR 1.78 (95% C I 1.21, 2.63)] and mortality was greatest for those with a GFR <70ml/min/1.73m^2.[147] Despite this, patients included those with diabetes and known coronary artery disease and over 80% of participants had subendocardial patterns of LGE in keeping with coronary artery ischaemia.[147] In essence, this was a clinical study of patients with ischaemic heart disease with secondary mild renal impairment.[3] Consequently, it is difficult to attribute the presence of LGE solely to a reduction in GFR with these other confounding variables at play.

In a retrospective study in our own unit, however, LGE was still present in 34% of the 159 with pre-dialysis CKD that were studied.[3] In contrast to the study of Dandamudi et al. participants with diabetes, diagnosed coronary artery disease and LV dysfunction were excluded in order to reduce the effects of coexistent atherosclerotic disease.[3] In this study ventricular insertion point and mid-wall patterns were the most frequent (51% and 33% respectively), see **Figure 1.9**.[3] The subendocardial pattern of LGE seen in the majority of patients studied by Dandamudi et al. accounted for only 9% in our cohort and was more in keeping with the study of ESRD subjects by Mark el al.[3, 91, 147] This suggests that those with early stage CKD may be subject to the same pathological processes in the myocardium as those with ESRD and it is unlikely to simply be attributable to atheromatous disease.[3]

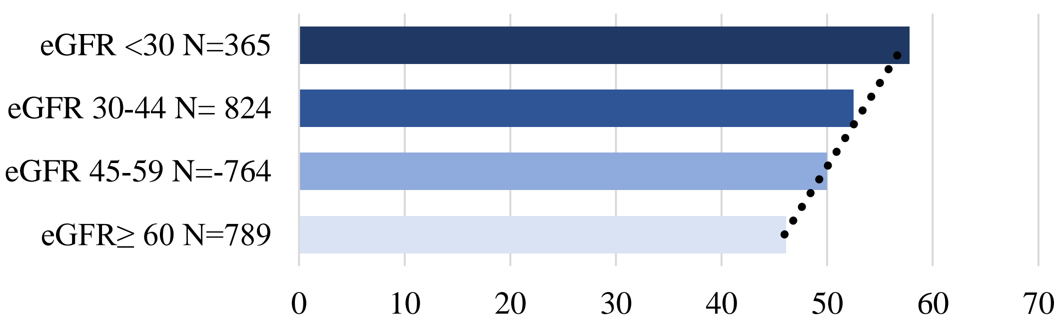

Figure 1.8. Mean left ventricular mass according to each GFR stage.
Adapted and reproduced with permission from Park et al. [145] Demonstrates a linear relationship between GFR and left ventricular mass.

eGFR: Estimated glomerular filtration rate. LV; Left ventricular.

A

B

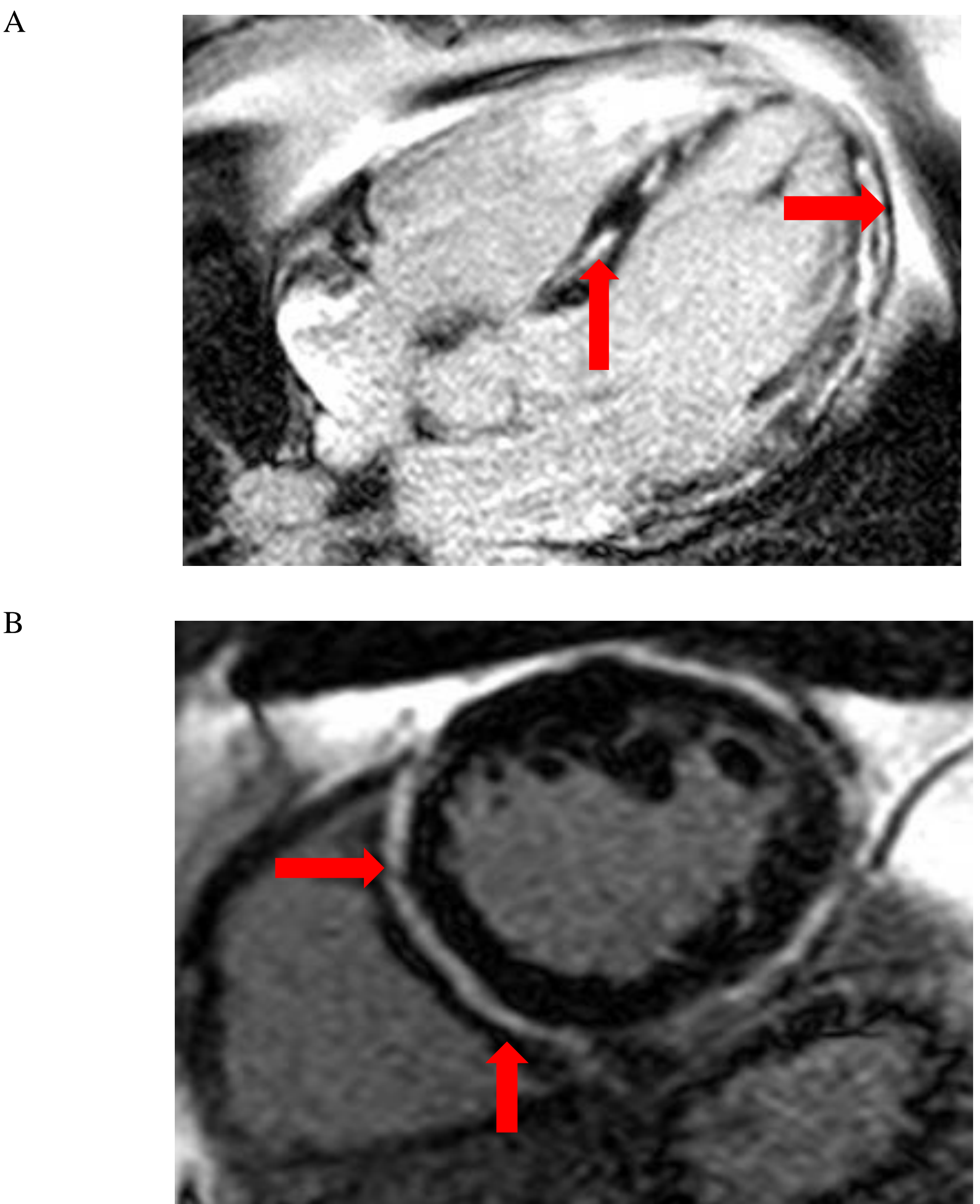

Figure 1.9. An example of mid- wall LGE in a patient with CKD
First published and reproduced under terms of the Creative Commons CC-BY license from Price et al.[3]

A. The image at the top is a horizontal long axis chamber view illustrating extensive mid-wall LGE. Areas of fibrosis appear bright white against the black background of normal myocardium (shown with red arrows).
B. The image at the bottom is a short axis view of the same patient. The underlying aetiology was Adult Polycystic Kidney Disease. The coronary angiogram was normal and the pattern does not relate anatomically to a single coronary territory. From these images it can be appreciated that LGE is often based on a subjective visual assessment and although quantification methods are available longitudinal comparison is challenging.[115]

Although studies of ESRD are limited to non-contrast/native T1 mapping because of safety concerns, those with earlier stages of CKD can receive gadolinium safely and consequently ECV can be calculated.[148] Reports of NSF in those with a GFR of >30ml/min/1.73m^2 are exceptionally rare.[149] These techniques give us further insight into the underlying pathology. In a prospective study Edwards et al. studied patients with stage 2-4 CKD compared to age and gender matched healthy controls and hypertensive controls.[148] T1 and ECV were significantly higher in those with CKD compared to controls (T1 time=986ms vs 955ms), (ECV= 28% vs 25%).[148] The hypertensive cohort had T1 and ECV levels similar to that of controls and lower than those with CKD.[148] Both native T1 times and ECV correlated with GLS despite there being no differences in LV volumes or LV mass between groups.[148] This suggests that increased interstitial fibrosis occurs early in the course of CKD proceeding the changes in mass and volumes seen in ESRD.[148] Even at this early stage, fibrosis may have functional consequences as it is associated with markers of reduced systolic function such as GLS.[148]

There is some evidence that fluid balance and oedema may have an impact on the appearance of the myocardium even in those who are not dialysis dependent.[134] In a retrospective multi-centre study of 154 patients with CKD (defined as less than 60ml/min/1.73m^2), 158 patients with hypertension and 158 with hypertrophic cardiomyopathy, T1 was elevated in all patients and was the best discriminator between patients and the 133 normotensive healthy controls studied.[134] T1 time in the CKD cohort was higher than either the control group or those with hypertension.[134] T2 time, however, was the only measure higher in the CKD cohort and the best discriminator between CKD and patients with hypertension.[134]

Furthermore, subtle changes in myocardial contractile dysfunction have been observed to proceed structural change and occur early in the course of CKD.[150] In a prospective study of 40 patients with CKD stage 2 or 3 without diabetes or known cardiovascular disease mean

GLS was significantly reduced compared to 30 healthy controls (-15% ± 4% vs. -17% ± 3%) using Doppler myocardial imaging.[151] Despite this, ejection fraction, was no different between the two groups.[151] This suggests that subtle changes in functional parameters are present even in the earliest stages of CKD whilst traditional measures of systolic function such as ejection fraction are preserved.[151]

Furthermore, evidence suggests subtle changes in cardiac function deteriorate in a stepwise manner in parallel to a decline in renal function.[150] In a cross sectional study of 106 patients with CKD (stage 1-5) and 38 controls with preserved ejection fraction (defined as a Simpsons biplane >55% and no regional motion wall abnormalities) Panoulas et al. found cardiac dysfunction was evident even in those with stage 1-2 CKD.[150] Participants underwent conventional transthoracic echocardiography and speckle tracking analysis.[150] GLS impairment (defined as >-16%) was seen with increasing prevalence as CKD progressed whilst LV twist and the number of segments with diastolic dysfunction increased as GFR deteriorated.[150] LV twist describes the base of the heart rotating clockwise and the apices rotating counter clockwise.[150] LV twist is thought to contribute to over a third of stroke volume and is a very early sign of myocardial dysfunction.[152] GFR was independently and inversely associated with both LV twist and the number of segments with diastolic dysfunction after adjustment for diabetes, systolic blood pressure, family history and body mass index (BMI).[150]

These subclinical functional abnormalities seen in the earlier stages of CKD are also predictive of adverse cardiovascular events.[139, 150] Panoulas et al. found that over a 12 month follow up period those with an impaired GLS (>-16%) had a greater risk of major cardiovascular events (event free survival in impaired GLS 88.5% vs. 93.7%, p=0.038).[150]

Diastolic dysfunction is not only limited to those with ESRD.[142] In a study of 40 healthy controls and 202 CKD participants with stage 2-5 CKD, mitral A velocity was reduced and the ratio of mitral E and A velocity was much higher in the healthy controls compared to all of those with CKD including the earliest stages of renal dysfucntion.[142]

In summary, there is evidence that those with early stages of CKD also develop structural changes to the heart including a rise in LV mass in a graded relationship as GFR declines.[145] Novel imaging techniques also indicate that those with early stage CKD share similar patterns of LGE to those with ESRD and also have elevated T1 times which have previously been associated with cardiac fibrosis.[3, 148] In addition functional abnormalities in early stage CKD include impaired myocardial deformation (impaired GLS), LV twist and diastolic dysfunction resembling heart failure with preserved ejection fraction (≥50%).[144]

As structural and functional cardiovascular changes occur in the earliest stages of CKD one may consider that CKD associated cardiomyopathy is a more accurate term than uraemic cardiomyopathy as many of these patients cannot be considered significantly uraemic.[153] It also raises the question of whether a modest reduction in GFR in isolation, as a result of living kidney donation may have similar effects on the cardiovascular system.

1.6 Potential causes of uraemic cardiomyopathy and therapeutic targets

1.6.1 Haemodynamic overload

Anaemia, erythropoietin and iron

In those with CKD, anaemia has been associated with LV dilatation, hypertrophy and mortality.[154] In an echocardiography study of over 400 patients with ESRD, Foley et al. found anaemia was independently associated with dilatation of the LV and new diagnoses of heart failure.[155] Whilst early studies of the treatment of anaemia in CKD with erythropoietin have demonstrated regression of LV mass more recent trials have shown no significant cardiovascular benefits, see **Table 1.2**. [156, 157] In order to mitigate potential risks of using erythropoietin but treat anaemia there has also been recent focus on the treatment of iron deficiency.[158]

1.6.2 Hormonal and circulating factors

Disturbance in bone and mineral metabolism

In CKD, the inability to effectively excrete phosphate leads to dysregulation of the hormonal feedback loops which control calcium and phosphate homeostatsis.[159] This results in elevated fibroblast growth factor-23 (FGF23), phosphate and parathyroid hormone (PTH) levels with correspondingly low levels of 1, 25-dihydroxyvitamin D and calcium. All of which have been linked to adverse cardiovascular outcomes and the focus of many randomised clinical trials, see **Table 1.3**.

One of the main strategies for limiting phosphate levels is the use of phosphate binders, however, trials have been disappointing.[159] In 2018 an extensive Cochrane review of 104 studies there was no clear beneficial effect of Sevelamer, Lanthanum, iron and calcium-based phosphate binders on all-cause mortality, myocardial infarction or coronary artery calcification of those with stage 2-5.[160] In addition clinical trials of calcimimetics such as Cinacalcet have been undertaken to examine the effect of PTH suppression on cardiovascular

outcome.[47] The results, however, have also been discouraging and Cinacalcet has not been demonstrated to reduce risk of major cardiovascular events.[161] Furthermore, in a recent systematic review and meta-analysis, Lu et al. found that although in 21 observational studies vitamin D and its analogues were associated with reduced all-cause and cardiovascular mortality, current randomised trials did not show sufficient evidence of a beneficial effect.[162]

FGF23 and klotho axis

Elevated FGF 23

It is not clear whether FGF23 has a direct cardiovascular toxic effect or is a bystander and merely associated with other deranged biochemistry mediating pathology. [163] There is emerging evidence, however, that FGF23 is associated with LV disease.[164] In a larger study of over 3000 participants who took part in the Chronic Renal Insufficiency Cohort study, FGF23 levels were three times higher in those with CKD compared to that expected in the general population.[165] The prevalence of normal LV geometry on echocardiography decreased with each increasing quartile of FGF23 and those with normal blood pressure had over a 4 fold increased risk of developing a new diagnosis of LVH with each unit increase of FGF23.[165]

Faul and colleagues have conducted extensive animal studies on the effects of FGF23 on the heart.[165] In a study using neonatal rat ventricular cardiomyocytes treated with FGF23, Faul et al found rising levels of α-actinin on immunocytochemical analysis (suggesting increased sarcomeres) and increases in cell surface area.[165] Taken together this indicates cardiac hypertrophy was in direct response to exposure to FGF23.[165]

α-klotho deficiency

CKD is considered a condition of profound α-klotho deficicency.[166] It was previously thought that the co-dependent relationship between α-klotho, FGF23 and phosphate homeostasis meant that cardiac remodelling was likely a consequence of uncontrolled high FGF23 and phosphate levels rather than the absence of α-klotho itself.[167] Recent studies, however, have

suggested that soluble α-klotho has its own independent effects.[167] Mice that are heterozygous for klotho deficiency develop cardiac hypertrophy and poor cardiac function as measured by CMR imaging and dietary measures instigated to correct FGF23 and phosphate did not reverse this.[167] Mice were then injected with a transgene which encoded soluble α-klotho .[167] Compared to mice injected with an empty vector those receiving the transgene had a reduction in heart to weight body ratio and percentage of cardiac fibrosis.[167] This suggests there could be a future therapeutic avenue.

Hyperuricemia

Although historically the role of uric acid in renal progression and cardiovascular disease in CKD has been somewhat dismissed due to strong collinearity with traditional risk factors, there has been some renewed interest.[168, 169] In a recent meta-analysis of 27,081 with CKD those with the highest levels of serum uric acid were at over 40% greater risk of cardiovascular mortality than those with the lowest levels.[170] A one unit change in uric acid levels equated to a 12% increased risk of cardiovascular mortality. [170]

The use of allopurinol has also a beneficial effect on cardiovascular structure and function.[171] Kao et al. conducted a randomised, double-blind, placebo-controlled study of patients with CKD stage 3 and LVH diagnosed by echocardiography.[171] After 9 months of receiving 100mg of allopurinol there was a significant reduction in LV mass compared to the control group.[171]

Disturbance of the renin-angiotensin system

The pathophysiology of the renin-angiotensin-aldosterone system (RAAS) has a central role in progression of CKD, cardiovascular disease and hypertension.[172] Therapeutic targets of the RAAS system have also been the cornerstone of therapy for both renal disease and heart failure and have included angiotensin converting enzyme inhibitors, direct renin inhibitors and angiotensin receptor blockers.[173] Despite the beneficial effects of angiotensin converting

enzyme (ACE) inhibition a ‘resistance’ appears to develop.[172] This has been attributed to aldosterone breakthrough in which excess aldosterone secretion occurs in response to a rise in potassium levels as a result of ACE blockade.[174] There is growing evidence that aldosterone has more direct actions on cardiovascular structure and function.[175] Trials in those with CKD, however, have not been consistent, see **Table 1.4**.

1.6.3 Molecular factors

Chronic inflammation and oxidative stress

Age related diseases are frequently linked to chronic levels of low grade inflammation and have previously been extensively investigated in other chronic diseases.[176, 177] CKD, is frequently referred to as a disease of 'persistent inflammation' and has been recognised as a contributing factor in cardiovascular disease since the 1990s.[178-180] Inflammation also plays a strong central role in cardiovascular pathology and in many cases cardiovascular disease can be considered an age related disease.[181]

CKD has consistently been associated with elevated inflammatory biomarkers and, in particular, a mismatch between pro and anti-inflammatory cytokines.[182] In a study of over 200 participants on haemodialysis over two thirds had an abundance of cytokines considered pro-inflammatory (Interleukin (IL)-1, IL-6 and Tumour necrosis factor (TNF)-alpha) compared to anti-inflammatory cytokines (IL-2, IL-4, IL-5, IL-12).[182] Furthermore those with a greater level of mismatch between pro and anti-inflammatory cytokines had an increased risk of mortality [HR 2.26 (95% CI 1.44, 3.53)].[182]

Table 1.2. Key randomised clinical trials with cardiovascular end points targeting anaemia.

Intervention	Study design	Reference and study acronym	Study population	Cardiovascular outcome measure	Study result
Treatment targets for haemoglobin and management of anaemia (erythropoietin and iron)					
Target haemoglobin of 120-130 g/L vs. 90-100 g/L.	Open-label	Roger et al 2004.[183]	n=155 CKD	Change in LVM index.	No difference in LVM. No difference in SBP. No effect on the development of left ventricular hypertrophy
Target haemoglobin of 120-140g/L vs. 90-105g/L	Open-label	Levin et al 2005.[184]	n=172 CKD	Change in LVM index from baseline to 24 months.	No difference in LVM or left ventricular growth.
Target haemoglobin of 95-115g/L vs. 135-145g/L	Double-blind	Parfrey et al 2005.[185]	n=596 Haemodialysis No symptomatic cardiovascular disease	Left ventricular volume index	No difference in left ventricular volume or LVM.
Target haemoglobin of 130-150g/L and early treatment vs. 105-115g/L and delayed treatment	Open-label Parallel design	Drueke 2006[186] CREATE	n=603 GFR 15-30ml/min/1.73m^2	Composite of eight cardiovascular events. LVM.	No difference between those with early complete correction of anaemia and those with delayed treatment.
Target haemoglobin of 135g/L vs. 113g/L.	Open-label	Singh 2006[187] CHOIR	n=1432 CKD GFR 15-50ml/min/1.73m^2	Composite of death, MI, hospitalisation for congestive heart failure and stroke.	A higher haemoglobin target was associated with increased risk of death.
Darbepoetin alfa vs. placebo	Placebo controlled	Pfeffer 2009.[188] TREAT	n=4038 CKD Type II diabetes	Composite outcomes of death or cardiovascular event and death or end-stage renal disease.	No difference in mortality between groups.
High dose vs. low dose intravenous iron	Open-label Blinded end point analysis	Macdougall et al 2019[158] PIVOTAL	n=2141 Haemodialysis	Composite of non-fatal myocardial infarction, non-fatal stroke, hospitalisation for heart failure or death.	Low dose iron given in a reactive fashion to declines in haemoglobin was associated with greater risk of cardiovascular events.

CHOIR; Correction of haemoglobin and outcomes in renal insufficiency. CREATE; Cardiovascular reduction early anaemia treatment epoetin beta. CKD; Chronic kidney disease. LVM; Left ventricular mass MI: Myocardial infarction. PIVITOL; Proactive intravenous iron therapy in haemodialysis patients. SBP; Systolic blood pressure. TREAT; Reduce cardiovascular events with Aranesp therapy. UK HARP-III: UK Heart and renal protection.

Table 1.3. Key randomised clinical trials with cardiovascular end points targeting bone and mineral metabolism.

Intervention	Study design	Reference and study acronym	Study population	Cardiovascular outcome measure	Study result
Therapeutic targets on bone and mineral metabolism					
Sevelamer vs. calcium-based phosphate binders	Open-label Active control Parallel-group	Suki et al 2007.[189] DCOR	n=2103 Haemodialysis	All-cause mortality. Cause-specific mortality (cardiovascular, infection, and other) All-cause hospitalisation.	No difference in all-cause mortality. No difference in cardiovascular mortality.
Cinacalcet plus low dose vitamin D sterols vs. vitamin D sterols	Open-label Active control	Raggi et al 2011[190] ADVANCE	n=360 Haemodialysis PTH>150pg/mL On calcium-phosphate binders	Percentage change in coronary calcification score from baseline to Week 52	Calcification scores were reduced in those on Cinacalcet.
Cinacalcet vs. placebo	Double-blind Placebo controlled	Chertow et al 2012.[161] Wheeler et al. 2012[47] EVOLVE	n=3883 Haemodialysis with moderate to severe hyperparathyroidism	Fatal and non-fatal cardiovascular events.	Cinacalcet did not significantly reduce the risk of death or major cardiovascular events Secondary outcomes found Cinacalcet was associated with reduced rates of sudden cardiac death and heart failure.
Paricalcitol (active vitamin D) vs. placebo	Double-blind Placebo controlled	Thadhani et al 2012[191] PRIMO	n=227 CKD GFR 15-60mL/min/1.73m^2	Changes in LVM over 48 weeks	No change to LVM. No change to measures of diastolic dysfunction.
Sevelamer vs. placebo	Double-blind Placebo controlled	Chue et al. 2013[192]	n=120 CKD Stage 3	LVM. Systolic and diastolic function. Carotid-femoral PWV.	No difference in LVM, systolic and diastolic function or PWV. Sevelamer associated with lower serum FGF23 levels.
Paricalcitol (active vitamin D) vs. placebo	Double-blind Placebo controlled	Wang et al. 2014[193] OPERA	n=60 CKD with left ventricular hypertrophy Stages 3-5	Change in LVM index over 52 weeks	No difference in LVM, left ventricular volume, LVEF or measures of diastolic dysfunction. Paricalcitol reduced cardiovascular-related hospitalisations and PTH.
Cholecalciferol vs. placebo	Double-blind Placebo controlled	Banerjee et al 2019[194]	n=48 CKD Stage 3-4 Non-diabetic Vitamin D <75nmol/L On RAAS blockade High/normal LV mass	Change in LVM index over 52 weeks.	No difference in LVM, LVEF, or ventricular/atrial volumes.

ADVANCE; A randomised study to evaluate the effects of Cinacalcet plus low dose vitamin D on vascular calcification in subjects with chronic kidney disease receiving haemodialysis. CKD; Chronic kidney disease. DCOR: Dialysis clinical outcomes revisited. EVOLVE: Evaluation of Cinacalcet hydrochloride therapy to lower

cardiovascular events. LVEF; Left ventricular ejection fraction. LVM; Left ventricular mass. OPERA; Oral Paricalcitol in stage 3-5 CKD. PRIMO; Paricalcitol capsules benefits renal failure induced cardiac morbidity in subjects with chronic kidney disease stage 3 and 4. PTH; Parathyroid hormone PWV; Pulse wave velocity.

Table 1.4. Key randomised clinical trials with cardiovascular end points targeting the renin-angiotensin aldosterone system.

Intervention	Study design	Reference and study acronym	Study population	Cardiovascular outcome measure	Study result
Therapeutic targets on the renin-angiotensin-aldosterone system					
Spironolactone vs. placebo	Double-blind Placebo controlled	Edwards et al.2009 [103]	n=112 Stage 2-3 CKD On RAAS blockade	LVM Arterial stiffness (PWV/aortic distensibility) at 40 weeks	Reduction in LVM Reduction in arterial stiffness.
Spironolactone vs. placebo	Double-blind Placebo controlled	Vukusich et al. 2010[195]	n=53 Haemodialysis Non-diabetic Not on RAAS blockade	Carotid intimal thickness	Reduced progression of carotid intimal thickness compared to placebo.
Spironolactone vs. no spironolactone	Open-label	Ito et al 2014[196]	n=158 Peritoneal dialysis On RAAS blockade	Rate of change in LVM index	Reduction in LVM and improvement in ejection fraction with spironolactone.
Spironolactone vs. no spironolactone	Open-label	Matsumoto et al 2014[197] DOHAS	n=309 Haemodialysis	Composite of death from cardiovascular and cerebrovascular events. Hospitalisation for cardiovascular and cerebrovascular events	Reduced the risk of cardiovascular and cerebrovascular morbidity and death with spironolactone.
Spironolactone vs. placebo	Double-blind Parallel design Placebo controlled	Hammer et al 2019[198]	n=97 Haemodialysis	Change in LVM index from baseline to 40 weeks.	No difference in LVM or LVEF. No difference in ambulatory blood pressure No difference in 6-minute walk test distance or New York Heart Association functional class.
Spironolactone vs. placebo Multiple dosage trial	Double-blind Placebo controlled	Charytan et al 2019[199] SPIN-D	n=129 Haemodialysis	Diastolic dysfunction	No difference in diastolic function between groups.

CKD; Chronic kidney disease. DOHAS: Dialysis outcomes heart failure Aldactone study. LVEF; Left ventricular ejection fraction. LVM; Left ventricular mass PWV; Pulse wave velocity. RAAS; Renin angiotensin aldosterone system. SPIN-D; Safety and cardiovascular efficiency of spironolactone in dialysis-dependent end stage renal disease.

1.7 Living kidney donation

The evidence for increased cardiovascular mortality and morbidity in CKD is extensive, consistent, comes from multiple sources and is apparent even in the early stages of CKD. If cardiovascular disease is directly and independently related to a decrease in GFR in a stepwise manner then living kidney donors would be expected to be susceptible to the same disease processes as those seen in CKD: atherosclerosis, arteriosclerosis and uraemic cardiomyopathy.

Living kidney donors are ideal candidates to investigate the relationship between GFR and cardiovascular disease. Firstly, they undergo strict criteria for donation at baseline and are usually healthy with a normal cardiovascular system (without coexisting conditions seen in CKD such as diabetes and uncontrolled hypertension). Secondly, at 5 years after donation, up to a third of patients can be expected to have an GFR of less than 60ml/min/1.73m^2 (equivalent GFR of those with stage 3 CKD).[200] Finally, they have a reduction in GFR at a known time point meaning they can be studied prospectively (which is seldom possible in those with CKD).

Studies of living kidney donors therefore enables a structured approach to disentangling the complex association of renal and cardiovascular disease thus allowing important pathophysiological information on the mechanisms of cardiovascular disease in CKD to be gained.[2]

1.7.1 Living kidney donation in the UK

The cost of CKD has a huge financial impact on the UK National Health Service (NHS).[201] It is estimated that around £1.6 billion per annum is spent on the treatment and complications of CKD in the UK and accounts for 1.3% of overall National Health Service (NHS) spending.[201] A large proportion of spending on CKD is on dialysis, estimated to cost over £500 million per year, yet serves only 2% of those with CKD.[201] Whilst the use of dialysis has become

more mainstream, it is still associated with a high symptom burden, high rates of mortality and poor quality of life.[202, 203] Renal transplantation has been shown to be a more cost-effective strategy for the NHS when compared to the alternative of long term dialysis.[21, 201] Transplantation is considered the gold standard renal replacement therapy for patients with ESRD as it bestows improvements to both quality of life and survival in comparison to dialysis. [204, 205]

The UK, however, is currently facing an organ donation crisis and has the highest organ donation refusal rate in Europe.[206] The major imbalance between the supply of deceased donor organs and demand combined with improved pharmaceuticals and technology has led to the expansion of living kidney donation. [206-209] In the last 20 years living kidney donation has increased substantially.[210] There are now wider acceptable criteria for donation, inclusion of altruistic donors, complex multiple chain donations and donation for those with ABO incompatibility.[210] As a result almost half of all transplanted kidneys in the UK are now from living kidney donors (~1000 living kidney donors per year).[205, 208, 211] In the last seven years however, living kidney donation rates in the UK have plateaued with small declines in recent years.[212]

The speed of change in transplantation however, has exceeded the acquisition of knowledge about the pathophysiological effects of donating a kidney. There is much controversy over the long term consequences of donation.[213 214] This is in part due to a paucity of long term registry data and partly due to some of the unique challenges of designing research studies in living kidney donors such as selecting a comparable control group and eliminating bias.[215]

1.7.2 Mortality and cardiovascular events

Findings from multiple studies with up to 40 years of follow up have shown no evidence of reduced survival compared to the general population and some have reported better life expectancy, see **Table 1.5**.[216-220] Most are single centre reports and describe health event rates

far lower than the general population, although importantly, the control data were often derived from populations containing large numbers of subjects who would not have been fit to donate. [218] In an attempt to overcome this, Garg et al. used a matched cohort study to compare donor death and cardiovascular event rates with the 'healthiest general population' and excluded those with conditions that would have precluded donation.[221] Reassuringly, the combined end points of death and adverse cardiovascular events were lower in donors than controls and the risk of cardiovascular events alone was not significantly different.[221] Further support comes from a large study using US registry data comparing survival in over 80,000 donors to that of a matched cohort of 9364 participants without CKD (1:1 matched based on comorbidities) drawn from the third National Health and Nutrition Examination Survey (NHANES).[216] Over a median follow up of 6.3 years, mortality amongst donors was not different to controls stratified by age, sex, and race. [216]

There are a number of limitations of these studies. Firstly, the short duration of follow up means that increased long term cardiovascular risk cannot be excluded. To date, most studies have median follow up periods of 6-8 years. [216, 221, 222] Secondly, the influence of race on cardiovascular outcomes after kidney donation is unclear. Most of the outcome data are based on predominantly Caucasian populations such as those in Canada and Norway.[221, 223] There is a need for mortality studies on black, Hispanic and Asian patients especially given the increased risk of hypertension in these groups.

Concerns relating to possible long term adverse effects of donation arose in 2014 in a paper examining 15 year outcomes in 1901 Norwegian donors and 32,621 control participants who were potentially eligible for donation. [223] The hazard ratios for all-cause death 1.30 (95% CI 1.11-1.52), cardiovascular death 1.40 (95% CI 1.03-1.91) and ESRD 11.38 (95% CI 4.37-29.63) were significantly increased in donors with curves diverging after about 10 years. [223] Limitations of this study include: exclusion of marginal donors with co-morbidity such as

obesity, an older donor group (8 years) than controls, and longer follow up of donors compared to controls.[223, 224] In addition, the rural area of Norway used to conduct the study has an unusually high life expectancy and most donors (including all who developed ESRD with its attendant high cardiovascular risk) were genetically related to the recipient.[224] Nevertheless, these data are at least cause for concern and should give rise to more intensive long term follow up of donor populations around the world. It is impossible to exclude with certainty that a reduction in GFR of any cause, including donation, may lead to an increase in adverse cardiovascular events.

A major problem with studies using non-mortality end points in living kidney donors is surveillance bias. [222] Higher rates of hypertension and proteinuria in donors may be a result of more intensive medical review and may lead clinicians to more readily commence anti-hypertensive agents leading to cases being 'overstated'.[222, 225] Reese et al. found that donors made more visits to primary care and had more diagnosed non-melanoma skin lesions, both findings are suggestive of this form of bias and suggests this is a complicating factor when studying donors.[222] This reinforces the need for well controlled prospective studies of adequate duration.

Table 1.5. Summary of mortality studies in living kidney donors. First published in Price et al.[2]

Reference	Date	Patient numbers	Control group	Matched controls?	Follow up	Ethnicity/Sex/Age of donors	Single centre ?	Outcome	Mortality in donors
Garg.[218]	2008	Donors=1278 Controls=6359	Health administration data	Yes-age, sex, income and number of physician visits.	Mean 6.2 yrs.	92% Caucasian 5% Asian <3% black Canada	No	No differences in either mortality or cardiovascular disease events (1.3% vs. 1.7%; hazard ratio 0.7)	Unchanged
Ibrahim.[217]	2009	Donors=3698	Life table data	Yes- Age, sex and race. 255 matched 1:1	40 yrs.	United States	Yes	Survival was similar to controls	Unchanged
Segev.[216]	2010	Donors=80,347 Controls= 9364	Third cohort of National Health and Nutrition Examination Survey (NHANES III)	Yes-1:1 based on co-morbidities.	Median 6.3 yrs.	13.1% black 12.3% Hispanic United States	No	Mortality amongst donors was no higher than controls even when stratified by age, sex, and race	Unchanged
Mjoen.[219]	2012	Donors=2269 Controls=6807	General population statistics	Yes-Age, gender and year of birth. 3:1 match	Median14.3 yrs.	Mean age 47yrs 41% male Norway	Yes	Both overall and cardiovascular mortality was lower for donors	Lower
Garg.[221]	2012	Donors=2028 Controls=20280	'Healthiest general population' Excluded those with a condition that precluded donation.	Yes- age, sex, income and residence 10:1 match	Median6.5 yrs.	Median age 43yrs Likely Caucasian Ontario, Canada	Yes	Risk of death or major cardiovascular events was lower in donors with a hazard ratio of 0.66.	Lower
Reese.[222]	2014	Donors=3368 Controls=3368	Healthy older patients in the Health and Retirement Study	Yes- Based on patient reported health	Median 7.8 yrs.	Mean age 59yrs Only 7% black 41% male United States	No	Donors were not at an increased risk of death or cardiovascular disease.	Unchanged
Mjoen.[223]	2014	Donors=1901 Controls= 32,621	Health Study of Nord-TrØndelag (HUNT) population study	No. Controls were considered fit to donate.	Median 15.1 yrs.	Mean age 46yrs All Caucasian Norway	Yes	Increased risk of all-cause and cardiovascular death	Higher
Rizvi.[220]	2016	Donors =90 Controls=90	Siblings of donors	Yes-siblings paired.	Mean 5.8 yrs.	Mean age 37yrs 70% male Pakistan	Yes	No difference in rates of ischaemic heart disease	Unchanged

1.7.3 Risk of ESRD

Previous studies of the risk of ESRD in donors have compared donors to non-donating siblings who share the same genetic and environmental factors which may predispose them to future kidney disease.[226] Whilst such studies proved reassuring with no change in blood pressure or proteinuria in up to 20 year follow up this idealist approach led to a number of studies with very small numbers. [227, 228]

The best data comes from two large scale matched cohort studies. In 2014, Muzaale et al. compared 96,217 donors with 20,024 healthy matched controls from the NHANES data with a median follow up of 7.6 years.[229] The risk of developing ESRD in donors was 30.8 per 10,000 compared to 3.9 per 10,000 in the matched control group at 15 years after nephrectomy.[229] Absolute risk, however, was small with a lifetime risk of 90 per 10,000 for donors.[229] In contrast in same year, Mjoen et al. compared 1901 kidney donors and a matched cohort of 32,621 controls considered suitable for donation.[223] The risk of ESRD was markedly higher in donors than controls with a hazard ratio of ESRD after imputation for missing values of 11.38 (95% CI 4.37-29.6) although the alarming high risk may be overestimated due to underlying genetic predisposition in the donor group.[223] All donors developing ESRD were family members and all the causes were immune mediated.[223]

Similar findings were observed by Matas et al. who noted that the 16% of known causes of ESRD in donors had a similar aetiology to the recipient.[230] However many of those developing ESRD were male, donated at a younger age and had a history of smoking.[230] Consequently, the majority of causes were linked to diabetes and hypertension. [230] Health promoting behaviour and adequate risk stratification of donors is likely to be important to mediate the risk both before and after donation.[226, 230, 231] More recently risk stratification tools have been developed in response to this discrepancy to predict individuals lifetime risk of ESRD.[232]

1.7.4 Vascular changes

Hypertension

Most patients with CKD are hypertensive but it is not clear if this is a universal finding when GFR is reduced. There has been suspicion for many years that donors have excess rates of hypertension and albuminuria but the quality of evidence is poor and reports are inconsistent.[225] A meta-analysis of 48 studies found that it was not possible to assess the risk of hypertension requiring treatment as none of the primary studies had an adequate sample size to detect a 1.5-fold increase in risk after donation with at least 80% statistical power.[233] Thus, change in blood pressure (mmHg) is frequently used as an intermediary marker for increased risk of hypertension. [233] Of the ten studies that had a control group and a follow up of over 5 years, there was an increase in blood pressure of about 6mmHg systolic and 4mmHg diastolic when compared to healthy adults with similar age, sex and ethnicity.[233] Garg et al. also found that donors were more likely to be diagnosed with hypertension (defined using diagnostic codes on outpatient or discharge paperwork) than controls (16.3% vs. 11.9%, hazard ratio 1.4) however, there is a strong possibility of surveillance bias.[218]

There are many flaws in these studies: most were retrospective and few used contemporaneous control groups that were followed up in a similar way to donors.[225] The transplant community can be criticised for a lack of quality prospective long term studies of blood pressure in living kidney donors but there are significant obstacles. Not only are such studies expensive and difficult to perform, particularly with respect to finding appropriate controls, but live donor transplants are often carried out in large hospital centres involving long travelling times. [223, 225] In Korea for example, just 11% of patients were followed up despite over 80% of kidney transplantation in that country involving live donors.[234]

Data from 24-hour (hr) ambulatory blood pressure studies are mixed. In a prospective controlled observational study, Kasiske et al. found no statistical difference in ambulatory

blood pressure values or in night time ‘dipping’ at 36 months between 135 well matched controls and 126 donors.[235] In contrast, data from 1214 donors in the mandatory Swiss lifelong donor follow up has raised concern.[225] Among initially normotensive donors, 43.1% developed hypertension diagnosed by ambulatory blood pressure monitoring within the 10 year follow up period.[225] Hypertension was defined as a systolic of greater than 140mmgHg and/or a diastolic of greater than 90mmHg or the use of an anti-hypertensive medication.[225] There was no control group so conclusions are difficult to draw but using the Framingham hypertension risk score it was estimated that by 12 months, nephrectomy had increased the risk of hypertension by 3.64 times.[225] In addition the authors could not exclude the possibility of surveillance bias as the addition of anti-hypertension medication into the definition means that some normotensive patients on an ACE inhibitor for proteinuria for example would be included.[225]

The influence of race on rates of hypertension and other morbidities requires much more investigation. To date, the best data comes from a retrospective US study of 4650 living kidney donors.[236] Post nephrectomy events were compared to NHANES data from the general population with a median follow up of 7.7 years.[236] Thirteen percent of the group were black and 8% Hispanic.[236] The overall prevalence of hypertension at 5 years was 17.8% but this was increased by 52% for black and 36% for Hispanic donors compared to white donors exceeding what would be expected in the general population in both Hispanic and black patients over the age of 55.[236] The definition of hypertension was based on billing claims, pharmacy claims and anti-hypertensive drug category codes.[236]

In a number of studies blood pressure variability rather than blood pressure alone has been linked to cardiovascular mortality and progression of renal disease.[237, 238] In a systematic review and meta-analysis variability in systolic blood pressure was associated with an increased risk of all-cause and cardiovascular mortality (HR 1.15 and 1.18 respectively).[239]

Ternes et al. studied 193 donors and 196 controls as part of the prospective ATOLD study.[240] There was no difference in blood pressure coefficient of variance 12 month post nephrectomy compared to controls.[240]

In summary, despite years of study, it is still not possible to draw safe conclusions on whether the reduction in GFR caused by kidney donation causes an increase in blood pressure. This may be because there is no renal cortical damage or ischaemia in kidney donors; the circulating renin angiotensin system is probably not activated.[241, 242] This lack of association between living kidney donors and increased risk of hypertension benefits studies investigating the influence of a reduced GFR on the cardiovascular system as it eliminates the possible confounding effects of high blood pressure. The caveat, however, is that if blood pressure is a major distinguishing feature between donors and patients with CKD, findings in kidney donors may not apply to those with CKD.

Pre-eclampsia and gestational hypertension

Patients with CKD are at higher risk of developing pre-eclampsia during pregnancy and at an increased severity compared to controls.[243] This is of importance with respect to long term cardiovascular health as pre-eclampsia confers a 12 fold increased future risk of cardiovascular disease.[244] Studies investigating risk of pre-eclampsia in living kidney donors are mainly retrospective, observational and reliant on patient self-reporting. Ibrahim et al. reported on 1085 living kidney donors with 3213 pregnancies.[245] Pregnancies after donation were associated with a lower rate of full-term deliveries (73.7% vs 84.6%).[245] Donors also had higher rates of gestational hypertension (5.7% vs 0.6%) and pre-eclampsia (5.5% vs 0.8%) after donation compared to pre donation.[245] Gestational hypertension was defined as a need for treatment during pregnancy only (not before or after).[245] Maternal, foetal and pregnancy outcomes were, however, similar to the general population and the influence of patient bias recall cannot be discounted.[245] In a similar study, Reisaeter et al. also used

questionnaires to review over 100 living kidney donors and found higher pre-eclampsia rates after donation than before (5.7 vs 2.6%) although maternal age, a major confounder, could not be entirely accounted for in multivariable modelling due to the low event rate.[246] As the pregnancy complications were recorded by clinicians this data may be more accurate.[246] In a retrospective cohort study of 85 female living kidney donors and 131 pregnancies, Garg et al. matched donors with controls in a 1:6 ratio for number of pregnancies, time to pregnancy, age, income and urban/rural background.[247] Gestational hypertension and pre-eclampsia (defined by diagnostic codes following clinical assessment) were more than twice as common in living kidney donors than controls.[247] In a systematic review by the KDIGO work group, Slinin et al. concluded that women of child bearing age should be informed of an increased risk as part of the consent process.[213] On current evidence it appears that kidney donation, like CKD, increases the risk of pre-eclampsia.

Arterial stiffness

PWV is the gold standard non-invasive measure of aortic stiffness.[248] It is elevated in CKD and a strong predictor of cardiovascular risk in CKD and a variety of other diseases.[249] There are several studies of the effects of kidney donation on arterial stiffness but many are small uncontrolled pilot studies from which safe conclusions cannot be drawn. Fesler et al. showed no change in PWV or any other marker of arterial stiffness in a study of 45 donors before and 1 year after donation without a control group.[248] In contrast, a cross sectional study of 101 Lebanese kidney donors demonstrated that PWV was 10% higher than healthy controls with a similar age and sex distribution (though not screened to be ‘donor eligible’).[250]

It is estimated that the required sample size to adequately power a study to determine a 0.4m/s change in PWV is over 350 patients per group.[249] Since there are no studies of this size it is unsurprising that the literature is inconsistent.

An alternative method of measuring arterial stiffness is to use aortic distensibility. This has been used in a number of studies and is of prognostic value.[241] In a prospective controlled study, distensibility was reduced in donors compared to controls at 12 months from nephrectomy.[241]

1.7.5 Biochemical changes

Lipids and glucose tolerance

In a prospective study of 182 donors compared to 173 controls (also suitable for donation) there was no significant difference in lipid profiles including high density cholesterol, low density cholesterol, triglycerides or lipoprotein at 3 years.[235] The subjects also underwent both a Haemoglobin A1c and 'the homeostasis model assessment of insulin resistance' (HOMA-IR).[235] Although both increased over time, there was no difference between the donors and controls. [235]

Proteinuria

Proteinuria is an independent risk factor for cardiovascular mortality in the general population and CKD.[15] Recent studies have also demonstrated an increased prevalence of microalbuminuria.[225, 241] Thiel et al. for example found that ACR increased from 1.2±2.7 to 1.9±10.7 mg/mmol in donors and the prevalence of microalbuminuria increased from 4.8% to 10.4% over 10 years with a strong association with the development of hypertension.[225] Moody et al. also found that donors had a significantly raised prevalence of microalbuminuria compared to healthy controls at 12 months [odds ratio, 3.8 (CI 1.1–12.8); P=0.04].[241] This effect may be progressive; in a three year prospective study of living kidney donors and matched controls, Kasiske found a gradual rise in ACR in donors which did not occur in controls.[235]

Renin-angiotensin abnormalities

Living kidney donors show no evidence of elevated concentrations of circulating renin or aldosterone and yet have evidence of cardiovascular damage including increased LV mass

and reduced aortic distensibility, see **Table 1.6**.[241, 251] Although circulating levels of renin and aldosterone have not been identified there is some evidence of intrinsic activation.[252] Kendi et al. used a novel method of investigating activation of the RAAS in living kidney donors by studying Urinary angiotensinogen (AGT) before and after donation.[252] Urinary AGT is considered a marker of intrarenal RASS activation and was five times higher at 12 months post donation compared to baseline.[252] The study, however only included 20 patients and there was no control group.[252]

Metabolic bone abnormalities

In a prospective controlled study, biochemical changes were examined in 201 donors and 198 controls at six months after donation.[253] There was a large (23%) increase in PTH in this cohort; this increase was confirmed by Moody et al. in their prospective study of donors at 12 months.[241]

FGF23 has been found to increase both after nephrectomy and compared to controls in a number of donor studies [241, 254-256] although there are some inconsistencies which may be related to the use of different assays, see **Table 1.7**.[257, 258]

There have been two small studies investigating the effect of kidney donation on α-klotho with divergent results. Ponte et al. found an acute reduction in circulating klotho levels after serial measurements at 0, 1, 2, 3 days post donation in 27 living kidney donors.[258] Klotho levels remained lower than baseline at both 180 and 360 days after donation but had risen since the immediate post-operative period.[258] In contrast, in a cross sectional study of 35 subjects at 5 years after donation, Thorsen et al. found no difference compared to healthy controls .[257, 258] Taken together these studies suggest that klotho levels may decline acutely after donation recovering to baseline in the long term but further studies are needed to draw firm conclusions.[257]

Uric acid

In kidney donors at 1, 2 and 3 years, serum uric acid was elevated compared to controls meeting criteria for donation.[235 241] In a small prospective cohort study of 20 living kidney donors, uric acid levels decreased immediately after nephrectomy only to subsequently rise and remain high throughout the 12 month study.[252] Over the long term, donors are more likely than controls to be newly diagnosed with gout and to be commenced on treatment with allopurinol or colchicine.[259] In a small study of 42 living kidney donors, uric acid correlated with indoxyl sulphate and p-cresyl sulphate.[260] These uraemic toxins have potential importance as they have been found to be associated with increases in carotid intima-media thickness and markers of endothelial dysfunction in donors.[260]

Novel cardiovascular biomarkers

A variety of other biomarkers of cardiac disease have been found to be deranged in CKD and associated with cardiac events, death and renal progression, see **Table 1.8-1.9**.[254, 261]

Table 1.6. Changes in the renin-angiotensin-aldosterone system. First published in Price et al.[2]

Reference	Date	Population	Study numbers	Control group	Study type	Outcome	In donors
Aldosterone and Angiotensin II							
Bellavia.[251]	2015	LKD	Donors=15 Controls=15	Italian donors. U.S age and gender matched controls.	Cross sectional. Measurements at least 5 years post donation.	No difference in either aldosterone or angiotensin II between donors and controls	No difference
Moody.[241]	2015	LKD and Healthy controls	Donors=68 Controls=56	Healthy controls meeting LKD criteria	Prospective Longitudinal Baseline and 12 months. Multi-centre.	No difference in aldosterone between donors and controls.	No difference
Renin							
Bellavia.[251]	2015	LKD	Donors=15 Controls=15	Italian donors. U.S age and gender matched controls.	Cross sectional. Measurements at least 5 years post donation.	No difference between donors and controls.	No difference
Moody.[241]	2015	LKD and Healthy controls	Donors=68 Controls=56	Healthy controls meeting LKD criteria	Prospective Longitudinal Baseline and 12 months. Multi-centre.	No difference between donors and controls.	No difference

LKD; Living Kidney Donors.

Table 1.7. Changes in FGF23 and α-Klotho. First published in Price et al.[2]

Reference	Date	Population	Study numbers	Control group	Study type	Outcome	In donors
α-Klotho							
Ponte.[258]	2014	LKD	Donors=27	No controls	Cross-sectional, observational. 0, 1, 2, 3, 180 and 360 after donation.	Circulating klotho levels remained lower over a sustained period.	Decrease
Thorsen.[257]	2016	LKD CKD stage Healthy controls	Donors=35 CKD 3=22 CKD 4= 18 CKD 5= 20 Controls=35	Colleagues and friends of the authors.	Cross-sectional, observational, single-centre.	No difference between donors and controls. Lower levels seen in patients with advancing CKD.	No difference
Fibroblast growth factor-23 (FGF 23)							
Young.[256]	2012	LKD	Donors=198 Controls=98	Known to the LKD. Health status based on patient recall.	Cross sectional. Multi-centre.	Serum FGF23 was increased in donors compared to controls (38.1 vs 29.7pg/mL).	Increase
Huan.[254]	2013	LKD	Donors=34	No controls	Prospective Longitudinal Baseline and 6 months	FGF23 levels increased at 6 months compared to baseline 54.0±27.9 RU/ml vs 70.0±32.9 RU/ml.	Increase
Ponte.[258]	2014	LKD	Donors=27	No controls	Cross-sectional, observational. 0, 1, 2, 3, 180 and 360 months after donation.	No change significantly post donation. At 180 days there was no change in FGF23 levels compared to baseline.	No difference
Moody.[241]	2015	LKD and Healthy controls	Donors=68 Controls=56	Healthy controls meeting LKD criteria	Prospective Longitudinal Baseline and 12 months. Multi-centre.	Increase significantly from 67-84 RU/ml post donation.	Increase
Thorsen.[257]	2016	LKD CKD stage Healthy controls	Donors=35 CKD 3=22 CKD 4= 18 CKD 5= 20 Controls=35	Colleagues and friends of the authors.	Cross-sectional, observational, single-centre.	Non-significantly higher in donors compared to controls. Increased as renal function deteriorated.	No difference
Kasiske.[255]	2016	LKD	Donors=182 Controls=173	Matched controls	Prospective, longitudinal. Baseline, 6 months and 36 months post donation.	Serum FGF-23 levels at 6 and 36 months were higher than controls.	Increase

CKD; Chronic Kidney Disease. LKD; Living Kidney Donors.

Table 1.8. Markers of inflammation. First published in Price et al.[2]

Reference	Date	Population	Study numbers	Control group	Study type	Outcome	In donors
C-reactive protein (CRP)							
Kielstein.[261]	2011	LKD	Donors=24	No controls	Cross-sectional. 1, 6, 12, 24, 72, and 168 hrs post nephrectomy	Increase post operatively significantly at 6 hours. Peaked at 3 days and then began to decline. Still above baseline at 7 days post nephrectomy.	Increase
Huan.[254]	2013	LKD	Donors=34	No controls	Longitudinal Baseline and 6 months	No significant difference between baseline and 6 months.	No difference
Kasiske.[253]	2013	LKD and Healthy control	Donors=201 Controls=198	Healthy siblings of LKD approached first. Healthy controls meeting LKD criteria.	Prospective. Observational cohort study. Baseline and 6 months.	No difference between donor and controls	No difference
Moody.[241]	2015	LKD and Healthy controls	Donors=68 Controls=56	Healthy controls meeting LKD criteria	Longitudinal Baseline and 12 months. Multi-centre.	Increased serum high sensitivity CRP in donors compared to controls at 12 months 1.90mg/dl vs 1.00.	Increase
Interleukin-6 (IL-6)and Tumour necrosis factor alpha (TNFα)							
Kielstein.[261]	2011	LKD	Donors=24	No controls	Cross-sectional. 1, 6, 12, 24, 72, and 168 hrs post nephrectomy	Increases prior to CRP. Elevated at 1 hour post operatively then began to decline. Still about baseline at 7 days post nephrectomy.	Increase
Huan.[254]	2013	LKD	Donors=34	No controls	Longitudinal Baseline and 6 months	No significant difference in IL-6 or TNFα post donation.	No difference

LKD; Living Kidney Donors.

Table 1.9. Markers of myocardial fibrosis and left ventricular hypertrophy. First published in Price et al.[2]

Reference	Date	Population	Study numbers	Control group	Study type	Outcome	In donors
N-terminal prohormone of brain natriuretic peptide (NT-pro BNP)							
Bellavia.[251]	2015	LKD	Donors=15 Controls=15	Italian donors. U.S age and gender matched controls.	Cross sectional. Measurements at least 5 years post donation.	No difference between donors and controls	No difference
Moody.[241]	2015	LKD and Healthy controls	Donors=68 Controls=56	Healthy controls meeting LKD criteria	Prospective Longitudinal Baseline and 12 months. Multi-centre.	No difference between donors and controls.	No difference
Altmann.[242]	2017	LKD	Donors=23	No controls	Prospective, cohort study. Baseline, 4 months and 12 months.	No difference following donation.	No difference
High sensitivity-Troponin							
Moody.[241]	2015	LKD and Healthy controls	Donors=68 Controls=56	Healthy controls meeting LKD criteria	Prospective Longitudinal Baseline and 12 months. Multi-centre.	Increase in detectable serum hs-cTnT≥5 ng/L in donors 21% vs 2%	Increase
Amino-terminal peptide of procollagen III (PIIINP) and Procollagen type I N terminal propeptide (PINP)							
Bellavia.[251]	2015	LKD	Donors=15 Controls=15	Italian donors. U.S age and gender matched controls.	Cross sectional. Measurements at least 5 years post donation.	Elevated PIIINP levels seen in donors 5.8 (5.4–7.6) µg/L) vs (1.1 (0.9–1.3)mg/dL.	Increase
Kasiske.[255]	2016	LKD	Donors=182 Controls=173	Matched controls	Prospective, longitudinal. Baseline, 6 months and 36 months post donation.	PINP concentrations were higher at 6 months than paired normal controls (24.3% and 8.9%). No difference at 36 months.	Increase
Altmann.[242]	2017	LKD	Donors=23	No controls	Prospective, cohort study. Baseline, 4 months and 12 months.	Increase in PIIIP donors seen at 12 months 0.45±0.11ng/ml vs 0.56±0.14ng/ml	Increase

Hs-cTnT; High sensitivity cardiac troponin T. LKD; Living Kidney Donors. PIIINP; Procollagen type III N-Terminal-is involved in fibroblast activation. PICP; Procollagen type I N-Terminal propeptide -indicates collagen synthesis.

1.7.6 Cardiac structure and function

Several studies have investigated whether human kidney donation causes structural and functional change in the LV.[241, 242, 251] Moody et al. studied 68 donors and 56 equally healthy controls (many of whom were worked up for donation but did not donate) as part of the Chronic Renal Impairment in Birmingham (CRIB)-DONOR study . [241] At 12 months there was an increase in LV mass measured by CMR in donors but not controls.[241] GCS was also decreased indicating early changes in systolic dysfunction.[241] There was no change in blood pressure measured by ambulatory monitoring and no association between change in LV mass and changes in blood pressure.[241] In a similar but uncontrolled and smaller study also using CMR, Altmann et al. studied 23 living kidney donors and found that LV mass had increased at 12 months without change in office blood pressure.[242] In a small cross sectional echocardiographic and CMR study, 15 Italian donors were compared to age and sex matched healthy controls from the US at a median of 8.4 years (minimum of 5 years) from donation.[251] Most measures of LV geometry and function were not different in donors and controls but donors did exhibit abnormalities of LV apical rotation and torsion.[251] In contrast, Hewing et al. also studied 30 living kidney donors at baseline and 12 months following donation using 2D speckle tracking echocardiography and found no significant differences in left or right ventricular function.[262]

In summary, there are few studies investigating cardiac structural and functional change after kidney donation. The studies that do exist have small sample sizes. Current evidence indicates that kidney donation results in small changes in cardiac structure and function. Whether these changes are sustained and are associated with an increase in cardiovascular risk is not known. Well controlled follow up studies with serial cardiac investigations are required.

1.8 Summary

In summary, CKD is associated with increased risk of both all-cause and cardiovascular mortality compared to the general population.[15] Cardiovascular mortality and cardiovascular events in CKD are not easily predicted by conventional risk modelling and the increased risk of cardiovascular mortality persists despite careful adjustment for traditional risk factors.[48] Furthermore, many cardiovascular deaths are not attributable to occlusive coronary disease with increasing rates of arrhythmia and sudden cardiac death as renal function declines.[33] Taken together, cardiovascular morbidity and mortality in CKD cannot be attributed solely to atheromatous disease and is likely to be related to other forms of arterial disease and cardiac muscle disease.

Over the last 20 years a combination of post mortem studies, echocardiography and more recently novel imaging techniques using CMR have given us insight into the structural and functional changes of cardiac muscle observed in ESRD.[104, 130, 165] LV abnormalities include increases in LV mass, LGE and elevated T1 times suggestive of myocardial fibrosis.[130] In addition diastolic dysfunction, impaired myocardial deformation and a picture consistent with heart failure with a preserved ejection fraction emerges.[130] The term 'uraemic cardiomyopathy' is now better recognised and better defined.

There is now strong evidence from multiple sources that structural and functional changes begin to occur in earlier stages of CKD. Even those with stage 2 or 3 CKD have shown subtle changes to GLS and increases in LV mass.[145] Moreover, these changes have often been shown to occur in a graded relationship with GFR.[145] These findings have two important implications. Firstly, if changes are evident early in CKD then perhaps uraemic cardiomyopathy is a misnomer and CKD cardiomyopathy is a much more appropriate term. Secondly, if cardiovascular changes are occurring early in CKD and are independent of

traditional risk factors then it would be expected that the same processes are occurring in living kidney donors who lose approximately a third of their baseline renal function.

Living kidney donors account for approximately half of all UK transplants. [205, 208, 211] The UK Organ Donation and Transplantation Activity Report for 2019-2020 has indicated that numbers of living kidney donations have remained relatively static over the last seven years.[212] Overall there was a small reported fall from a peak of 1,148 living kidney donors in 2013-2014 to 1,001 in 2019-2020.[212] Despite this there remains some doubt over the very long term consequences of donation. Several reports have suggested that donors share many of the same predispositions to disease as those with CKD such as hypertension, gout and pre-eclampsia.[2] One prominent epidemiological study has also reported increasing rates of cardiovascular mortality in donors.[223] Despite the study's flaws, further smaller cohort studies have also suggested cardiovascular changes including small increases in LV mass, reduced aortic distensibility, abnormalities in apical torsion and changes to GCS in donors compared to controls.[241, 242, 251]

Living kidney donors are an intriguing group to study. Research into the cardiovascular consequences of living kidney donors not only expands our knowledge of the long term safety of living kidney donation but it might provide us with valuable insights into the pathophysiology of cardio-renal disease by allowing examination of the isolated effects of a reduction in GFR on the cardiovascular system, see **Figure 1.10**.[2]

This thesis aims to investigate the effects of a reduction in GFR after nephrectomy on cardiovascular structure and function over a 5 year period.

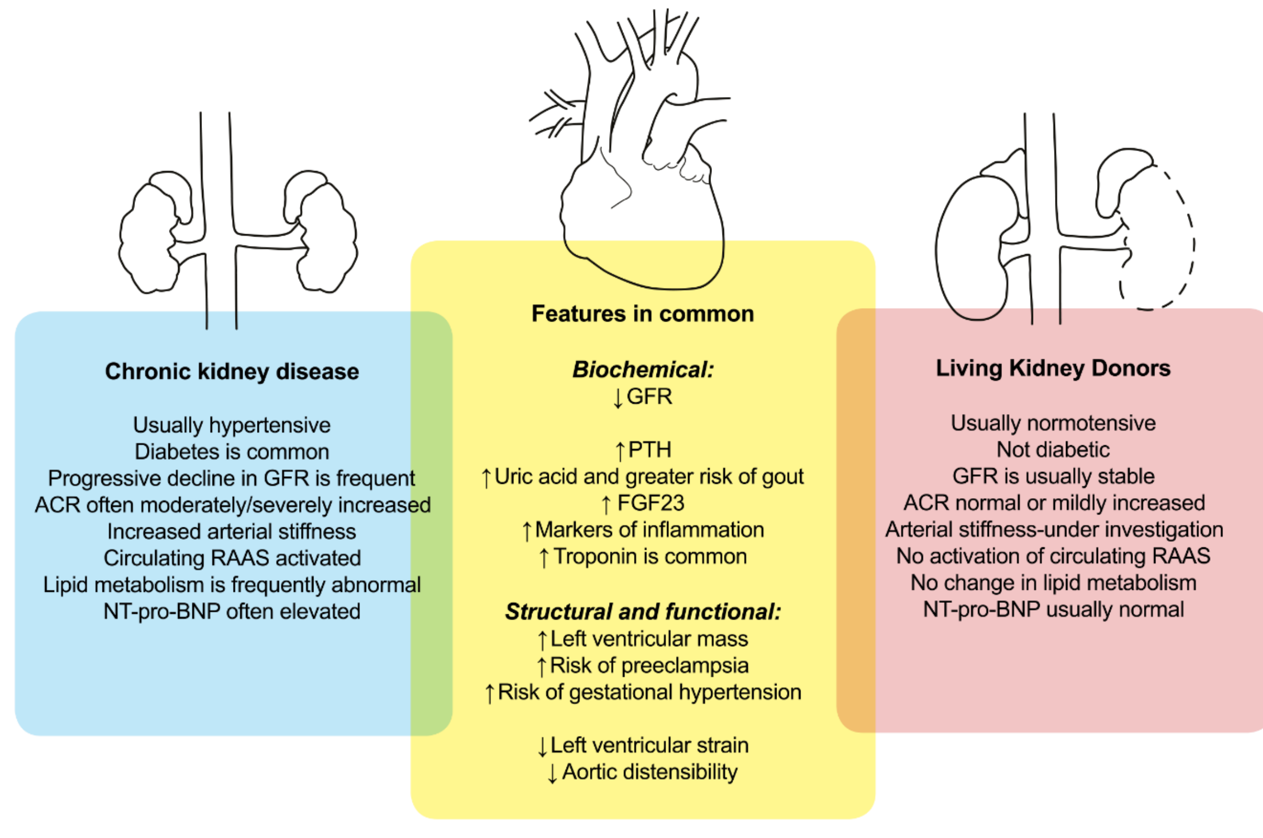

Figure 1.10. A comparison of donors and patients with CKD. Features in common give us valuable mechanistic information for possible mediators of cardiac disease.[200, 217, 225, 235, 241, 242, 245-247, 249, 251, 253-256, 259, 261, 263] First published in Price et al.[2]

PTH; Parathyroid hormone. RAAS; Renin-angiotensin-aldosterone system. GFR; Glomerular filtration rate. NT-Pro BNP; N-terminal pro-brain natriuretic peptide. FGF23; fibroblast growth factor-23. ACR; urine albumin to creatinine ratio.

1.9 Aims and hypothesis

1.9.1 Aims

1. To determine whether the reduced GFR after kidney donation results in adverse effects on blood pressure and arterial function at 5 years from donation.
2. To determine whether the reduced GFR after kidney donation results in adverse effects on myocardial structure and function at 5 years after nephrectomy.
3. To determine whether the reduced GFR after kidney donation results in adverse effects on myocardial tissue characterisation and biomarkers suggestive of inflammation and fibrosis at 5 years after nephrectomy.

1.9.2 Hypothesis

A reduction in GFR following living kidney donation is associated with adverse cardiac and vascular effects detectable at 5 years.

Adverse effects will include:

1. An increase in LV mass.
2. Impaired LV systolic function.
3. Increased LV interstitial fibrosis (elevated T1 and ECV).
4. Reduced aortic distensibility.
5. Increased systolic but not diastolic blood pressure.
6. Increases in markers of inflammation and biomarkers suggestive of myocardial fibrosis and LV stretch.

CHAPTER 2

GENERAL METHODS AND MATERIALS

2.1 Study population

Living kidney donors were recruited for studies within this thesis according to national guidelines published by the Joint Working Party of the British Transplantation Society and the Renal Association for living kidney donors.[264] These guidelines set out the inclusion and exclusion criteria for living kidney donors.[264, 265] In addition, subjects also required an age specific estimated glomerular filtration rate (eGFR) high enough to be suitable to donate in accordance with British Transplantation Guidelines.[264] All healthy controls were also required to meet the same criteria, see **Figure 2.1** Subjects recruited for the CRIB-DONOR II study were recruited from May 2017 to May 2019.

2.2 Clinical assessment

2.2.1 Basic demographics

Basic demographics of the subjects were recorded. Subjects were asked to recall past medical history and any medical events since their previous visit. Medication history, including dates when medication was commenced or stopped were also included. Height and weight were recorded for calculation of BMI and body surface area.

2.2.2 Office blood pressure

A BpTRU™ (BPM_100 model) blood pressure device, validated by the British Hypertension Society was used for measures of office blood pressure.[266, 267] The BpTRU device blood pressure measurements have been found to closely correlate with ambulatory blood pressure recordings.[268] Office blood pressure was recorded when the patient was sitting at rest for at least 5 minutes. Blood pressure was recorded using an appropriate cuff size on the non-dominant arm. The BpTRU uses an automated cycling setting to record five consecutive blood pressures and heart rates at one minute intervals and then generates an average. The average was used for the

analysis. The same process was followed for supine blood pressure, ensuring the subject had been lying for at least 5 minutes before commencing measurements.

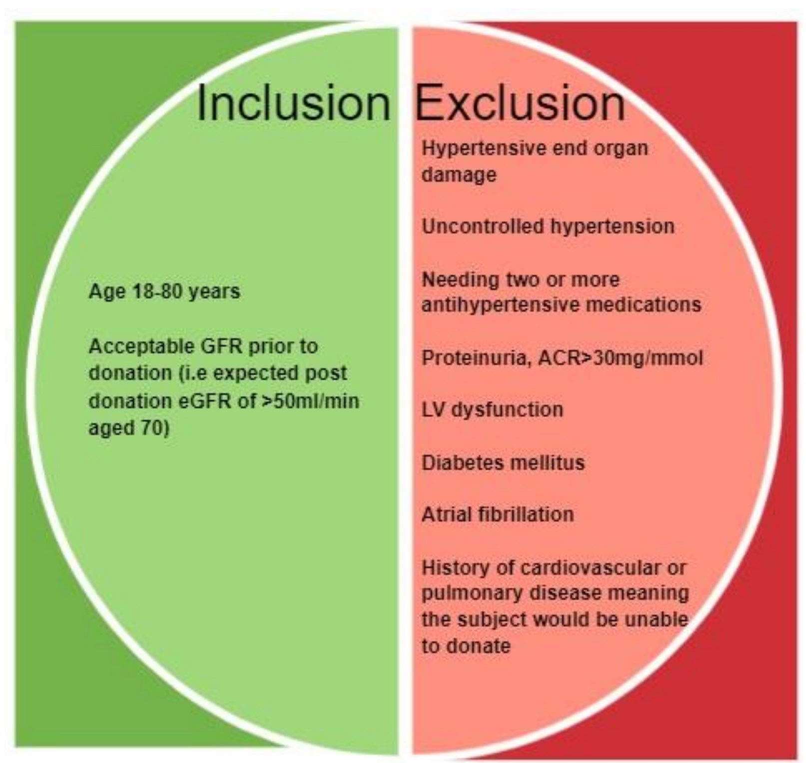

Figure 2.1. Inclusion and exclusion criteria. Adapted from Moody et al.[249]

GFR; Glomerular filtration rate. LV; Left ventricle.

2.2.3 Electrocardiogram

A standard 12-lead electrocardiogram (ECG) was taken during the visit. The ECG was assessed for arrhythmia, changes in the ST segment and T wave inversion.

2.2.4 Ambulatory blood pressure monitoring

Following the study visit, subjects were fitted with 24-hr blood pressure monitors. A Mobil-O-Graph®, PWA machine was used. An appropriate sized cuff was attached to the non-dominant arm. The monitor was set to record peripheral blood pressure every 30 minutes from 8:00am-21:59pm and every hour from 22:00pm-7:59am. Subjects were encouraged to relax their arm during a recording and go about normal activities but to avoid heavy exercise.

The 24-hr recordings were evaluated for both patient compliance and artefact. Adequate recordings were in accordance with the European Hypertension Society Guidelines which states at least 14 valid day time recordings and at least 7 night time recordings are required.[269]

2.3 Biochemical tests and biomarkers

2.3.1 Laboratory blood and urine samples

Blood and urine samples were taken for standard laboratory tests including a full blood count (haemoglobin, white blood cells, and platelets), urea and electrolytes (sodium, potassium, urea, creatinine and bicarbonate), liver function tests (alkaline phosphatase, bilirubin) and bone profile (phosphate and corrected calcium). Blood samples were also taken for PTH, aldosterone, renin, vitamin D, urate, thyroid function, magnesium, total cholesterol (non-fasting), random glucose and glycated haemoglobin. Blood was taken to the laboratory immediately after being drawn and processed within an hour of being taken. A urine sample was also sent for ACR. In addition, two blood samples taken in serum separator tubes were allowed to clot for 10-30 minutes and then centrifuged at 4°C at 1500g for 15 minutes. The serum supernatant was aliquoted into 1ml

cryovials and stored at -80°C. Two samples taken in ethylenediamine tetraacetic acid (EDTA) tubes were also centrifuged at 4°C at 1500g for 15 minutes. The plasma supernatant was aliquoted and stored at -80°C. Urine was centrifuged twice at 4°C for 15min at 1500g then one aliquot of 2ml was stored at -80°C.

2.3.2 Assessment of glomerular filtration rate

Estimated glomerular filtration rate

An eGFR is essential in the assessment of living kidney donors before donation.[270] Assessment for elective nephrectomy however, provides challenges for all current methods of eGFR using endogenous filtration markers as they tend to be more inaccurate at the ranges of GFR encountered in donors.[271] The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2009 equation is the current recommended method of estimating GFR for those with high or normal renal function such as living kidney donor candidates and was therefore the chosen equation used for this study.[270]

Measured glomerular filtration rate

The 2018 British Transplantation Society guidelines for kidney donation recommend that prospective kidney donors undergo a measured GFR (mGFR) using exogenous filtration markers prior to donation.[272] There are a range of exogenous filtration markers that can be employed. In the UK and specifically in our centre, ^{51}Cr-EDTA is used most often and considered the 'gold standard'.[273, 274]

During this study only living kidney donors underwent an isotopic GFR (iGFR). This was in part due to financial constraints and in part due to the ethical considerations of repeat exposure to ionising radiation in a healthy control group. The dose of radiation per individual however, is estimated to be 0.004mSv and the background radiation exposure per person in the UK is

estimated to be 2.3 mSv per year.[275] Consequently, the additional dose is less than the amount of natural background radiation a person would be exposed to in one day.

Ethical approval and an Administration of Radioactive Substances Advisory Committee (ARSAC) research certificate were obtained for the radioactive GFR measurement.

Study subjects were asked to eat a light breakfast on the morning of the GFR measurement visit. They were advised to avoid protein rich meals throughout the test as these can lead to a higher GFR with ^{51}Cr EDTA.[276] Normal hydration was encouraged. The iGFR was carried out within the Nuclear Medicine Department by specialist technologists and analysed by Clinical Scientists with support from a Medical Physics Expert.

In line with the local departmental policy to comply with the Ionising Radiation Medical Exposure Regulation (IRMER) 2000 and 2017, women aged up to 55 years were asked if their menstrual period was overdue.[277] If the menstrual period was not overdue the iGFR was performed. This is known as the "missed period rule" and is based on guidance from the National Radiological Protection Board because conception is unlikely in the first 10 days of the menstrual cycle.[277] If it were not overdue the iGFR was performed. If she could not exclude pregnancy and her period was overdue the iGFR could proceed provided a negative urine human chorionic gonadotropin pregnancy result was obtained on the day of the test.
An estimated GFR within the last three months was required to decide on specific blood sample timing following administration of the radioisotope. Due to annual living kidney donor follow up being in the subject's local hospital this was not always available and in such cases a venous sample of serum was sent urgently for eGFR on the day of the study visit and prior to injection of the radioisotope. All other blood samples required for the study were taken before isotope administration for consistency.

Following safety checks a single dose of 1.85 MBq ^{51}Cr-EDTA in 2.5ml was injected into a large vein within antecubital fossa. This was followed by at least 10ml of saline flush through the cannula. The precise amount of ^{51}Cr-EDTA was determined by weighing the syringe before and after administration to the patient using an analytical balance. Venous administration (rather than extravasation) was confirmed by using a Series 900 monitor with Type 44A scintillation detector (Mini Instruments Ltd. UK) to measure the gamma ray count rate from ^{51}Cr at the injection site and the contralateral arm. Three 10ml venous blood samples in heparinised tubes were taken from the contralateral arm to ^{51}Cr-EDTA administration to avoid contamination at 2, 3 and 4 hours post administration if the eGFR was >60ml/min/1.73^2 or 2, 4 and 6 hours if the eGFR was between 30-60ml/min/1.73m^2 . The exact time of each sample was noted.

The blood samples were stored at room temperature overnight.[278] The following day, blood samples were centrifuged and equal volume aliquots of plasma pipetted from each tube for gamma counting. A standard solution was prepared by diluting 0.185 MBq (0.25ml) of the same ^{51}Cr EDTA batch as injected into the patient, to 1000ml with water. Aliquots of the standard (same volume as the plasma samples) were pipetted into two standard tubes for gamma counting. Two background tubes containing water, the two ^{51}Cr EDTA standard tubes and the three plasma samples were then counted for 10 minutes using a Cobra Auto Gamma Counter (Packard Ltd.) with energy window 240-400 keV to bracket the ^{51}Cr gamma ray at 320 keV.

Rates of plasma clearance were determined, taking into account background radiation using a slope-intercept method. A linear fit was applied to the log of the background-corrected count rate from the three plasma samples.[278] The GFR was calculated for a 1.73m^2 body surface area and the Brochner-Mortensen correction was applied as recommend by the British Nuclear Medicine

Society.[278-280] The calculation was routinely performed using a Visual Basic programme on the Cobra Auto Gamma counter (Packard Ltd.).

2.3.3 Enzyme-linked immunosorbent assays

Fibroblast growth factor-23

FGF23 levels were analysed using the Human FGF23 carboxyl-terminal 2nd generation enzyme-linked immunosorbent assay (ELISA) kit manufactured by Immutopics, Inc, San Clemente, California (Catalogue number: 60-6100).[281] It was chosen specifically as it matched that used in the original CRIB-DONOR study and has a lower reported intra-individual variability than intact FGF23.[241, 282] The detection of c-terminal FGF23 uses a sandwich ELISA principle in which the antigen within the sample is bound to the coated plate. The capture antibody and enzyme (in this case horseradish peroxidase (HRP)) conjugated antibody bind to the antigen and thus once the substrate is added, enzymatic activity (measured by timed absorbance), is relative to the sample concentration.

Three plates with the same serial and expiry number were ordered from the same manufacturer (lot number: 142958). Analysis for all the 5 year samples were conducted over consecutive days in the same temperature controlled laboratory. No identifying information was provided on sample cryovials and therefore I was blinded to donor/control status. Consequently, each plate contained a random selection of healthy controls and donors. A 'biological control' was analysed on each consecutive plate to ensure accuracy and consistency across the plates. This consisted of the same subject sample being tested on each plate.

The plasma samples (from EDTA tubes) had been frozen at -80°C and only used once (no thaw-refreeze cycle). Samples were gently thawed on ice whilst the following materials were prepared.

Reagents:

All reagents were stored at 4°C and brought to room temperature prior to preparation as follows:

- Human FGF23-standards: Six standards of human FGF23 (40-6131 to 40-6136) were provided within the kit with the concentration provided by the manufacturer. Standard 1 (0 RU/ml) was reconstituted with 2.0ml of deionised water. The remaining standards were reconstituted with 1.0ml of deionised water each: standard 2 (19 RU/ml), 3 (50 RU/ml), 4 (151 RU/ml), 5 (419 RU/ml) and 6 (1383 RU/ml). A timer was started to allow the vials to stand for at least 20 minutes. Vials were inverted on occasions to ensure they were well mixed.
- Human FGF23-controls: Two vials, controls 1 and 2 (40-6141 and 40-6142) were provided with known concentrations. Control 1 had a known concentration of 26-44 RU/ml and control 2 had a known concentration of 208-347 RU/ml. Each vial was reconstituted with 1.0ml of deionised water and again left to stand for 20 min with occasional inversion until thoroughly mixed.
- ELISA wash concentrate (40-0041): The contents (20ml) was diluted with 480ml of deionised water.
- 'Working antibody' solution: The biotinylated human FGF23 antibody (40-6110) and HRP conjugated human FGF23 antibody (40-6120) provided in the kit were mixed with equal volumes immediately prior to use.

Assay procedure: Streptavidin coated microtiter plate strips were placed in the holder and 100µl of each standard, control and thawed subject sample were carefully pipetted into the designated well. One blank well was left with substrate only. All samples were analysed in duplicate. A volume (50µl) of the working antibody solution was subsequently pipetted into each well, the plate was then sealed and covered in aluminium foil before being incubated at

room temperature for three hours on a horizontal rotator set to 180-220rpm. The plate was then washed gently with 350 µl of wash solution per well; this step was repeated five times. ELISA HRP substrate (150µl) was added to each well, the plate was sealed and covered with foil then incubated at room temperature for 30min on a horizontal rotator at 180-220 rpm. Once the incubation had finished an absorbance was initially read at 620nm within 5 mins. ELISA stop solution (sulphuric acid, 50µl) was then added to each well and mixed on a horizontal rotator for further 1 min before a second absorbance reading at both 450nm and 620nm was taken within 10min of the addition of stop solution. The absorbance for each duplicate was averaged and then the absorbance recorded from the blank well. The concentration of the sample was derived from a calibration curve of the standards determined at 450nm. Where the sample values were measured to be between the 5th and 6th standard the concentration was calculated from a calibration curve determined from the initial reading at 620nm.

Soluble α-klotho

Soluble α-klotho levels were analysed using the human soluble α-klotho kit manufactured by Immuno-Biological Laboratories Co, Ltd, Japan (Catalogue number: JP27998).[283, 284] A sandwich ELISA method has been developed using a monoclonal antibody with an affinity to human α-klotho and is one of the more widely used commercial assays currently available.[285]

In a similar method used when conducting the FGF23 assays, three plates with the same serial and expiry date were ordered from the same manufacturer (lot number: 1E-809). All the samples at 5 years were analysed on consecutive days in the same laboratory. Again samples were anonymised and analysed in a random order and a biological control was used across plates to ensure consistency.

All reagents were stored at -4°C and brought to room temperature prior to use.

Preparation:

- Wash buffer concentration: A 50ml of wash buffer concentrate containing 0.05% Tween20 in phosphate buffer provided in the kit was diluted with 1950ml of deionised water.
- Labelled antibody preparation: The antibody provided was a HRP conjugated anti-human klotho mouse IgG monoclonal antibody and was diluted with the solution for labelled antibody (containing 1% bovine serum albumin, 0.05% tween phosphate buffered saline) in a 30x dilution. This was done immediately before use.
- Preparation of standard concentrations: The standard solution provided is 0.5ml of 12000 pg/ml soluble α-klotho. This was diluted with 0.5ml of deionised water. For the standard curve, eight micro centrifuge tubes were then labelled 1-8. 230µl of EIA buffer was pipetted into each tube. Then 230µl of the standard solution was the pipetted into tube one. The tube was vortexed to ensure it was completely mixed. 230µl was removed from tube 1 and pipetted into tube 2. The process was repeated until tube 7 to create a series dilution. Tube 8 remained a test sample 'blank' with only 230µl of EIA buffer. The following concentrations were achieved: Tube 1- 6000 pg/ml, Tube 2- 3000 pg/ml, Tube 3- 1500 pg/ml, Tube 4- 750 pg/ml, Tube 5- 375 pg/ml, Tube 6- 187.5 pg/ml, Tube 7- 93.75 pg/ml and Tube 8- 0 pg/ml.

The subject samples used were plasma (from EDTA tubes) and stored at -80°C. They were only used once (no thaw-refreeze cycle). Samples were gently thawed on ice and centrifuged prior to use.

Assay procedure: The plate provided is pre-coated with anti-human klotho mouse IgG monoclonal antibody. A volume of 100µl EIA buffer was pipetted into each well. The standard curve was created by pipetting a volume of 100µl from tube 1-8 in consecutive wells. The same volume of each subject sample was transferred into each well. All samples were analysed in duplicate. The plate was sealed, covered in aluminium foil and left to incubate at room temperature for 60 minutes. Following incubation, the plate was washed gently using the wash solution prepared, this step was repeated 7 times. A volume of 100µl of the labelled antibody was then pipetted into each well. The plate was sealed and covered in aluminium foil for a second incubation of 30 minutes. The plate was washed again gently, this step was repeated 9 times. Chromogen (100µl) was added to each well. The plate was then left for a third 30 minute incubation wrapped in aluminium foil. Sulphuric acid (100 µl) was added to each well to stop the reaction. The plate was then read at 450nm within 30 minutes against the blank. The absorbance of the blank well was subtracted from the recorded absorbance to give the true absorbance. To calculate the sample concentration the standard solutions were plotted against the true absorbance recorded. Each subject sample was averaged and the absorbance of the blank was subtracted. The concentration was then calculated using the standard curve.

N-terminal-pro B type natriuretic peptide

N-terminal-pro B type natriuretic peptide (NT-pro BNP) was measured using serum samples previously stored at -80°C at laboratories within University Hospitals Coventry and Warwickshire, UK. Detection was via electrochemiluminescence immunoassay sandwich principle (Elecsys, Cobas®, Roche diagnostics, Mannheim).[286] The lowest limit of detection for NT-pro BNP was 0.6pmol/L.

High sensitivity Troponin T

Highly sensitive Troponin T (hsTrop T) was measured using serum samples previously stored at -80°C at laboratories within University Hospitals Coventry and Warwickshire, UK. Detection was via electrochemiluminescence immunoassay sandwich principle (Elecsys, Cobas®, Roche diagnostics, Mannheim).[287] The lowest limit of detection for hsTrop T was 5ng/L.

High sensitivity C-reactive protein

High-sensitivity C-reactive protein (CRP) was measured using serum samples previously stored at -80°C at laboratories within Birmingham Heartlands Hospitals, UK. A latex immunoassay principle was used for immunoturbidimetric quantification of high sensitivity CRP (MULTIGENT CRP vario assay®, Sentinel Diagnostics, Milan). A 1:10 dilution was used to determine high sensitivity as per manufacturer guidelines. The lowest limit of detection was 0.01mg/dL.

2.3.4 Multiplex magnetic immunoassays

Two custom premixed multiplex magnetic bead-based immunoassay kits were used (Human Magnetic Luminex® multi-analyte assay, R&D systems, Bio-Techne brand, Minneapolis). One 4-Plex assay kit (catalogue number: LXSAHM-04) and a further 12-Plex assay kit (catalogue number: LXSAHM-12), see **Table 2.1**. All assay kits were ordered from the same manufacturer and had the same lot number and expiry data (4-Plex Lot: 1573578 and 12-Plex Lot: L133365).

Table 2.1. Analytes and units for each immunoassay kit.

4-Plex	12-Plex
Galectin 3 (pg/ml)	Monocyte Chemoattractant Protein-1 (pg/ml)
Matrix Metallopeptidase-9 (pg/ml)	Interleukin 6 (pg/ml)
Neutrophil Gelatinase-Associated Lipocalin (pg/ml)	Tumour Necrosis Factor Alpha (pg/ml)
Uromodulin (pg/ml)	Interleukin 8 (pg/ml)
	Interleukin 10 (pg/ml)
	Interleukin 1 receptor antagonist (pg/ml)
	Angiopoietin-2 (pg/ml)
	Vascular Endothelial Growth Factor (pg/ml)
	Leptin (pg/ml)
	Soluble Suppression of Tumourigenicity-2 (pg/ml)
	Atrial Natriuretic Peptide (pg/ml)
	Kidney Injury Molecule-1 (pg/ml)

The Human Magnetic Luminex® multi-analyte assay allows quantification of multiple human biomarkers simultaneously within a single biological sample. In short, subject samples are added to a well and analyte-specific antibodies are added that have been pre-coated on magnetic beads (microparticles) with embedded fluorophores, to allow the antibodies to bind with any of the specific analytes present in the sample. This type of ELISA based assay is termed a 'sandwich-based' ELISA, as it 'sandwiches' the analyte of interest between two antibodies. Following this, a biotinylated antibody combination specific to the chosen analytes is added. A streptavidin-phycoerythrin (PE) conjugate is added, which binds to the biotinylated antibody. Magnets within the analyser capture the magnetic beads and light emitting diodes excite both the fluorophores within the beads and the PE conjugate to allow determination of how much analyte has bound to the microparticle and which analyte it relates to. The resultant florescence emissions are used to plot a standard curve for each analyte.

Samples of serum were anonymised and analysed at random with a biological control and a blank (negative control) on each plate to ensure quality and consistency. Subject samples were analysed neat. A standard curve was also run on each plate in duplicate. All samples were tested in duplicate and the mean fluorescence intensity (MFI) was used minus the blank MFI, to remove any non-specific background fluorescence. All samples were analysed on consecutive days in the same laboratory.

All reagents were stored at -4°C and brought to room temperature prior to use.

Preparation of kit components:

- Wash buffer: 20ml of the wash buffer concentrate (buffered surfactant) was diluted in 480ml of deionised water.

- Reconstitution of standard cocktails: Standards in the kit were unique to the analytes chosen (for the 4 plex, 4 were provided, for the 12 plex, 6 were provided). Each standard cocktail was reconstituted with calibrator diluent RD6-52 according to the standard cocktail lot information and reconstitution volume and allowed to dissolve for at least 15 minutes.
- Preparation of standard curve: Seven 1.5ml polypropylene tubes were labelled standard 1-7. In tube 1, 100µL of each standard cocktail was added. Calibrator diluent RD6-52 was added to ensure the total volume in tube 1 was 1000µL (for the 4 plex 600µL was added, for the 12 plex 400µL added). In tube 2-7, 200µL of calibrator diluent RD6-52 was added to each tube. A 3-fold dilution series was then produced by removing 100µL from tube 1 and pipetting it into tube 2. Tube 2 was vortexed then 100µL was removed from tube 2 and pipetted into tube 3 and so on. Tube 7 remained a blank with calibrator diluent only.
- Diluted microparticle cocktail preparation: The vial was put in a water bath for sonication for 15 minutes. The vial was then centrifuged at 1000g for 30 seconds. The microparticle cocktail (500µL) was diluted with calibrator diluent RD6-52 (5ml) immediately before use and stored in an amber bottle protected from light.
- Diluted biotin-antibody cocktail: The vial was centrifuged at 1000g for 30 seconds and gently vortexed. Biotin-antibody cocktail (500 µL) was diluted with 5ml calibrator diluent RD6-52 and vortexed to mix.
- Streptavidin PE preparation: The vial was centrifuged at 1000g for 30 seconds and gently vortexed. Streptavidin concentrate (220 µL) was diluted with 5.35ml of prepared wash buffer immediately before use and stored in an amber bottle protected from light.

Assay procedure: Serum samples, stored at -80°C, were gently defrosted on ice, then centrifuged at 16,000g for 4 minutes to pellet any unwanted cell debris. A volume of 50µL of either the standards controls, or participant samples were added to each well, according to a specific plate layout. The microparticle cocktail was sonicated and gently vortexed prior to being added to each well (50µL). The plate was sealed with a foil plate sealer and left to incubate at room temperature on a horizontal plate shaker set at 800rpm (Corning® LSE™ Digital microplate shaker) for 240 minutes. After incubation, wells were washed three times with 100µL of wash buffer using a magnetic wash station (Bio-Plex Pro™ Wash station, Bio-Rad, California), to ensure no magnetic microparticles were removed from the wells. A volume of 50µL of biotin-antibody cocktail was then added to each well, the plate sealed with a foil plate sealer and left for a second incubation at room temperature for 60 minutes on a plate shaker at 800rpm. Following incubation, the wells were washed again three times with 100µL of wash buffer using the Bio-Plex Pro™ Wash station. Streptavidin-PE (50µL) was added to each well. The plate was sealed with a foil plate sealer and allowed to incubate at room temperature for 30 minutes on a plate shaker at 800rpm. A final three washes were carried out using 100µL of wash buffer and the Bio-Plex Pro™ Wash station. Wash buffer (100µL) was added to each well to resuspend the microparticles and the plate was agitated gently for 2 minutes on a plate shaker at 800rpm with a foil lid to prevent unwanted photobleaching prior to quantification. Florescence emissions were measured immediately (Bio-Plex Luminex®200™, Bio-Rad, California). A standard curve was created using the Bio-Plex Software Manager™, version 6.1 management system, using a five parameter logistic curve fit to calculate concentrations.

2.4 Non-invasive vascular assessment

2.4.1 Measures of arterial stiffness and its surrogates

A non-invasive vascular assessment of each subject was carried out using the SphygmoCor system®, Atcor medical Australia, which utilizes applanation tonometry, a widely used 'gold standard' method of assessment.[288] This included measurements of both pulse wave analysis (PWA) at the radial artery and carotid-femoral PWV using a high fidelity micromanometer (SPC-301; Millar Instruments, Houston, TX).[249]

Pulse wave analysis

PWA is a non-invasive method of utilizing peripheral arterial waveforms, peripheral blood pressure and validated transfer functions to calculate central aortic blood pressure and surrogate indices of systemic arterial stiffness such as augmentation index (AIx). [289-292] This technique has been used over the last two decades in both CKD and ESRD to predict cardiovascular events and mortality.[293, 294] It is highly reproducible in these cohorts and has often been used as outcome measures in a number of randomised controlled trials in CKD.[103, 295, 296]

To obtain a peripheral waveform reading subjects were asked to lie supine for at least 15 minutes in a temperature controlled room prior to assessment.[249] The elbow of the non-dominant arm was rested on a pillow with the wrist in a dorsiflex position.[249] Peripheral supine brachial systolic and diastolic blood pressure (measurement as previously described) was entered into the SphygmoCor software system prior to analysis. The subject was asked to relax and not to talk during the analysis. The tonometer was placed on the radial artery with enough pressure to visualise a waveform without occlusion. The tonometer position was adjusted to ensure consistent, smooth uniform waveforms. At least 15 seconds of continuous waveforms were recorded before the data was captured. Inbuilt SphygmoCor software provides an index indicative of the quality and reproducibility of waveforms and other quality control metrics

including waveform height and variation in shape. An acceptable operator index was considered to be >90% and the quality control metrics (i.e. pulse height >100 and diastolic variation <5) generated from the software were reviewed before being accepted.[297] At least three high quality recordings were taken. Both the average central blood pressure and AIx generated from the recordings were used for statistical analysis.

AIx is one of the primary outcome measures generated from PWA yet it is frequently referred to as only a surrogate marker of systemic arterial stiffness as it is the result of a multitude of factors in which vascular stiffness is just one.[298, 299] Its determination is based on both a combination of forward flow from the ventricle and backward flow generated from reflection in peripheral vasculature, see **Figure 2.2**.[298] The discrepancy between peripheral systolic blood pressure (forward flow) and aortic systolic blood pressure (reflected flow) is the augmentation pressure. Augmentation pressure is calculated as a percentage of pulse pressure, giving an AIx.[288, 298] Consequently, the timing of the reflected waveform and therefore heart rate has a major influence on AIx.[298, 300] For comparative purposes, particularly in this longitudinal study, AIx was corrected for a heart rate of 75 beats per minute by the SphygmoCor system using regression (based on a 4.8% reduction in AIx for every 10bpm increase), see **Figure 2.3**.[301, 302,288]

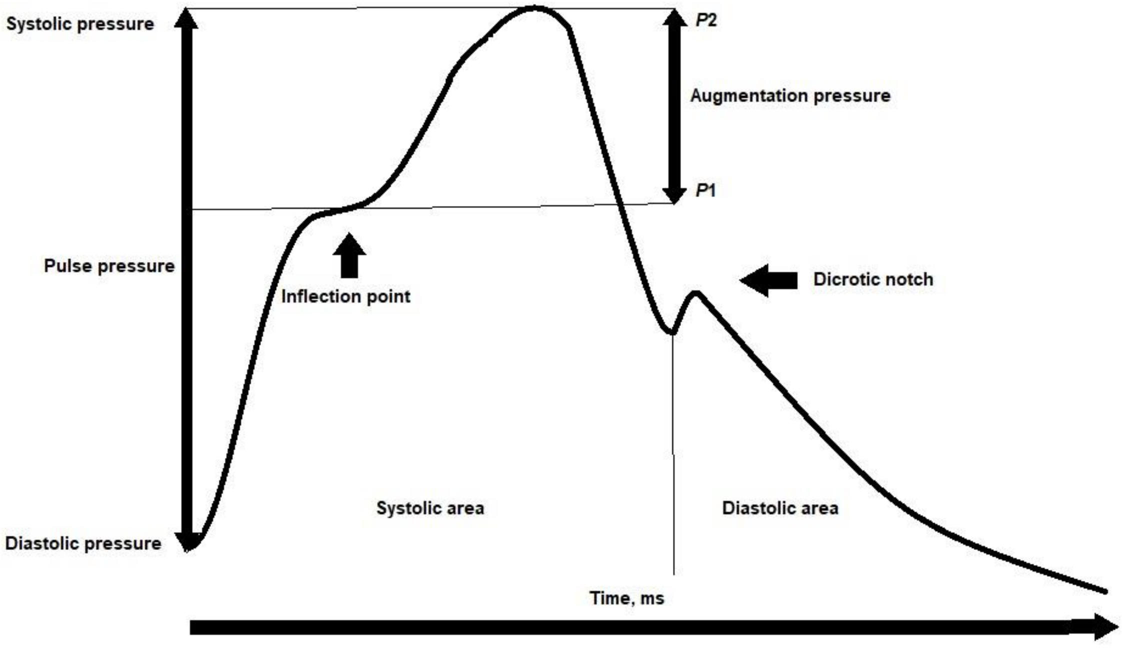

Figure 2.2. Diagram of an aortic pressure waveform. Adapted from Stoner et al.[288]
The forward and reflected wave are demonstrated. The difference between them is known as the augmentation pressure. Augmentation index is the augmentation pressure given as a percentage of pulse pressure.

P1; First systolic pressure. P2; Second systolic pressure.

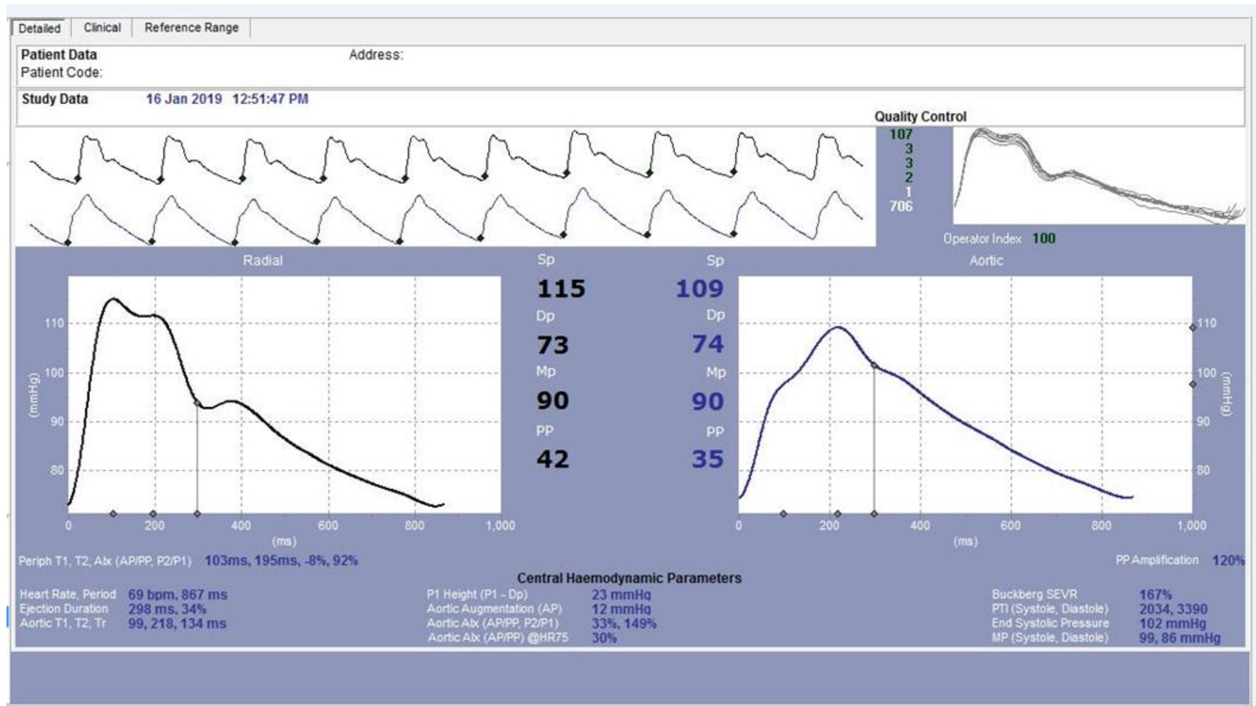

Figure 2.3. A typical recording from radial artery applanation tonometry.
At the top a series of individual waveforms can be seen. In the top right panel the waveforms are overlaid to demonstrate quality and reproducibility. An operator index is given along with quality control metrics. The large waveform on the left is that of the radial artery waveform. The peripheral blood pressures entered at the time of the examination include the brachial systolic and diastolic blood pressure shown in black (115/73mmHg). The wave form on the right is the central pressure waveform generated using transfer functions along with derived central blood pressure readings in blue (109/74mmHg). Central haemodynamic parameters are given below and include augmentation pressure, augmentation index and augmentation index corrected for heart rate.

Carotid-femoral pulse wave velocity

Carotid-femoral PWV has been demonstrated to be a reliable, reproducible and valid non-invasive assessment of regional arterial stiffness in the general population and those in CKD.[295, 299, 303] Carotid-femoral PWV has been used in a wide range of longitudinal studies.[294, 304]

Prior to assessment a supine peripheral blood pressure was entered into the SphygmoCor system software. The distance between the sternal notch and the femoral pulse (distal distance) and the distance between the sternal notch and the carotid artery (proximal distance) also entered into the software were measured using a tape measure after identifying anatomical landmarks. The distance used to calculate PWV was the difference between the distal distance and the proximal distance.[299, 305] The algorithm used for calculating PWV was intersecting tangents. Electrodes were placed on the patient's chest for a 3-lead electrocardiogram trace ensuring an R wave with a good amplitude. The subject was assessed after 15 minutes supine rest and was asked to rotate their head laterally and point their chin into the air.[306] The tonometer was placed where the carotid pulse felt strongest. The carotid waveforms were reviewed for quality and at least 15 seconds of waveforms were recorded. The same was then repeated with the femoral pulse. Following at least a 15 second capture of the femoral waveform the PWV was calculated. The electrocardiogram and waveforms were reviewed for quality and consistency ensuring that the upstroke of the R wave is clear. At least three readings were taken but readings were repeated if there was a discrepancy of >0.5m/s between readings or the standard error of the mean was >1.0m/s, see **Figure 2.4**. An average of three readings was recorded.

PWV is calculated using the time from the peak of the R-wave to the foot of both the carotid and femoral pulse.[302] Arterial path length is calculated using body surface measures as previously

described.[302] The difference between the time from R-waves to each pulse was then divided by the distance of the arterial path to give carotid-femoral PWV.[302]

In accordance with the American Heart Association (AHA) scientific statement for standardising vascular research, PWV was adjusted for both heart rate and mean arterial pressure.[299] PWV was adjusted using a general linear regression model to determine unstandardized residuals which were added to the predicted PWV using the constant and unstandardized β coefficient from the equation.

Figure 2.4. Example of SphygmoCor output for carotid-femoral pulse wave velocity. The peripheral blood pressure (158/77mmHg), distance (370mm) and algorithm (intersecting tangent) used are listed in the top panel. Site A refers to carotid waveforms and a series of carotid waveforms are shown in the middle panel with the corresponding electrocardiogram shown below. The foot of the pulse and the R-wave is highlighted as a green dot. Those highlighted in red are not included in the final analysis. Site B is shown in the bottom panel and refers to the femoral pulse. The pulse wave velocity and standard deviation is shown in the middle at the bottom of the screen. A reference range for normal subjects according to age can be seen in the graph in the bottom right panel.

2.4.2 Carotid intima-media thickness

CIMT is the distance measured between the luminal-intimal interface and the medial-adventitial interface, see **Figure 2.5.**[307] Detection of the intimal-media thickness using ultrasound is well correlated with histology.[307, 308] Increased thickening of the intima can be considered indicative of early subclinical atherosclerosis.[307] Furthermore, this non-invasive technique of CIMT measurement has been demonstrated to improve risk stratification of cardiovascular events in the general population and in those with CKD. [58, 309]

For adequate measurement it was vital the subject was supine, comfortable and with their head positioned at 45°. Subjects were asked to extend their neck with their chin pointing upwards and turned away from me. A 3-lead electrocardiogram signal was obtained by attaching electrodes to the bony prominences.

A Philips iE33 ultrasound machine was used with a L9-3Mhz linear array transducer recommended for vascular scanning. The ultrasound was set to B-mode system for 2-dimensional scanning in black and white. The probe was placed horizontally at the maximal position of the carotid pulse to identify the carotid artery and jugular vein in a cross-sectional view. The artery was both pulsatile and non-compressible. Once the vessels were seen the ultrasound was rotated to a 90° degree angle to obtain a longitudinal view of the artery. The ultrasound probe was moved superiorly or inferiorly until the bifurcation of the common carotid artery into the external and internal carotid was seen in the middle of the screen, see **Figure 2.6**. Once the anatomical position was identified the focus depth was adjusted to improve clarity of the vessel wall, usually 30-40mm. The screen brightness and then gain was adjusted to optimise edge detection ensuring adequate contact with minimal pressure. Excessive pressure will

obliterate the view of the artery. After adequate amplification of the signal, the carotid intima-media thickness appears illuminated as a white double-line. The two lines delineate anatomical boundaries of the lumen-intima interface and the media-adventitia interface, see **Figure 2.7**. Images of adequate clarity were captured and analysed immediately. Software which allows semi-automated wall tracking by means of edge detection system was employed to measure the interface between the lumen-intima and the media-adventitia using (Automated; Intima-media-thickness (IMT), QLAB, Philips, UK).[310] Automated tracking has been demonstrated to produce lower values than manual tracking and reduce inter-observer variability which is particularly pertinent for longitudinal studies.[310]

Measures were taken from the far wall only, 1cm proximal to the carotid bulb prior to the bifurcation of the common carotid artery and >0.5cm from its end.[311, 312] The carotid bulb was defined as the point at which the common carotid was no longer parallel with the distal carotid.[310] At least three measures were taken on the right and left carotid arteries. As it has previously been established that values are generally higher on the left side, an average of both sides was used in the final analysis and this approach is considered more reproducible.[313, 314]

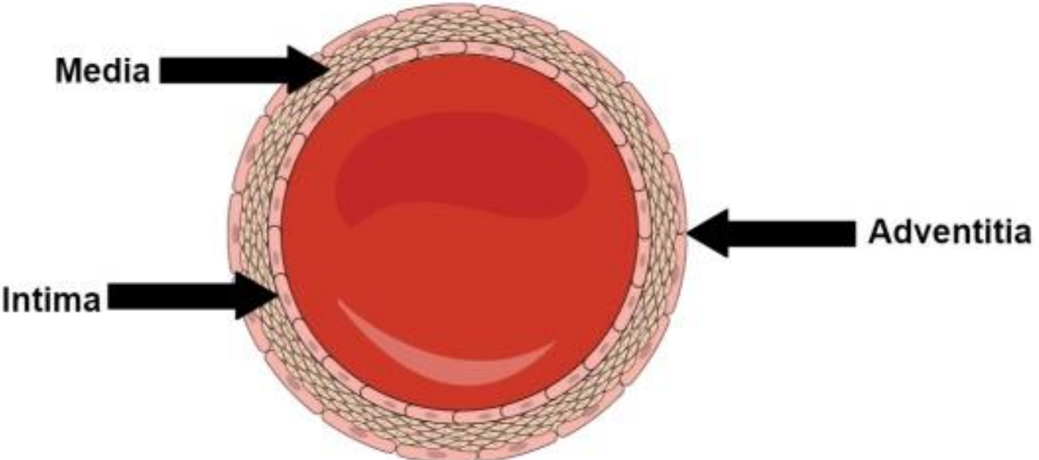

Figure 2.5. Cross sectional diagram of the carotid artery.
A cross sectional view of the carotid artery indicates the outer adventitia, the muscular media within the centre of the arterial wall and the inner intima. The intima-media thickness is the thickness between the lumen-intima interface and the media-adventitia interface shown as bright white lines on ultrasound.

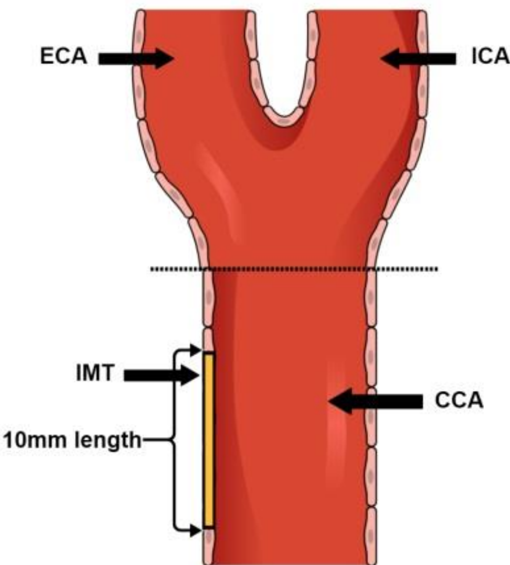

Figure 2.6. Diagram of the carotid tree. Adapted from Touboul et al.[312]
The common carotid artery bifurcates into the external and internal carotid artery. The origin of the internal carotid artery is the carotid bulb (not shown). The dotted line indicates the bifurcation. A 10mm length approximately 1cm proximal from the bifurcation is used to measure intima-media thickness.

CCA; Common carotid artery. ECA; External carotid artery. ICA; Internal carotid artery IMT; Intima-media thickness

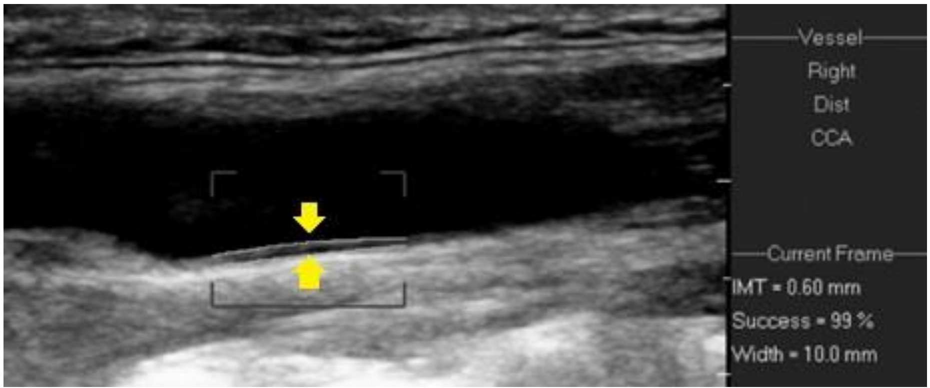

Figure 2.7. Example of intima-media thickness measurement using ultrasound. Following ultrasound of the common carotid artery of a study subject the image was assessed in real time using QLAB plug in software. The carotid bulb is seen and the origin of the internal carotid artery indicative anatomically of the bifurcation. Two white lines are highlighted within the box indicating the intima-media thickness has been detected, see yellow arrows. The thickness is calculated as 0.60mm.

2.5 Cardiac magnetic resonance imaging acquisition

2.5.1 Scanner details and shimming considerations

CMR studies were carried out using the same 3 Tesla scanner (Magnetom, Skyra, Siemens, Erlangen, Germany) in all subjects unless there were specific contraindications to high field strengths in accordance with published MRI safety guidance.[315, 316] In subjects who had contraindications to high field strengths a 1.5 Tesla scanner (Avanto, Siemens, Erlangen, Germany) was used. A 1.5T scanner is frequently used for clinical cardiac CMR because of its ability to achieve high quality, reproducible steady-state free precession (SSFP) cine images owing to reduced sensitivity to flow related artefact.[317] Higher field strengths however, have distinct advantages including increased signal to noise ratio, higher temporal resolution and altered relaxation times improving contrast enhanced scans.[318] There are some disadvantages at higher field strengths such as B0 and B1 inhomogeneity which can lead to higher rates of band, flow and susceptibility artefact across the myocardium.[318, 319] These were offset in this study with high order volume selective B0 shimming over the heart, B1 radiofrequency shimming and reviewing each image before proceeding.[318, 320] This approach is recommended by the 2017 consensus statement by the Society of Cardiovascular Magnetic Resonance (SCMR) for T1 and T2 mapping at higher field strengths.[320] Localised volume-selective shimming was used in combination with frequency scouts to optimize image quality.[318, 319, 321, 322] For dark banding artefacts, frequency scouts spanning resonance frequencies from -300hz to 300hz were routinely used.[317, 323] Images were reviewed in 50hz intervals and the frequency was selected according to the optimum position of artefact away from the region of interest.[317, 323]

2.5.2 Overview of general CMR imaging protocol

A summary of the CMR imaging scanning protocol and timing of sequences is shown in **Figure 2.8**.

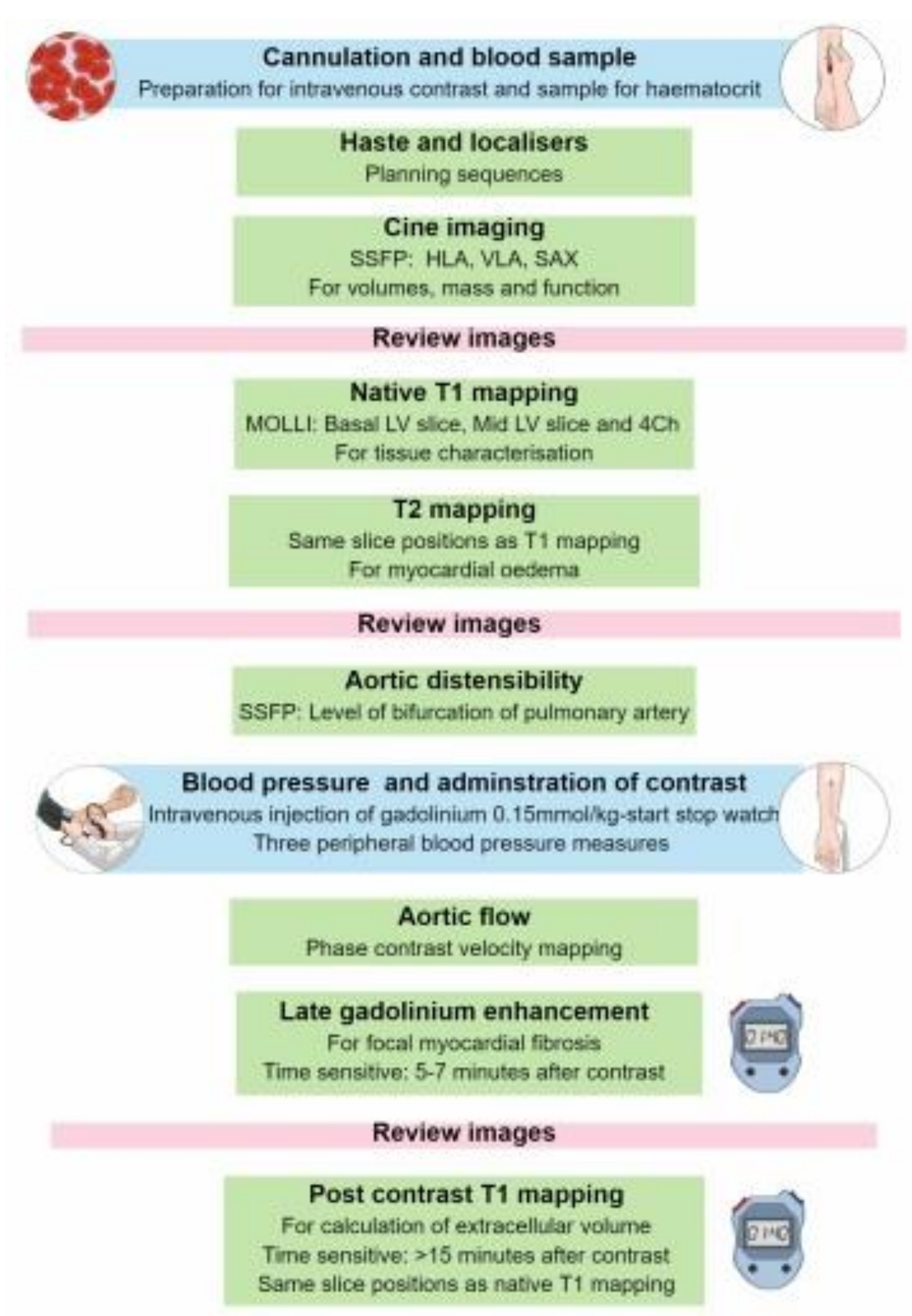

Figure 2.8. A summary of the CMR imaging study protocol.
HLA; horizontal long axis. MOLLI; Modified look-locker inversion recovery. SAX; Short axis stack. VLA; vertical long axis. SSFP; steady-state free precession.

2.5.3 Sequences and protocols

Patient preparation

Following MRI safety checks, subjects had an intravenous cannula inserted into the antecubital fossa and a sample of blood was taken in an EDTA tube for haematocrit levels immediately before the scan.[320] The haematocrit level was required for later calculation of ECV.[321] On the other arm, a blood pressure cuff was attached with the lead left free and unattached. Electrodes were applied to bony prominences for a 3-lead electrocardiogram, ensuring a good quality trace.

Standard localisers and steady-state precession

Standard multi-plane breath held localisers (sagittal, axial and coronal) were used for sequence planning and to ensure the heart was at the magnet isocenter.[322] SSFP cine imaging (retrospective electrocardiographic gated) of the vertical long axis (VLA) and horizontal long axis (HLA) of both the right and left ventricle were used to pilot the LV short axis stack (SAX). SSFP sequences were taken during the end-expiratory phase of a breath hold to reduce inconsistency and respiratory motion. Serial contiguous cines were obtained of the left ventricle starting from the atrioventricular junction and positioned parallel to the apex to formulate the LV stack and thus parallel to the true LV reducing partial volume errors.[324] Typical scan parameters were as follows: repetition time 45.48ms (TR); echo time 1.69 (TE); flip angle (FA); 65°; field of view (FOV) 340mm with a slice thickness of 7mm with a 3mm gap over 25 phases per cardiac cycle. [325] Slice and gap thickness were chosen in keeping with the previous CRIB-DONOR study protocol and previously well described.[241, 326] At this point all images were reviewed to ensure cines were of a good quality before proceeding.

Pre-contrast modified Look-Locker inversion recovery sequence for native T1 mapping

The MOLLI sequence was the first sequence that enabled T1 mapping of the whole of the myocardium but it has since undergone improvement and modification with a wide range of acquisition protcols.[115, 127] In this study, a breath-held SSFP motion corrected MOLLI sequence was used for native T1 mapping at the basal and mid ventricular SAX level and a single HLA view in diastole as recommended for the study of diffuse disease.[320] The basal slice was defined as the first slice of the LV stack with 100% myocardial rim visible throughout the cardiac cycle, without outflow tract to avoid the blood pool. The mid slice was two slices below the basal slice with the presence of papillary muscles. The slice positions were noted and the same image positions were used for post contrast T1 and T2 mapping. Following inversion pulses, serial single shot images were taken in diastole at consecutive heart beats.[327] Sampling for T1 recovery was taken using a 5(3)3 scheme over a total breath hold of 11 R-R intervals, leading to the acquisition of 8 T1 weighted images as previously described.[322] That is in contrast to the older MOLLI scheme of 3(3)3(3)5 which acquired 11 T1 weighted images over a 17 R-R interval.[127] The shorter and therefore faster acquisition time has the advantage of shortening the subjects breath hold so improving image quality and reliability.[328] Typical parameters for the MOLLI sequence were: TR 280.56ms, TE 1.12ms, FA 35° and FOV 360mm. In accordance with the SCMR and CMR Working Group of the European Society for Cardiology consensus statement the scan protocol and parameters remained consistent throughout the study.[321] Acquisition of MOLLI sequences were reviewed throughout the scans for adequate ECG gating and monitoring sequence sounds for mis-trigged or skipped heart beats.[320] All source images were reviewed for cardiac and respiratory motion and artefacts.[320] Suboptimal images were repeated with care not to change any major parameters.[320]

T2 preparation pulse-based sequences

T2 mapping sequences were bright-blood T2-preparation pulse-based sequences.[133] These sequences are less susceptible to blood flow artefact than the alternative dark-blood turbo spin-echo (TSE) sequences.[133, 329] Three single shot T2 weighted SSFP images were acquired at the following T2 preparation times 0ms, 30ms and 55ms at exactly the same slice positions chosen for MOLLI sequences.[329]

Aortic distensibility sequence and protocol

A transverse SSFP cine of the ascending aorta at the level of the bifurcation of the pulmonary artery during a breath hold was chosen for adequate anatomical assessment of the ascending and descending aorta throughout the cardiac cycle. The sequence was retrospectively ECG-gated over 25 cardiac phases. Typical acquisition parameters were TR 45.90ms, TE 2.47ms, FA 12◦, FOV 340mm and voxel size 1.8x1.8x6.0mm. Following the transverse SSFP sequence three peripheral blood pressures were taken in succession and recorded whilst the subject remained on the scanner table (necessary for later calculation of aortic distensibility). A 3 Tesla compatible non-invasive blood pressure monitor by Tesla^{Duo}® (Mammendorfer Institut f*ü*r Physik und Medizin, Mammendorf) was used to determine blood pressure and attached to the cuff already in situ. After blood pressure recordings were taken a bolus of 0.15mmol/kg of gadolinium contrast (Gadovist®, Bayer Healthcare Pharmaceuticals) was injected followed by a 10ml saline flush.[330] The dose remained consistent throughout the study.[320] At the time of injection a stop watch was started before proceeding.

Aortic flow assessment

In the time after gadolinium administration a retrospective ECG gated through-plane phase contrast velocity mapping sequence perpendicular to the ascending aorta was taken to assess aortic forward flow during a breath hold.[331] Typical parameters for the sequence were: TR

37.12ms, TE 2.47ms, FA 20°, FOV 340mm and velocity encoding 150cm/s. The phase cine was reviewed for pixilation in the aorta, evident of background noise and aliasing.[331] Suboptimal images were repeated after adjustment for encoding velocity.[331]

Late gadolinium enhancement protocol

At 5-7 minutes after the administration of gadolinium, scanning commenced for the assessment of LGE. The first scout image was taken using a cine-inversion recovery-SSFP positioned at the mid ventricular slice, in which the normal myocardium is nulled. The inversion time selected was based on the image where the myocardium appears black.[332, 333] Subsequent sequences for assessment of LGE were phase sensitive inversion recovery images of the VLA, HLA and LV SAX in late diastole.[333] The inversion time was adjusted accordingly throughout scanning to ensure the magnetisation level of normal myocardium was nulled. Due to time sensitivity of the sequences, all images were reviewed in real time. If any image was suspected to have evidence of LGE the image was repeated but with the alternative phase encoded direction (i.e. anterior to posterior or right to left) to ensure the abnormality seen is not artefact projected over the myocardium. LGE is sensitive at detecting areas of focal fibrosis and for over twenty years has been a well-accepted standard for assessing viable myocardium .[334, 335] For LGE the magnetisation level of normal viable myocardium is nulled and appears dark, thus areas of focal fibrosis with high concentrations of gadolinium are highlighted as bright white.[328, 336]

Post contrast MOLLI protocol

A contrast bolus as opposed to an infusion has been demonstrated to provide consistent values for ECV across a broad range of cardiac pathology.[330] Post contrast MOLLI images were acquired using identical slice positions as native images using a 4(1)3(1)2 T1 recovery sampling scheme over 11 RR intervals to obtain 9 T1 weighted images no sooner than 15 minutes after the administration of gadolinium.[327]

2.6 Cardiac magnetic resonance analysis

2.6.1 Anonymization technique

CMR images were anonymised using an anonymisation code and analysed consecutively over a two month period. The only information that remained attached to the scan was height and weight for indexing mass and volumes to body surface area. The localisers and hastes were not reviewed as they had been previously reported for any extra cardiac findings. Therefore I was blinded to temporal sequence, control/donor status and all clinical details until all CMR analysis was complete. All analysis was performed using post processing software cvi42® (version 5.3.4 Circle Cardiovascular Imaging, Canada) and in accordance with the SCMR Board of Trustees Task force on Standardized post processing.[337]

2.6.2 Ventricular mass and volumes

CMR is a highly accurate, non-invasive method of determining LV mass with high congruity to true LV mass seen at human post mortems.[324, 338] It has also been demonstrated to be more precise and more reliable than echocardiography.[339, 340]

Blinded analysis of ventricular volumes was performed using the contiguous SAX cine images. The positions of the atria and ventricles were confirmed by reviewing the VLA and HLA cine through all cardiac phases. The most basal slice was selected if over 50% of myocardium was present.[341] For volumetric assessment, both the end diastolic and end systolic phase were determined by selecting the largest and smallest cavity respectively, see **Figure 2.9**.[325] Delineation of trabeculations and papillary muscles were performed using thresholding to determine the endocardial border.[325 337] Papillary muscles were excluded from blood pool volumes yet included in calculations of LV mass as previously described.[325 337] The epicardial border was drawn manually then smoothed and defined as the visual detectable boundary

between the myocardium and extra cardiac space and the middle of the chemical shift artefact line.[325, 337] The same principles were repeated for the endocardial border of the right ventricle, for assessment of right ventricular (RV) volumes in which manual contours were traced in end systole and end diastole, see **Figure 2.10**.

LV mass was calculated automatically within post processing software as the total epicardial volume subtracted by the endocardial volume and then multiplied by 1.05g/ml, the specific density of myocardium.[337] Both endocardial and epicardial volumes are determined by the total sum of the slice thickness and slice gap multiplied by the cross sectional areas.[337] To ensure LV volumetric data was precise several criteria were satisfied before accepting the values, provided there were no valve abnormalities:

a. LV stroke volume and RV stroke volume should be within 10ml of each other.
b. The LV stroke volume should be within 10ml of the aortic forward flow.
c. Finally, end systolic LV mass should be within 10g of end diastolic LV mass.

Body surface area, calculated using the Mosteller formula was then used to index all volumetric measures and mass.[342]

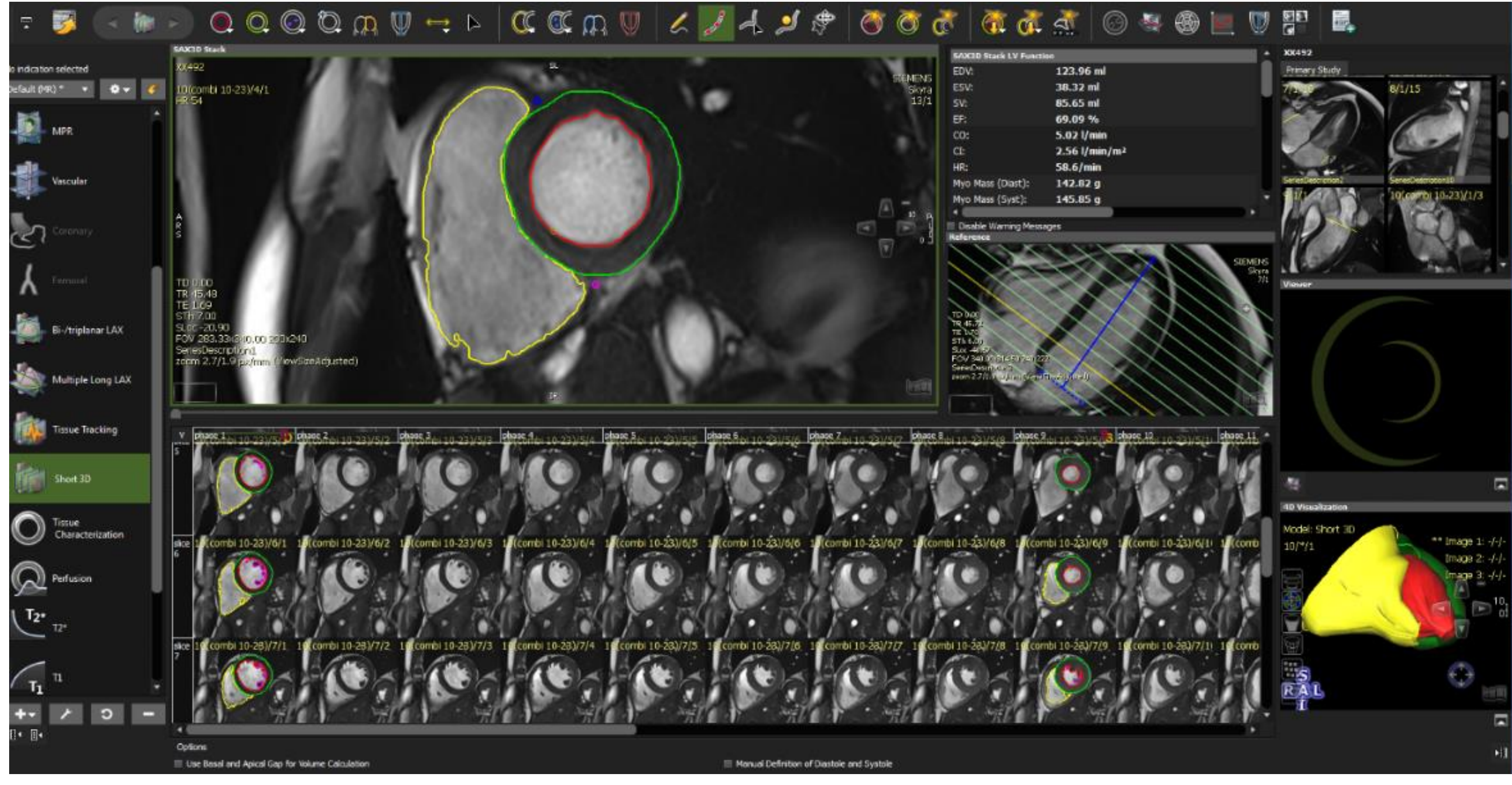

Figure 2.9. Analysis of ventricular mass and volumes.
A screen shot of the user interface of Circle Cardiovascular Imaging software, version 5.3.4 for the assessment of ventricular mass and volumes is shown. Each slice of the LV stack is displayed in thumbnails at the bottom of the screen from top to bottom (base to apex) with the 25 cardiac phases for each slice running from left to right (diastole to systole). In the main display a short axis slice in diastole can be seen with endocardial (red), epicardial (green) and right ventricular contours (yellow). For orientation, on the right of the main display, the HLA cine is shown with corresponding horizontal lines indicating short axis slice positions. Contours have been traced for phase 1 (diastole) and phase 9 (systole). A 3D model has been generated from the contours in the bottom far right hand corner. The ventricular mass and volumes have been calculated by the software and displayed at the top of the screen. HLA; horizontal long axis.

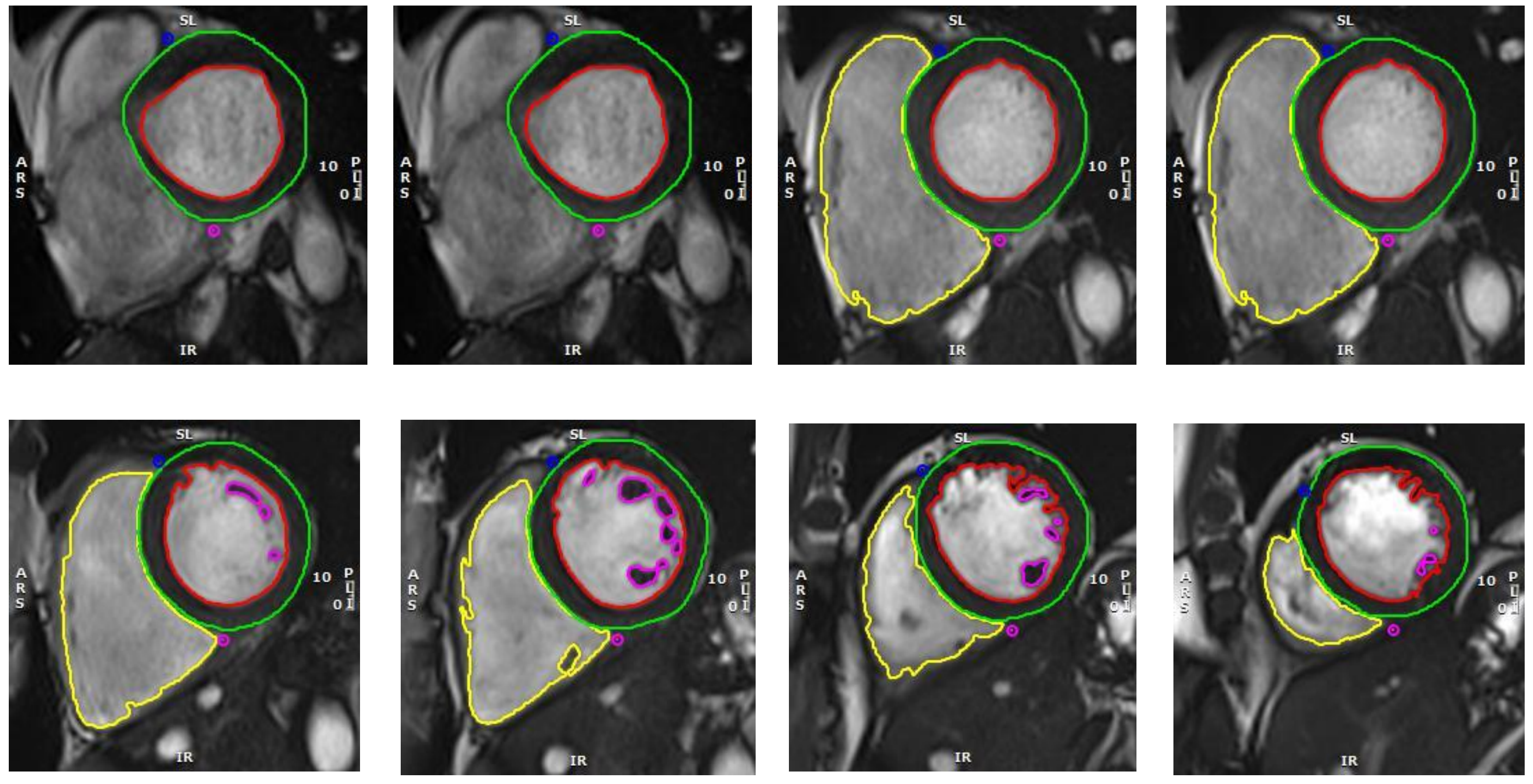

Figure 2.10. An example of analysis of the left and right ventricle.
Serial contours of the short axis stack in diastole are shown. Red contours indicate the endocardial border with pink contours excluding papillary muscles from calculation of volume. Green contours represent the epicardial border. Yellow contours are traced around the right ventricle.

2.6.3 Left ventricular geometry calculations

The wall thickness was determined by segregating the myocardium into segments as per the AHA 17 segment model with the exception of the apex.[343] A wall thickness was obtained for each segment in the end diastolic phase. The largest segmental thickness was deemed the maximum wall thickness. The mass/volume ratio was calculated as LV mass divided by end diastolic volume.[344]

2.6.4 Left atrial volumes

Left atrial (LA) volume was measured applying the biplane area-length method for end diastolic and end systolic volumes from HLA and VLA cines.[345] The cardiac phase was determined by observing the opening of the mitral valve and selecting the systolic phase accordingly.[346]

2.6.5 Myocardial strain

Three dimensional (3D) feature tracking calculating myocardial strain allows assessment of myocardial deformation beyond ejection fraction, it was assessed using standard SSFP cine images.[347] Feature tracking has been demonstrated to have good intra-observer and inter-observer reproducibility.[348] Furthermore, 3D feature tracking is more reproducible than 2D tracking in healthy subjects.[347] To track the myocardium, endocardial and epicardial rounded contours were drawn in end diastole only for the VLA cine, HLA cine and all slices of the short axis stack with the exception of the most basal slice if the LV outflow tract was visible. A 3D dimensional incompressible deformation model was fitted to each cine image using a validated algorithm to determine strain.[349] The degree of deformation, represented as 'boundary points' on the endocardial and epicardial border was reviewed to ensure accuracy of tracking

throughout the cardiac cycle, see **Figure 2.11**.[347] Three dimensional GCS, GLS and global radial strain (GRS) were measured. Strain rates for each parameter were also obtained (S' peak systolic strain, E' early diastolic strain and A' late diastolic strain rate), see **Figure 2.12**. Segmental strain was not assessed due to previously reported poor reproducibility.[350]

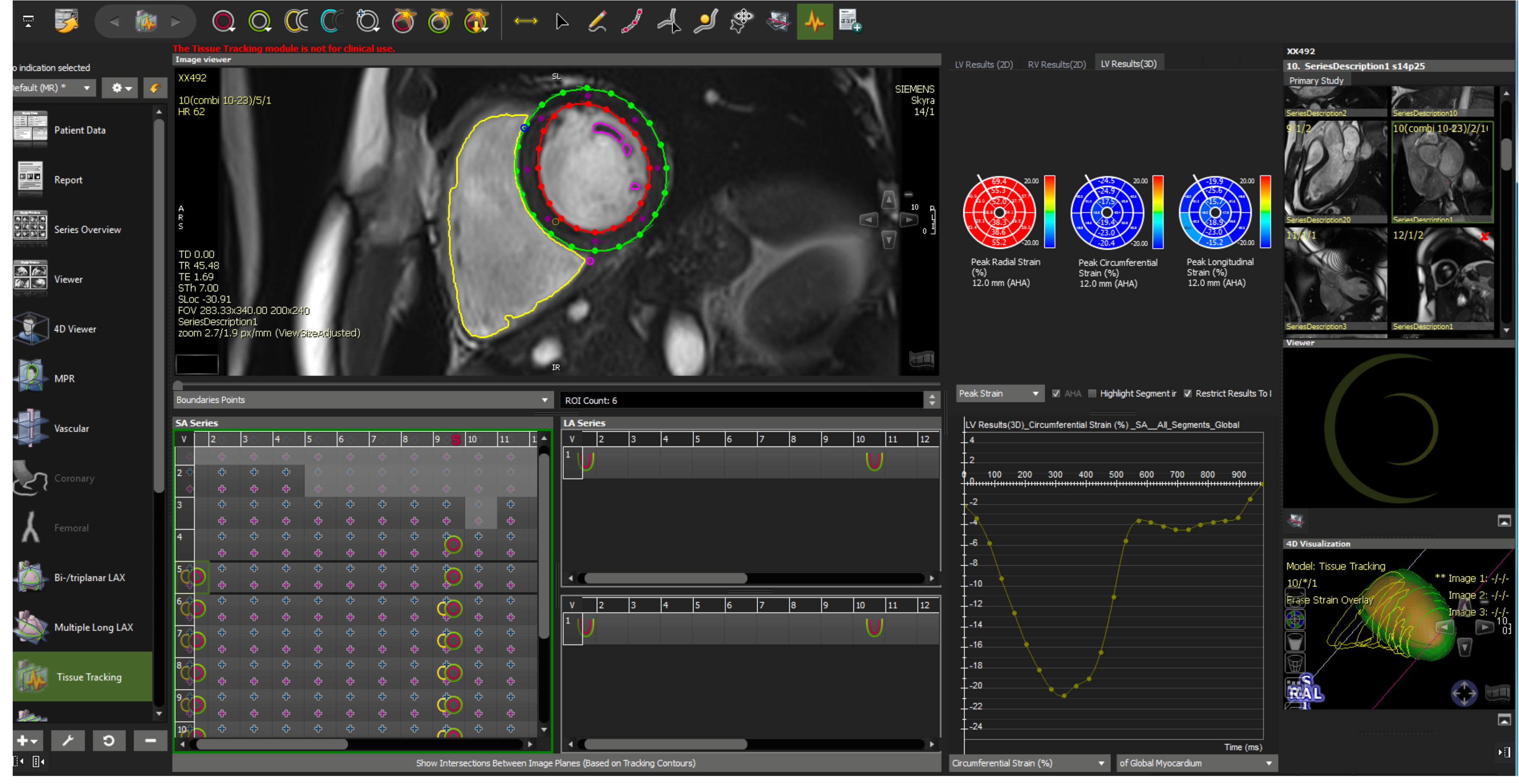

Figure 2.11. Analysis of systolic function and myocardial strain.
A screen shot of the user interface of Circle Cardiovascular Imaging software, version 5.3.4 for 3 feature tracking is shown. The SAX, HLA and VLA cines have been used. Unlike assessment for volumes and mass, contours are rounded. Boundary points are viewed as dots and can be reviewed through all phases of the cardiac cycle to ensure suitable tracking. Results for 3D global circumferential strain are shown on the graph. HLA; horizontal long axis. SAX; Short axis stack. VLA; vertical long axis.

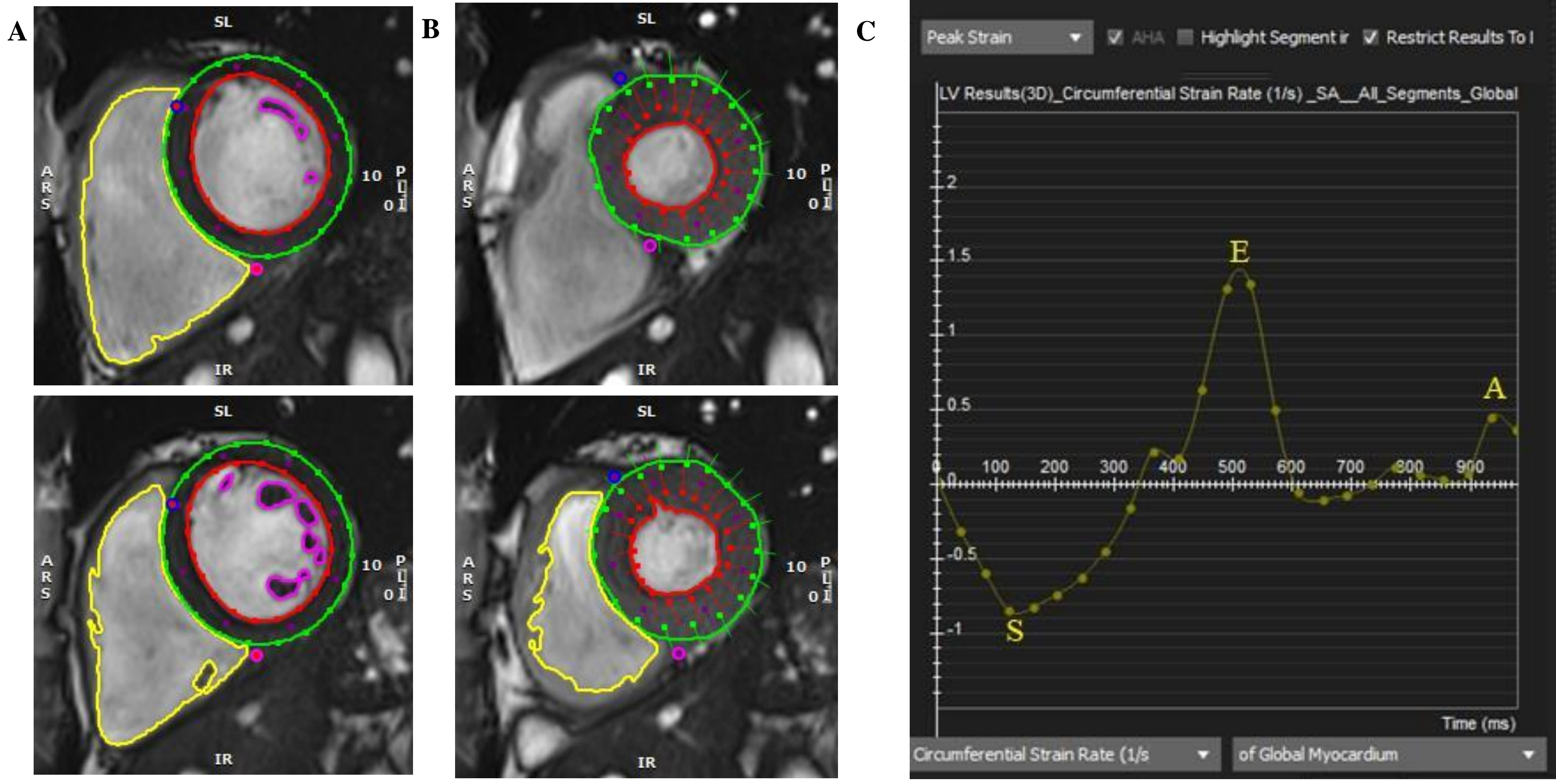

Figure 2.12. Feature tracking throughout the cardiac cycle and typical myocardial strain rate output.

A. Two SAX slices are shown in diastole with boundary points observed.
B. Corresponding slices are shown in systole where boundary points can be tracked throughout the cardiac cycle.
C. Following 3D feature tracking analysis strain rates can also be generated. In this case strain rates can be seen for circumferential strain and include S' peak systolic strain (the only negative rate), E' early diastolic strain (largest positive rate) and A' late diastolic strain rate.

SAX; Short axis stack.

2.6.6 T1 mapping analysis and calculation of extracellular volume

Prior to analysis of T1 images, the LGE sequences were reviewed to identify any focal areas of fibrosis. Additionally, the uncorrected T1 raw images and motion corrected raw images were reviewed systematically for artefact, see **Figure 2.13a**. Areas of LGE and artefact are excluded from the final analysis consistent with recommended consensus methodology from the SCMR working group.[321] Endocardial and epicardial borders were traced onto raw images taking care to avoid inclusion of the blood pool using a 20% offset on both the endocardial and epicardial border.[351] A 20% offset has previously been demonstrated to be the most reproducible contouring method.[352]

A colour pixel-wise T1 map is generated according to T1 times and contours were transferred onto the map and subsequently reviewed. [351] The myocardium was segmented into 6 segments using inferior and superior insertion point references.[343] Several T1 times were recorded:

a. The global T1 time, which was an average of all viable segments.
b. A septal time, which was the average of segment 2 and 3 or 7 and 8 depending on slice position. In keeping with published recommendations, a region of interest was drawn within the myocardium of the septum taking care to choose an area with enough pixels.[320, 321]
c. A blood time was measured by contouring the largest circular area within the blood pool whilst avoiding papillary muscles.

The same process was repeated with the post contrast images taking care to ensure the slice positon was the same, see **Figure 2.13b** and **Figure 2.14**. Using pre and post myocardial and blood T1 times global and septal ECV values were was calculated, as per the following calculation.[115, 321, 353]

$$ECV = (1 - haematocrit) - \frac{\frac{1}{post\ contrast\ T1\ myo} - \frac{1}{native\ T1\ myo}}{\frac{1}{post\ contrast\ T1\ blood} - \frac{1}{native\ T1\ blood}}$$

In the calculation T1 myo refers to the T1 time of the myocardium and T1 blood refers to the T1 time of the blood pool. Haematocrit levels immediately prior to the scan were used in the calculation.

Although T1 time is known to vary with tesla strength and protocol, ECV is a more reproducible and consistent measure. [351]

A **B**

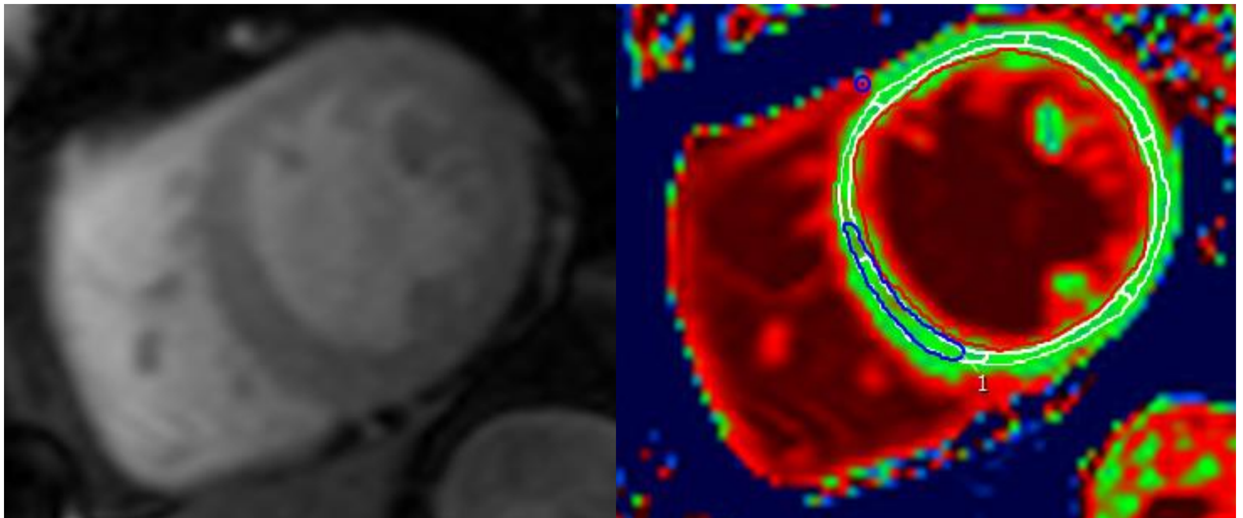

Figure 2.13. Analysis of T1 mapping. First published in Price et al.[354]

A. The grey scale image is a raw uncorrected MOLLI image of the mid ventricular short axis stack. There are eight pre-contrast raw images which were reviewed for artefact and cardiac and respiratory motion. Once satisfied the image is of sufficient quality the endocardial and epicardial borders were traced onto the raw image.
B. Contours were subsequently transferred to a pixelated colour map. In this case, for clarity, the T1 map has been colour coded according to T1 time: 800ms dark blue, 1000ms light blue, 1250ms green, 1400ms red and 2000ms dark red. Once insertion point markers are placed onto the image the myocardium is automatically segregated into 6 segments seen on the colour map in white. T1 times are generated for each segment. The 20% off set ensures generated segments are within the myocardium and are less likely to include blood pool. In addition a freehand manual contour of an area of interest, known as a region of interest has been drawn in blue within the septum.

MOLLI; Modified look-locker inversion recovery.

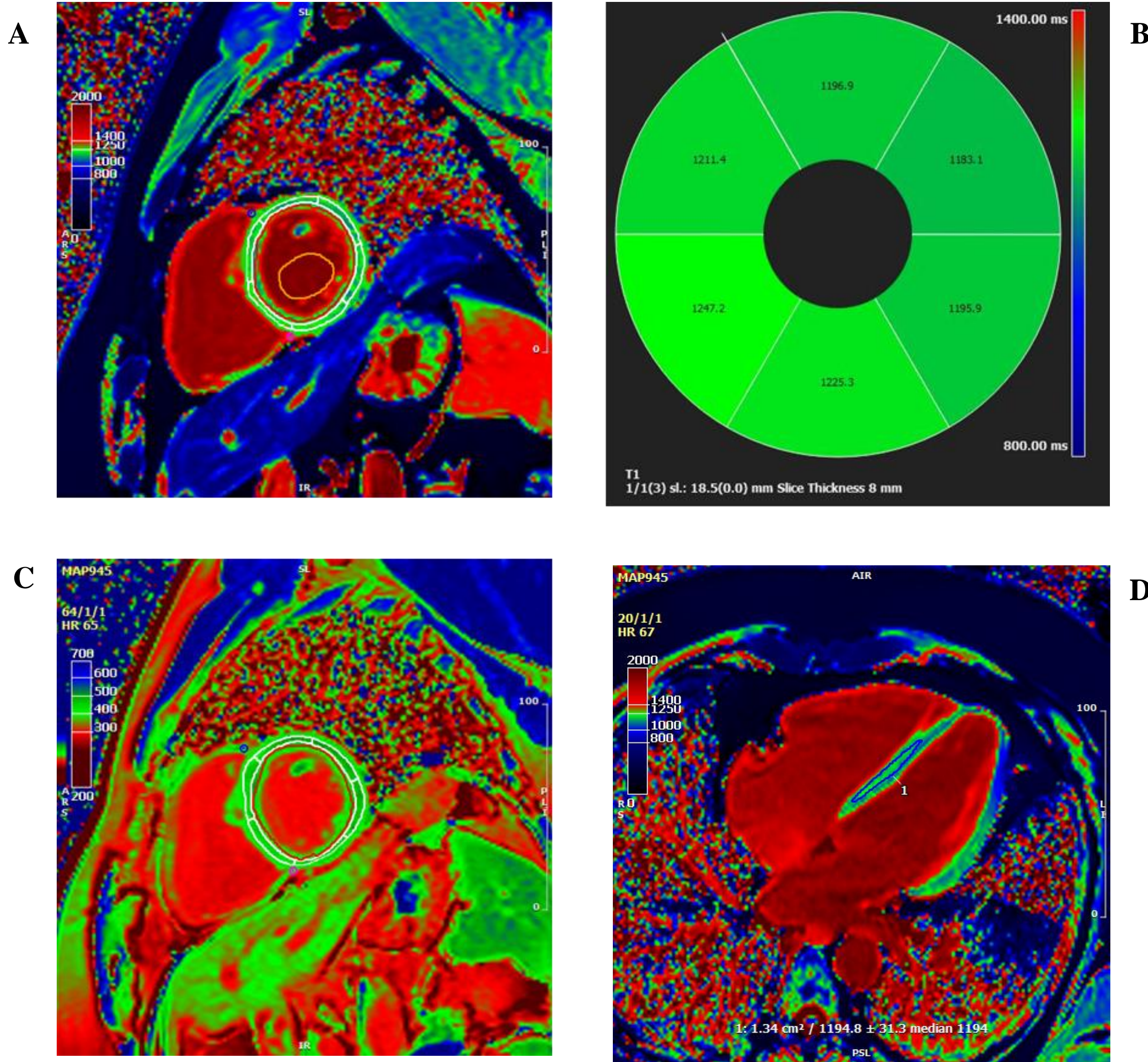

Figure 2.14. Examples of native and post contrast T1 mapping.

A. A typical example of native T1 mapping of the basal left ventricular slice. The orange contour represents the area selected for the determination for pre-contrast blood time.
B. Corresponding T1 times are given for each segment of the myocardium seen in image A.
C. A typical example of post contrast T1 mapping of the basal left ventricular slice.
D. An example of a native T1 mapping of the HLA. A blue contour in the septum indicates a region of interest.

HLA; horizontal long axis.

2.6.7 T2 mapping

A pixel wise T2 generated map based on curve fitting from a two-parameter equation was used.[329] The same methodology as per T1 maps, was employed to analyse T2 maps, see **Figure 2.15**.

2.6.8 Late gadolinium enhancement quantification

LGE was defined as presence of LGE on two contiguous cine images of the LV short axis stack or present on the LV short axis stack and a corresponding long axis view.[3] LGE also needed to persist following a repeat image with a change in phase encoding direction to rule out artefact.[3] LGE was quantified using full width, half max methodology as previously described and given as a percentage of overall LV mass, **Figure 2.16**. [355]

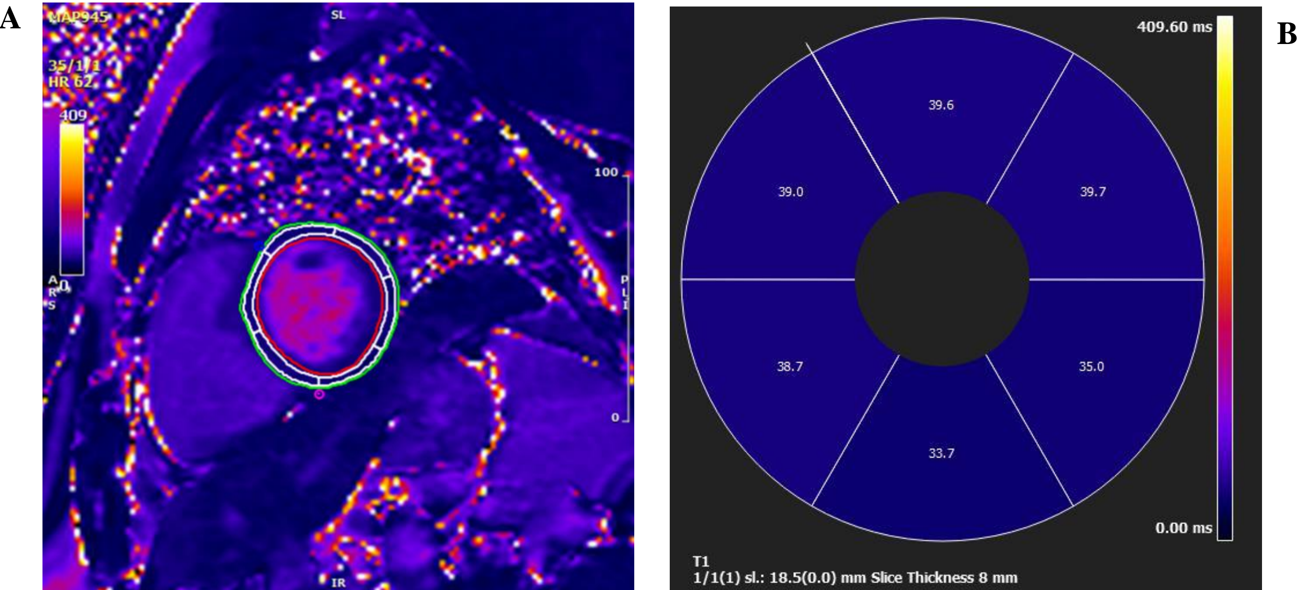

Figure 2.15. Analysis of T2 mapping.

A. A typical example of T2 mapping at the basal left ventricular slice. The myocardium has been segmented into American heart association segments using a 20% off set. Red and green contours display the endocardial and epicardial contours respectively.
B. Corresponding T2 times generated from image a.

A B

Figure 2.16. Analysis and quantification of late gadolinium enhancement. Reproduced under the Creative Commons Attribution license and first published in Price et al.[3]

A. Late gadolinium enhancement in the superior and inferior insertion point can be seen on this view of the SAX (see arrows). Areas of enhancement are shown as bright white in contrast to the dark myocardium.
B. On occasions where late gadolinium enhancement was seen it can be quantified and expressed as a percentage of left ventricular mass. Using full width half max methods pixelated areas of late gadolinium enhancement are shown along with endocardial (red) and epicardial (green) contours in order to calculate left ventricular mass.

SAX; Short axis stack.

2.6.9 Aortic distensibility

Aortic distensibility is related to the elastic properties of the aorta and is calculated as the difference in luminal area from diastole to systole divided by the pulse pressure.[356] It has been demonstrated to predict all-cause mortality and can be assessed non-invasively using non-contrast CMR.[357] Previous calculations of aortic distensibility relied on the use of manual contours throughout the cardiac cycle leading to high levels of variability. Even semi-automated methods subsequently developed still introduce variability.[358] In this study, I used a fully automated Aortic Distensibility Analysis (ADA) software developed by Biasiolli et al. at the University of Oxford which has already been validated on 5100 scans from the UK Biobank cohort.[356]

Analysis of aortic distensibility was conducted offline using the transverse SSFP cine of the ascending aorta at the level of the bifurcation of the pulmonary artery. Sequences were anonymised and exported as Digital Imaging and Communications in Medicine (DICOM) format files from cvi42® (version 5.3.4 Circle Cardiovascular Imaging, Canada) and analysis was conducted for all scans in a single time period. Sequences were initially reviewed for quality, in terms of adequate slice position and severity of any artefact. For adequate slice position both the ascending and descending aorta needed to be clearly visible at the level of the bifurcation of the pulmonary artery.[356] The sequences were also examined for artefact. If artefact disrupted the clarity of the vessel border at any point during the cardiac cycle the scan was excluded from analysis.

The DICOM files were analysed by the ADA standalone application using Matlab version R2017a (MathWorks). This application allows detection of both the ascending and descending aorta and tracking of the vessel wall throughout the cardiac cycle.[356] The software initially detects the vessels involved. Identification of the correct vessel was then manually confirmed before proceeding. On the rare occasion the algorithm fails to detect the correct vessel this can be altered or the contour can be hand drawn if required before continuing, see **Figure 2.17**.

Following confirmation of the correct vessel both the ascending aorta and proximal descending aorta were tracked throughout the cardiac cycle, see **Figure 2.18**. Individual automated contours for each cine image were reviewed. Discrepancies or errors could be manually altered if required. The maximum area was selected for systole (Amax) and minimum area selected for diastole (Amin). An average of three values were taken.

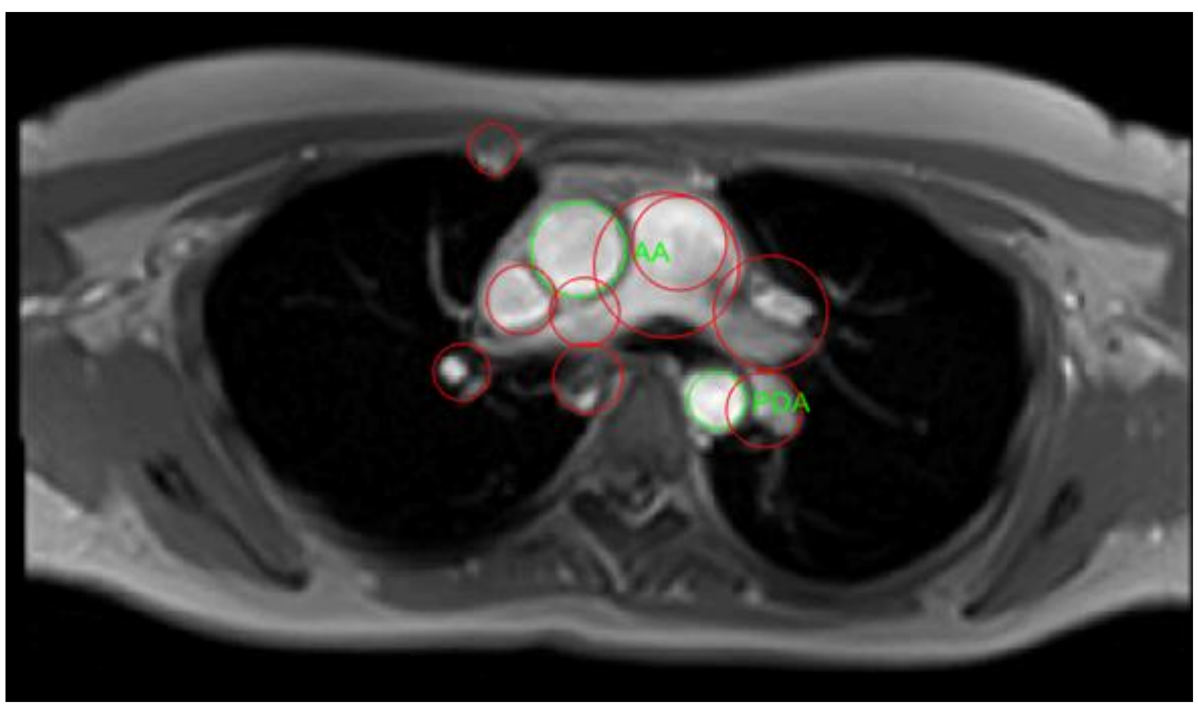

Figure 2.17. User interface of aortic distensibility software.
This is the user interface of software used for analysis. It demonstrates the automated detection of the proximal ascending aorta (AA) and the proximal descending aorta (PDA) on a transverse SSFP at the level of the bifurcation of the pulmonary artery. In this case, the software has correctly identified both arteries as green circles, the region of interest. Other 'vessels' potentially detected are indicated as red circles and can be chosen as alternatives. Only the vessels selected in green will be analysed.

Once the maximum and minimum luminal areas were determined aortic distensibility was calculated using the following equation. [359, 360]

$$\frac{\left(\frac{\text{Amax mm}^2\text{-Amin mm}^2}{\text{Amin mm}^2}\right)}{\text{Pulse pressure mmHg}} = \text{Aortic distensibility mmHg}^{-1}$$

Pulse pressure (diastolic blood pressure –systolic blood pressure) was determined from the average of three peripheral blood pressures taken immediately following the sequence as described earlier.

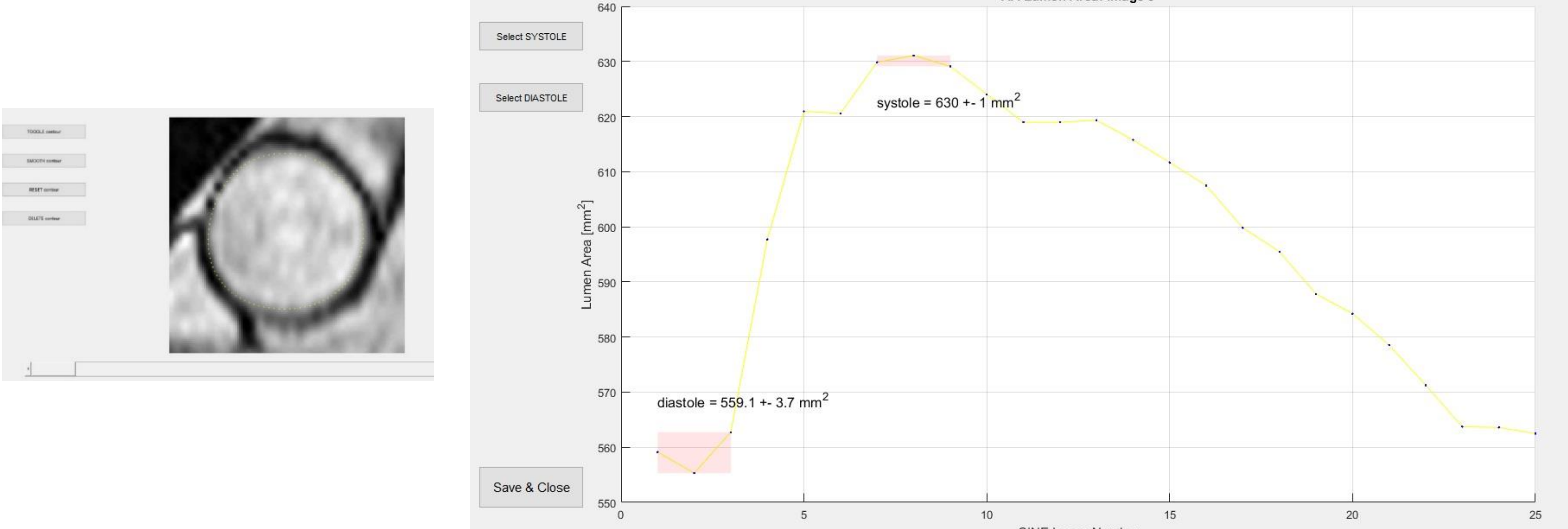

Figure 2.18. An example of automated tracking of the ascending aorta.
The first image generated is that of the region of interest, in this case the contour of the ascending aorta, seen on the left. A sliding bar allows visual assessment of all phases of the cardiac cycle. Any error in contours can be hand drawn, smoothed or adjusted if required. The calculated luminal area (mm^2) is then plotted against each cine number producing a graph of luminal area over time. In systole the aortic lumen is at its maximum, whilst in diastole it is at its minimum. Three points are selected on the graph for both systole and diastole which generates an average luminal area (see points shaded in pink for example calculation)

2.7 Quality and reproducibility in CMR imaging

2.7.1 Reproducibility in CMR imaging

For the 5-year study intra-observer variability was assessed in 10 randomly selected studies, which were analysed twice by myself two months apart whilst blinded to subject identity and control/donor status. I also assessed inter-study variability for LV mass in ten subjects undergoing a repeat CMR imaging within one week of the original study. Inter-observer variability for LV mass, T1 and T2 times were assessed between myself and another independent reporter blinded to patient data and study visit. To ensure consistency in assessment for the baseline and 12 month CMR studies further inter-observer variability for LV mass (primary end point) between myself and the original researcher for CRIB-DONOR were assessed in an additional 20 subjects.

2.7.2 Quality control for T1 mapping

To ensure quality and consistency of T1 times throughout the study quality assurance tests were carried out every three months or when any updates were made to the MRI system using a validated phantom containing multiple vials of agar at different T1 values (Eurospin T05 phantom Diagnostic Sonar, Scotland).[320] Scanning of the phantom used the same MOLLI sampling schemes as those used in the study 5(5)3 and 4(1)3(1)2 with a simulated heartrate. In addition basic TSE sequence was also used to account and adjust for any temperature fluctuations and/or any degradation of the phantom.

2.8 Statistical analysis

2.8.1 General statistical approach

Statistical analysis was carried out using SPSS©, version 23 (IBM, Armonk, New York, US) unless stated otherwise. Categorical variables are displayed as whole numbers and (percentages). In general, continuous variables were assessed graphically using histograms to determine normality. Variables of a normal distribution are reported as mean ± standard

deviation otherwise data are displayed as median [interquartile range]. All continuous non-parametric data was Log10 transformed prior to analysis and plotted graphically. If the logged values were normally distributed a parametric test was favoured otherwise a non-parametric test was used. Data analysed in a logged format is highlighted in the results tables. A p value of < 0.05 was considered significant and no adjustments were made for multiple comparisons. In general missing values were handled using a listwise deletion (complete-case analysis) approach.

For comparison of two different groups (donors vs. controls) independent samples t tests were used for continuous variables to estimate the mean difference and confidence intervals between groups. Where continuous variables of more than two groups were compared a one way analysis of variance (ANOVA) was carried out with post hoc Tukey test for comparisons between each group. For comparison of categorical variables between two different groups chi square or fishers exact tests were used. On occasions where the difference between values at baseline and follow up were required in a single group, paired t tests were used to estimate the mean difference and confidence interval between time points.

To assess the influence of confounding variables on the variable of interest multivariable linear regression models were used providing data was of a linear relationship, normally distributed and variables were not co-dependent. Associations between continuous variables were analysed using Pearson's correlations for parametric data.

For the longitudinal assessment of continuous and categorical variables over 5 years generalized estimating equations were used to compare change in variables over time. Pearson's residuals generated from the models were plotted and assessed graphically on Q-Q plots to ensure linearity (normality of residuals) was a reasonable assumption. A linear or binary, unstructured model was chosen with the variable of interest as the dependent variable

and with participants as the subject variable. Study time was selected as within-subject factor and group (donor/control) as a between-subject predictor. An interaction term between each group and study time point was included to assess change over all time points (p-value for interaction). Pairwise analysis were also generated as part of the generalized estimating equations giving comparisons between donors and controls at each corresponding time point. Actual time was not included as a covariate as there was no significant difference in time to follow up between the two groups.

For assessment of reproducibility of CMR parameters intraclass correlation coefficients were used for inter-study, intra-observer and inter-observer variability. Intraclass correlation coefficients were also used to assess consistency of T1 measures using the phantom.

2.8.2 Adjustment for variables

PWV was adjusted for mean arterial pressure (MAP) and heart rate (HR) as has been recommended by the AHA to standardise vascular research.[299] Adjustment for MAP and HR was performed using a general linear regression model (where PWV is the dependent variable and MAP and HR are independent variables i.e. a multivariable model).[361] Calculations were as follows:

- Predicted PWV= Constant (from regression equation) + [(Unstandardized B coefficient (from regression model equation)* mean MAP of cohort)] + [(Unstandardized B coefficient (from regression model equation)*mean HR of cohort)].
- Adjusted PWV for an individual= Predicted PWV (for mean MAP and HR) + Unstandardized residual for the individual (from regression model).

CHAPTER 3

EFFECT OF A REDUCTION IN GLOMERULAR FILTRATION RATE AFTER NEPHRECTOMY ON ARTERIAL STIFFNESS, BLOOD PRESSURE AND CENTRAL HAEMODYNAMICS. THE EARNEST STUDY

3. 1 Extent of personal contribution

This study was a multi-centre study for which I was responsible for the Birmingham cohort of subjects. The Birmingham site was the second largest recruiting site for the study, accounting for over a third of all those consented. The regulatory documents, audits and correspondence with the sponsor for the Birmingham site were my primary responsibility. I maintained the site file and dealt with and documented any protocol deviations and adverse events.

I organised and arranged the follow up visits for the Birmingham subjects with the assistance of a research nurse. I conducted the study visits myself which included office and ambulatory blood pressure, assessment of PWA, carotid-femoral PWV and central blood pressure using the SphygmoCor (Atcor Medical, Sydney, Australia). I took their blood and urine samples and arranged laboratory storage and booked the iGFRs.

Following study completion I wrote the final report to the funder and was responsible for all electronic data input for the whole study for the Birmingham site. I also led the data analysis, statistical plan and interpretation of the whole national dataset. The first draft of the manuscript was written by myself and I was responsible for all edits and revisions. This chapter is based on the published first author original article where this data was first published.[362] Price AM, Greenhall GHB, Moody WE, Steeds RP, Mark PB, Edwards NC, Hayer MK, Pickup LC, Radhakrishnan A, Law JP, Banerjee D, Campbell T, Tomson CRV, Cockcroft JR, Shrestha B, Wilkinson IB, Tomlinson LA, Ferro CJ, Townend JN. Changes in blood pressure and arterial hemodynamics following living kidney donation. *Clinical Journal of the American Society of Nephrology*. 2020;15 (9):1330-1339. DOI: https://doi.org/10.2215/CJN.15651219.

3.2 Abstract

The **E**ffect of **A R**eduction in Glomerular Filtration Rate after **NE**phrectomy on Arterial **ST**iffness and Central Haemodynamics (EARNEST) was a multi-centre, prospective, controlled study designed to investigate the associations of an isolated reduction in kidney function on blood pressure and arterial haemodynamics.

Prospective living kidney donors and healthy controls who fulfilled British Transplantation Society criteria for donation were recruited from seven UK centres with expertise in vascular research. Participants underwent office and ambulatory blood pressure measurements, assessment of arterial stiffness and biochemical tests at baseline and 12 months.

A total of 469 participants were recruited with 306 (168 donors and 138 controls) returning for follow up at 12 months. At follow up, the mean eGFR rate was -27 mL/min/1.73m^2 (95% CI -29, -26) lower in donors compared to a +2 mL/min/1.73m^2 (95% CI -0.4, 3.8) in controls. At 12 months, mean ambulatory day systolic blood pressure was +0.1mmHg (95% CI -1.7, 1.9) in donors and +0.6mmHg (95% CI -0.7, 2.0) in controls with no significant difference between groups [-0.5mmHg (95% CI -2.8, 1.7), p=0.626]. Mean carotid-femoral PWV was +0.3m/s (95% CI 0.1, 0.4) in donors and +0.2m/s (95% CI -0.0, 0.4) in controls at 12 months but again there was no significant difference between groups [+0.1m/s (95% CI -0.2, 0.3) p=0.49].

Changes in ambulatory peripheral blood pressure and carotid-femoral PWV in living kidney donors at 12 months after nephrectomy were small, not clinically significant and no different from a healthy control group.

3.3 Introduction

Arterial stiffness, corresponding rises in blood pressure and the ensuing haemodynamic stress on downstream vascular beds, are considered important key contributing factors to the development of cardiovascular disease in CKD.[363] Furthermore, PWV, the non-invasive 'gold standard' measure of arterial stiffness, has been demonstrated to be an important predictor of adverse outcomes and has improved risk stratification of those at highest risk in the general population.[304] Additionally, PWV is a strong independent risk factor for cardiovascular mortality, with a reported 15% increase risk of mortality per 1m/s increase in PWV and predictive of poor outcomes following cardiovascular events such as ischaemic stroke and peripheral vascular disease. [364] [294, 304]

CKD leads to an accelerated phase of vascular ageing shown by increases in PWV compared to the general population.[365] Increased arterial stiffness is both highly prevalent in the earlier stages of CKD and has been shown to be inversely correlated with GFR, with changes evident from stage 2 onwards.[366-368] It is unclear however, whether increased blood pressure and arterial stiffness in CKD are direct consequences of the reduced GFR or result from the multiple co-morbid conditions that tend to accompany CKD.

Living kidney donors provide an opportunity to prospectively examine the haemodynamic consequences of a reduction in kidney function without the confounding effects of co-morbid disease. The aim of this study was to determine the effect of the reduction in kidney function that occurs after kidney donation on arterial stiffness, blood pressure and central haemodynamics compared to an appropriately selected control group in a sample large enough to detect small differences.

3.4 Brief methods

3.4.1 Study design

The EARNEST study was a prospective multi-centre UK cohort study. The study aimed to recruit 440 controls and 440 donors over a two year period from seven centres recognised for performing high numbers of living kidney transplants, see **Table 3.1**.

Recruitment began in April 2012; the last follow up patient was studied in May 2016. Recruitment was terminated in May 2015 on pragmatic and financial grounds. This was principally due to unanticipated large numbers of recruited participants dropping out at follow up.

3.4.2 Study population

The inclusion and exclusion criteria for both donors and controls were in accordance with national guidelines disseminated by the Joint Working Party of the British Transplantation Society and the Renal Association for living kidney donors as previously described.[264]

Most healthy controls taking part in EARNEST were individuals undergoing workup for donation but who were ultimately unable to donate due to factors such as immunological mismatch or recipient illness. Alternatively, donor-related family members or volunteers donating blood at local blood donation centres were recruited. All healthy controls were screened in the same way and to the same standard as the living kidney donors with the exception of detailed evaluation of renal anatomy by further imaging.

Table 3.1. Centres involved with recruitment.

Hospital	Location
Queen Elizabeth Hospital	Birmingham
Addenbrooke's Hospital	Cambridge
Western Infirmary	Glasgow
Southmead Hospital	Bristol
St George's Hospital	London
Central Manchester University Hospitals	Manchester
Sheffield Teaching Hospitals	Sheffield

3.4.3 Study protocol

All participants were investigated at baseline (less than 6 weeks prior to nephrectomy for prospective living kidney donors) and at 12 months, see **Figure 3.1**. The protocol included measures of office and ambulatory blood pressure, assessment of PWA, carotid-femoral PWV and central blood pressure using the SphygmoCor (Atcor Medical, Sydney, Australia). All participants underwent blood and urine tests with donors also undergoing iGFR. Full details are within the methodology chapter. A detailed protocol has previously been published.[249]

3.4.4 Study centre training

All centres taking part in the study already had existing expertise in vascular research. To ensure uniformity of data collection, all centres received a detailed study manual with quality control criteria for acceptable data. In addition, those undertaking the measurements attended training in the use of SphygmoCor for the assessment of PWA and PWV to reduce inter-observer variability as much as possible. Mean inter-observer variability difference has been demonstrated to be as low as 0.3±3.2m/s in those with CKD and −0.30 ± 1.25 m/s in healthy controls following an introduction to the technique and standard quality control criteria.[295, 303]

3.4.5 Primary outcome measures

1. Mean change in carotid-femoral PWV.
2. Mean change in ambulatory systolic blood pressure.

3.4.6 Statement of ethics

Ethical approval was obtained in February 2013 from the South Cambridge Regional Ethics Committee (Integrated Research Application System Reference: 118797, Research Ethics Committee approval number 13/EE/0015).

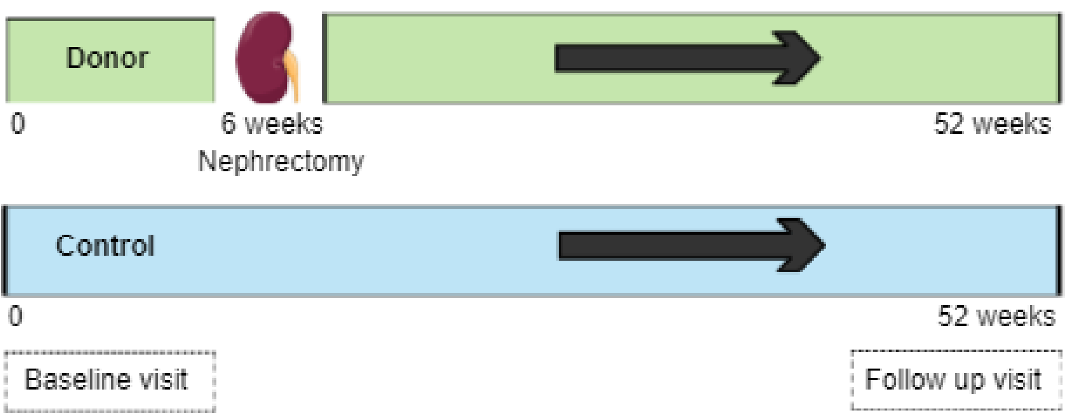

Figure 3.1. Summary protocol for the EARNEST study. Adapted from Moody et al.[249]

3.4.7 Power calculations and sample size

Using data from previous studies, the standard deviation of the within-patient changes was assumed to be 10 mmHg for blood pressure and 1.0 m/s for carotid-femoral PWV.[303, 369] A sample size of 800 participants (400 subjects per group) was planned in order to provide 80% power to detect a difference of 2.2mmHg in systolic pressure or 0.22 m/s in carotid-femoral PWV using a 2-sided *t*-test at the 2.5% significance level. Values for a sample size of 400 participants (200 subjects per group) have 92% power to detect a difference of 4 mmHg for systolic blood pressure and 0.4 m/s for carotid-femoral PWV, allowing for 15% loss to follow up at a significance level of 5%.

3.4.8 Statistical analysis

Statistical analysis was performed using Stata statistical software (release 15. StataCorp LCC, College Station, TX). A paired samples *t* test was used to estimate the mean change and 95% CI between baseline and follow up in each group (within-group change). An independent *t* test was used to estimate the mean change and 95% CI between within-group change in donors and within-group change in controls (between-group change).

Continuous variables at baseline for the whole cohort were compared using independent *t* tests. Categorical variables were compared using Chi squared or Fisher's exact tests. Multivariable linear regression was used to account for factors which may have confounded the relationship between kidney donation and change in carotid-femoral PWV (age, sex and smoking status).

A p-value of <0.05 was considered significant, no adjustments were made for multiple comparisons. Data presented includes subjects who returned for follow up. Missing data was dealt with by performing a complete case analysis. A further sub analysis was undertaken of those who remained in the study compared to those who were lost to follow up.

3.5 Results

3.5.1 Follow up and events

A total of 469 participants were recruited, see **Figure 3.2**. Following eligibility assessment there were 22 participants who consented to take part but were ultimately excluded; 20 lacked the minimal data set required for analysis and two were found to be ineligible after the initial visit. Those that were found to be ineligible were 'healthy controls' who did not meet criteria due to incidental findings; one was diagnosed with diabetes and one had an insufficient kidney function. Consequently, neither met living kidney donation criteria. Those who lacked the minimal data set were participants who consented to take part and withdrew prior to completing the baseline assessment. This was usually because of competing appointments during living kidney donor work up.

Recruitment was terminated after 3 years despite the lower than planned sample size due to financial constraints. Of the remaining 447 participants, there were 201 controls and 246 donors. Of these, a total of 38 controls and 46 donors were patients who originally consented into the CRIB-DONOR sub study, and re-consented to allow their data to be included.[241] One hundred and forty-one participants were unable to attend follow up at 12 months leaving 168 donors and 138 controls with complete paired carotid-femoral PWV data included in the final analysis. In addition, a further 49 donors and 27 controls who returned for follow up had incomplete ambulatory blood pressure recordings leaving 119 donors and 111 controls with complete paired ambulatory blood pressure data.

The commonest reasons for lack of study completion by participants were change of address or difficulty attending clinic visits due to travel distance, work and childcare commitments

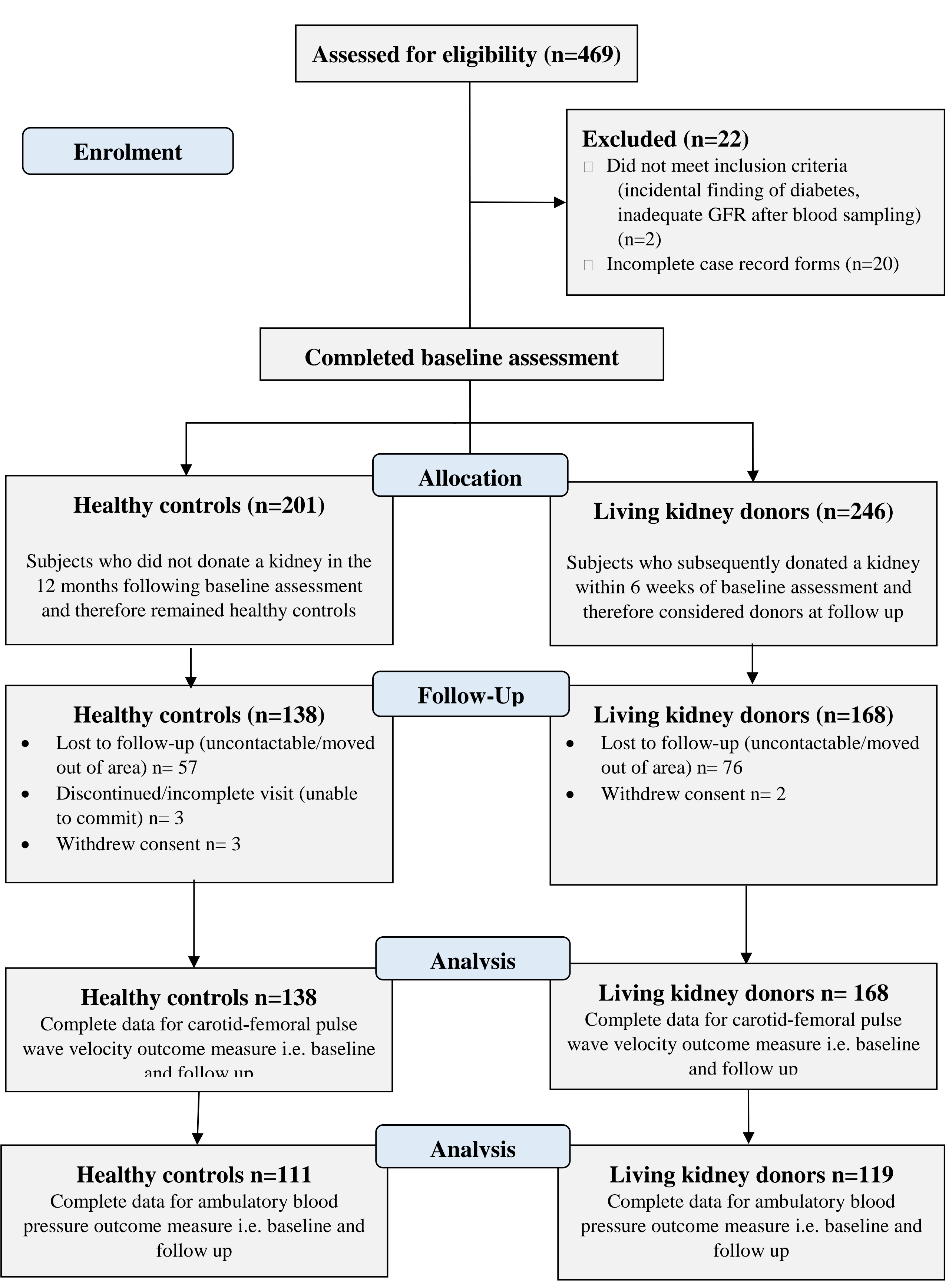

Figure 3.2. A flow chart illustrating those who were recruited and those lost to follow up. First published in Price et al.[362]

3.5.2 Patient characteristics

The demographics of living kidney donors and healthy controls who attended for both baseline and 12 month follow up visits were comparable with the exception of tobacco use, see **Table 3.2**. A higher proportion of donors had a history of smoking compared to controls. The baseline haemodynamic and biochemical characteristics are shown in **Tables 3.3, 3.4, 3.5, 3.6 and 3.8**.

Table 3.2. Baseline characteristics of participants who completed both baseline and 12-month evaluations. First published in Price et al.[362]

	Controls n=138	**Donors n=168**
Sex (male) *	57 (41)	78 (46)
Age (years) *	49 ± 14	51 ± 12
Race	Caucasian=127 (92) Non-white=8 (6) Unknown=3 (2)	Caucasian=158 (94) Non-white= 9 (5) Unknown= 1 (1)
History of hypertension	9 (7)	17 (10)
Anti-hypertensive usage*	9 (7)	18 (11)
ACE/ARB usage	4 (3)	5 (3)
Calcium channel blocker usage	4 (3)	6 (4)
Current or ex-smoker *	38 (28)	74 (44)
eGFR, categories ($mL/min/1.73m^2$)	<80= 25 (18) 80 to <90= 23 (17) >90 =88 (65)	<80= 38 (23) 80 to <90= 39 (23) >90= 91 (54)
eGFR ($mL/min/1.73m^2$)	94 ± 16	91 ±15
Normalised isotopic GFR ($ml/min/1.73m^2$) †	89 ± 13	89 ± 12

Categorical variables are presented as n (%) and continuous data are represented as mean ± standard deviation.

ACE; Angiotensin Converting Enzyme. ARB; Angiotensin receptor blocker. CKD; Chronic Kidney Disease. eGFR; estimated Glomerular Filtration Rate. GFR; Glomerular Filtration Rate.

*For the following categories: sex, age, anti-hypertensive usage and current or ex-smoker there were n=168 donors and n=137 controls due to an incomplete data set for one healthy control.

† For isotopic GFR, results from controls were part of the CRIB-DONOR sub study and included n=90 donors and n=22 controls.

3.5.3 Kidney function at follow up

At 12 months, eGFR fell by a mean of -27mL/min/1.73m^2 in donors with no significant change in controls, see **Table 3.3**. Although iGFR measurement was part of the protocol for living kidney donors, in practice few subjects consented to a 12 month iGFR due to concerns about the duration of the test and exposure to ionising radiation.

At follow up there were 6 living kidney donors whose eGFR fell into stage 3b CKD and one whose eGFR fell into stage 4 CKD according to the KDIGO.[11] KDIGO stages at follow up were as follows; stage 1 n=9, stage 2 n=86, stage 3a n=66, stage 3b n=6 and stage 4 n=1. Furthermore, only 16 patients (10%) reduced their eGFR by >40ml/min/1.73m^2 after nephrectomy. There was also no significant within-group or between-group change in urine ACR, see **Table 3.3**.

3.5.4 Comparison of blood pressure variables in living kidney donors and controls

Office and ambulatory blood pressure parameters at baseline and 12 months are given in **Tables 3.4 and 3.5**. Changes observed in office systolic blood pressure from baseline to 12 months in donors and controls were small, see **Table 3.4**. The mean change in office systolic blood pressure in donors (+1.8mmHg) was however, different to that in controls, in whom there was a mean reduction of -1mmHg [mean difference 2.8mmHg (95% CI 0.3-5.4), p=0.03]. The small increase in mean office diastolic blood pressure (+1.7mmHg) in donors was not significantly different to the (+0.7mmHg) value seen in controls. Minimal changes were seen in ambulatory blood pressure measures in both groups with no significant differences between donors and controls in any ambulatory blood pressure parameters at 12 months, see **Table 3.5.** The mean change in ambulatory heart rate however, was significantly greater in donors compared to controls at 12 months [mean difference 2.8 bpm, (95% CI 0.1-5.5), p=0.04].

3.5.5 Hypertension classification

Using current AHA ambulatory blood pressure criteria, 13 (9%) in the control group and 15 (9%) in the donor group developed hypertension over the 12 month period with no significant difference between the two groups, p=0.187.[370] Using the same criteria a similar number of participants had resolved hypertension, (i.e. met criteria for hypertension at baseline but did not at follow up) controls n=12 (9%) and donors n=12 (7%), p=0.186.

3.5.6 Determinants of a change in ambulatory blood pressure and sensitivity analysis

A further sensitivity analysis was carried out for change in ambulatory day systolic blood pressure. Participants were restricted to patients known to have no history of hypertension at baseline. For change in ambulatory day systolic blood pressure the mean change was -0.67mmHg in donors (n=105) vs. 0.81mmHg in controls (n=105), [mean difference -1.49 (95% CI -3.77, 0.78), p=0.197]. For change in ambulatory day diastolic blood pressure the mean change was -0.18mmHg in donors (n=105) vs. 0.99mmHg in controls (n=105), [mean difference -1.17 (95% CI -2.70, 0.35), p=0.133].

3.5.7 Comparison of haemodynamic variables in living kidney donors and controls

Changes in central systolic blood pressure were significantly greater in donors compared to controls [mean difference 3.3mmHg, (95% CI 0.3, 6.3), p=0.030] due to a mean increase in donors and mean decrease in controls at 12 months. Central diastolic blood pressure however, was not significantly different between groups. Small mean increases in adjusted carotid-femoral PWV and AIx were observed in both groups over the 12 month period although there was no significant between-group differences, see **Table 3.6**.

3.5.8 Determinants of a change in carotid-femoral pulse wave velocity and sensitivity analysis

There was no association between kidney donation and change in carotid-femoral PWV when accounting for age at baseline, sex and smoking history, see **Table 3.7**.

In recognition of the high proportion of smokers at baseline and the impact this may have on carotid-femoral PWV a further sensitivity analysis was conducted. After restricting the cohort to those who were current or ex-smokers the mean change in adjusted carotid-femoral PWV was 0.23m/s in donors (n=74) vs. 0.16m/s in controls (n=38), [mean difference 0.07m/s (95% CI -0.37, 0.52), p=0.748]. In those who had never smoked, the mean change was 0.33m/s in donors (n=94) vs. 0.22m/s in controls (n=100), [mean difference 0.11m/s (-0.17, 0.39), p=452]. Consequently a history of smoking had no major effect on the outcome measure.

Due to the participation of a small number of controls and donors with mild hypertension a further sensitivity analysis was carried out to account for this. Participants were restricted to patients with no history of hypertension at baseline. For participants with no history of hypertension the mean change was 0.29m/s in donors (n=147) vs. 0.69m/s in controls (n=128), [mean difference 0.12m/s (95% CI -0.12, 0.36), p=0.325].

Table 3.3. Changes in kidney function over 12 months. First published in Price et al.[362]

Variables	Patient group n=sample size	Single time point Baseline	12 months	Within-group*	Change Between-group†	*p-value* ‡
Urea (mmol/L)	Donors=167	5.1 ± 1.3	6.4 ± 1.7	+1.4 (1.2, 1.6)		
	Controls=136	5.0 ± 1.3	5.2 ± 1.4	+0.2 (0.0, 0.4)	+1.2 (0.9, 1.5)	<0.001
Creatinine (µmol/L)	Donors=168	75 ± 14	104 ± 21	+29 (27.0, 31.4)		
	Controls=136	72 ± 15	71 ± 15	-1 (-3.3, 0.8)	+30 (27.4, 33.4)	<0.001
eGFR (mL/min/1.73m^2)	Donors=168	91 ± 15	64 ± 14	-27 (-29, -26)		
	Controls=136	94 ± 16	96 ± 17	+2 (-0.4, 3.8)	-29 (-32, -26)	<0.001
Urine albumin: creatinine ratio (mg/mmol)	Donors=66	2.78 ± 4.66	2.61 ± 4.50	-0.2 (-1.1, 0.8)		
	Controls=69	2.27 ± 3.69	1.93 ± 3.61	-0.3 (-1.3, 0.6)	-0.2 (-1.1, 1.4)	0.807

Data is displayed as mean ± standard deviation or mean (95% lower CI-95% upper CI).
CI; Confidence interval, eGFR; estimated glomerular filtration rate

* Within-group change refers to change in values between baseline and follow up in each group.

† Between-group change refers to the difference between donors and controls within-group change.

‡ Comparison between controls and donors was made for within-group change using independent samples *t* tests.

Table 3.4. Changes in office blood pressure, weight and body mass index over 12 months. First published in Price et al.[362]

Variables	Patient group n=sample size	Single Time point Baseline	12 months	Change Within-group*	Between-group†	*p-value* ‡
Weight (kg)	Donors=168	75.4 ± 13.5	77.1 ± 14.7	+1.7 (0.4, 3.0)		
	Controls=136	74.7 ± 13.9	74.9 ± 13.8	+0.2 (-0.4, 0.8)	+1.5 (-0.12, 3.0)	0.070
BMI (kg/m^2)	Donors=168	26.2 ± 3.3	26.8 ± 4.6	+0.6 (0.1, 1.2)		
	Controls=136	26.0 ± 4.0	26.2 ± 4.0	+0.1 (-0.1, 0.3)	+0.5 (-0.1, 1.1)	0.094
Seated office systolic BP (mmHg)	Donors=168	125 ± 14	127 ± 12	+1.8 (-0.0, 3.6)		
	Controls=135	125 ±17	124 ± 17	-1.0 (-2.8, 0.7)	+2.8 (0.3, 5.4)	0.029
Seated office diastolic BP (mmHg)	Donors=168	78 ± 9	80 ± 8	+1.7 (0.4, 2.9)		
	Controls=135	77 ±10	78± 9	+0.7 (-0.8, 1.9)	+1.0 (-0.74, 2.9)	0.245

Data is displayed as mean ± standard deviation or mean (95% lower CI, 95% upper CI).
BMI; Body mass index. BP; Blood Pressure, CI; Confidence interval.

* Within-group change refers to change in values between baseline and follow up in each group i.e. mean weight in donors for baseline was 75.4 kg and at follow up was 77.1 kg giving a within-group change of 1.7 kg. The 95% confidence interval was estimated using paired sample *t* tests.
† Between-group change refers to the difference between donors and controls within-group change i.e. for weight, within group change for donors is 1.7 kg and 0.2 kg for controls giving a between-group change of 1.5 kg. The 95% confidence interval was estimated using independent *t* tests.
‡ Comparison between controls and donors was made for within-group change [i.e. mean change in weight of donors (1.7kg) vs. mean change in weight of controls (0.2kg)] using an independent samples *t* tests.

Table 3.5. Changes in ambulatory blood pressure parameters over 12 months. First published in Price et al.[362]

Variables	Patient group n=sample size	Single Time point Baseline	12 months	Within-group*	Change Between-group†	*p-value* ‡
Ambulatory day systolic BP (mmHg)	Donors=119	124 ± 10	124 ± 10	+0.1 (-1.7, 1.9)		
	Controls=111	122 ± 10	123 ± 12	+0.6 (-0.7, 2.0)	-0.5 (-2.8, 1.7)	0.626
Ambulatory day diastolic BP (mmHg)	Donors=119	79 ± 8	79 ± 8	+0.2 (-0.9,1.4)		
	Controls=111	77 ± 8	78 ± 9	+0.9 (0.0, 1.7)	-0.6 (-2.1, 0.9)	0.402
Ambulatory day HR (bpm)	Donors=65	73 ± 9	74 ± 10	+1.5 (-0.9, 3.9)		
	Controls=82	72 ± 9	71 ± 10	-1.3 (-2.8, 0.2)	+2.8 (0.1, 5.5)	0.041
Ambulatory night systolic BP (mmHg)	Donors=111	111 ± 11	112 ±11	+0.9 (-1.1, 3.0)		
	Controls=105	110 ± 10	110 ±12	-0.6 (-2.5,1.3)	+1.5 (-1.2, 4.3)	0.270
Ambulatory night diastolic BP (mmHg)	Donors=111	67 ± 9	69 ± 9	+1.4 (-0.2, 3.0)		
	Controls=105	66 ± 8	66 ± 9	+0.3 (-1.15, 1.7)	+1.1 (-0.9, 3.3)	0.270

Data is displayed as mean ± standard deviation or mean (95% lower CI, 95% upper CI).
BP; Blood Pressure, CI; Confidence interval, HR; Heart rate.

* Within-group change refers to change in values between baseline and follow up in each group.

† Between-group change refers to the difference between donors and controls within-group change.

‡ Comparison between controls and donors was made for within-group change using independent samples *t* tests.

Table 3.6. Changes in central haemodynamic and arterial stiffness parameters over 12 months. First published in Price et al.[362]

Variables	Patient group n=sample size	Single Time point Baseline	12 months	Within-group*	Change Between-group†	*p-value* ‡
Central systolic BP (mmHg)	Donors=105	113 ± 14	115 ± 14	+2.1 (-0.2, 4.4)		
	Controls=108	111 ± 17	109 ± 17	-1.2 (-3.1, 0.7)	+3.3 (0.3, 6.3)	0.030
Central diastolic BP (mmHg)	Donors=105	77 ± 9	78 ± 10	+1.3 (-0.7, 3.2)		
	Controls=108	75 ± 10	75 ± 10	-0.3 (-1.9, 1.1)	+1.5 (-0.9, 4.0)	0.220
Augmentation index, corrected for HR (%)	Donors=104	22.1 ± 12.0	25.6 ± 12.2	+3.4 (1.5, 5.3)		
	Controls=108	20.4 ±1 2.5	22.3 ± 12.0	+1.8 (-0.0, 3.6)	+1.6 (-1.0, 4.2)	0.230
Adjusted carotid-femoral pulse wave velocity (m/s)	Donors=168	7.0 ± 1.3	7.3 ± 1.4	+0.3 (0.1, 0.4)		
	Controls=138	7.0 ± 1.4	7.2 ±1.4	+0.2 (-0.0, 0.4)	+0.1 (-0.2, 0.3)	0.492

Data is displayed as mean ± standard deviation or mean (95% lower CI, 95% upper CI).
BP; Blood Pressure, CI; Confidence interval, HR; Heart rate.

These results are obtained from the SphygmoCor (Atcor Medical, Sydney, Australia).

* Within-group change refers to change in values between baseline and follow up in each group.
† Between-group change refers to the difference between donors and controls within-group change.
‡ Comparison between controls and donors was made for within-group change using an independent samples *t* tests.

Table 3.7. Linear regression model: association between 12 month changes in adjusted carotid-femoral pulse wave velocity and kidney donation, age, sex and smoking status. First published in Price et al.[362]

	Univariable analysis				Multivariable analysis			
	β	**CI**		***p***	**β**	**CI**		***p***
Donor	0.083	-0.155	0.323	0.492	0.098	-0.147	0.343	0.432
Age (years) at baseline	-0.003	-0.012	0.005	0.481	-0.003	-0.012	0.005	0.470
Female	-0.067	-0.307	0.172	0.579	-0.074	-0.318	0.170	0.551
Current or ex-smoker at baseline	-0.065	-0.312	0.182	0.605	-0.089	-0.345	0.165	0.490

Coefficients are given per unit change e.g. per year for age.
β;Beta coefficient; CI; Confidence interval. cfPWV; carotid-femoral pulse wave velocity.

Multivariable analysis shows mutually adjusted coefficients for each independent variable. Linear regression was used for all participants with both baseline and follow up data for cfPWV i.e. change in cfPWV (Living kidney donors n=168, Controls n=138). Carotid-femoral pulse wave velocity has been adjusted for mean heart rate and mean arterial pressure as previously described. Variables chosen for the multivariable model were based on clinical relevance and known factors influencing cfPWV.

3.5.9 Comparison of biochemistry in living kidney donors and controls

Results are shown in **Table 3.8**. At 12 months phosphate levels had fallen in donors by -0.1pmol/L but there was no change seen in the control group [mean difference -0.1pmol/L, (95% CI -0.1, -0.0), p=<0.001]. In contrast a significant increase in uric acid was seen in donors compared to controls [mean difference +51.7 µmol/L, (95% CI 39.5, 63.9), p=<0.001]. Mean albumin was lower at 12 months in donors compared to controls giving a significant, but clinically unimportant difference between groups [mean difference -0.7 (95% CI-1.5, -0.0), p=0.042). No significant changes were observed in corrected calcium or magnesium, see **Table 3.8**.

3.5.10 Sub analysis of participants lost to follow up

Due to a significant proportion of participants not returning to follow up a further sub analysis compared those who were lost to follow up to those that remained in the study, see **Table 3.9**. Minimal differences were observed in those who did not return for follow up at 12 months compared to those that completed the study. Participants who continued in the study, had a marginally lower eGFR, were more likely to be taking anti-hypertensive medications and less likely to have a history of smoking.

Patient demographics at baseline for all those recruited with valid data sets (n=447) are shown in **Table 3.10.** Donors had a higher mean age than controls; (51 yrs. vs 47 yrs., p=0.003) and were more likely to have a history of previous smoking; (46% vs. 33%, p=0.007). In all those recruited there was a greater use of anti-hypertensive usage in the donor group at baseline compared to controls (14% vs 7%, p=0.039). Baseline biochemical characteristics in all those recruited were comparable in donors and controls with the exception of a small clinically insignificant difference in albumin, see **Table 3.11**. Ambulatory blood pressure and measures of arterial stiffness were no different at baseline between donors and controls, **Table 3.12**. Only central systolic blood pressure was

marginally higher in the donor group at baseline compared to controls (113±13mmHg vs. 110±16mmHg, p=0.040).

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Table 3.8. Changes in biochemistry over 12 months. First published in Price et al.[362]

Variables	Patient group n=sample size	Single time point Baseline	12 months	Within-group*	Change Between-group†	*p-value* ‡
Albumin (g/L)	Donors=145	43 ± 4	42 ± 4	-0.4 (-1.0, 0.1)		
	Controls=135	42 ± 4	42 ± 5	+0.3 (-0.1, 0.8)	-0.7 (-1.5, -0.0)	0.042
Corrected calcium (mmol/L)	Donors=148	2.3 ± 0.1	2.3 ± 0.1	0.0 (-0.0, 0.0)	-0.0 (-0.0 – 0.0)	0.281
	Controls=136	2.3 ± 0.1	2.3 ± 0.1	0.0 (-0.0, 0.0)		
Phosphate (pmol/L)	Donors=130	1.1 ± 0.2	1.0 ± 0.2	-0.1 (-0.1, -0.1)	-0.1 (-0.1, -0.0)	<0.001
	Controls=121	1.1 ± 0.2	1.1 ± 0.2	0.0 (-0.0, 0.1)		
Magnesium (mmol/L)	Donors=77	0.9 ± 0.7	0.9 ± 0. 1	0.0 (-0.0, 0.0)	-0.0 (-0.0- 0.0)	0.944
	Controls=85	0.9 ± 0.7	0.9 ± 0.1	0.0 (-0.0, 0.0)		
Uric acid (µmol/L)	Donors=93	299 ± 73	349 ± 76	+50.5 (41.2, 59.8)	+51.7 (39.5, 63.9)	<0.001
	Controls=95	285 ± 66	284 ± 67	-1.3 (-9.3, 6.8)		

Data is displayed as mean ± standard deviation or mean (95% lower CI-95% upper CI). CI; Confidence interval

* Within-group change refers to change in values between baseline and follow up in each group.
† Between-group change refers to the difference between donors and controls within-group change.
‡ Comparison between controls and donors was made for within-group change using independent samples *t* tests.

Table 3.9. Baseline characteristics of patients who were lost to follow up compared to those who continued the study. First published in Price et al.[362]

Baseline characteristics	**Attended follow up**	**Lost to follow up**			***p-value****
	All=306 (68%)	All=141 (32%)	Donors n=78	Controls n=63	
Living kidney donor	168 (55)	78 (55)	-	-	0.934
Sex (male)	135 (44)	50 (35)	28 (36)	22 (35)	0.400
Age (years)	50 ± 13	47 ± 13	50 ± 12	44 ± 12	0.052
Race	Caucasian=285 (93) Non-white=17 (6) Unknown=4 (1)	Caucasian=108 (77) Non-white=17 (12) Unknown=16 (11)	Caucasian=64 (82) Non-white=4 (5) Unknown=10 (13)	Caucasian=44 (70) Non-white=13 (21) Unknown=6 (9)	0.040
eGFR (ml/min/1.73^2)	93 ± 15	97 ± 15	96 ± 15	99 ± 14	0.008
History of hypertension	26 (9)	9 (6)	7 (9)	2 (3)	0.080
Anti-hypertensive usage	27 (19)	8 (6)	6 (8)	2 (3)	0.039
Current or ex-smoker	112 (37)	68 (48)	39 (50)	29 (46)	0.006
ACE/ARB usage	8 (3)	2 (1)	1	1	0.295
Calcium channel blocker usage	10 (3)	4 (3)	3 (4)	1 (1)	0.628

Categorical variables are presented as n (valid %) and were analysed using Chi squared tests. Continuous data are represented as mean ± standard deviation. ACE; Angiotensin Converting Enzyme. ARB; Angiotensin receptor blocker. eGFR; estimated glomerular filtration rate.

* A comparison was made between all those lost to follow up n=141 compared to all those who returned for follow up n=306 using independent samples *t* test**.**

Table 3.10. Baseline patient demographics of the whole cohort recruited. First published in Price et al.[362]

Variable n=sample size	Controls	Donors	*p-value* *
Male sex Donors n=246 Controls n=201	79 (39)	106 (43)	0.400
Age, years Donors n=239 Controls n=195	47 ± 14	51 ± 12	**0.003**
Race Donors n=246 Controls n=201	Caucasian=171 (85) Non-white=21 (10) Unknown= 9 (5)	Caucasian=222 (90) Non-white=13 (5) Unknown=11 (5)	**0.040**
Previous history of hypertension Donors n=232 Controls n=194	11 (6)	24 (10)	0.080
Anti-hypertensive usage Donors n=173 Controls n=159	11 (7)	24 (14)	**0.039**
ACE/ARB usage Donors n=246 Controls n=201	5 (2)	7 (3)	0.679
Calcium channel blocker usage Donors n=246 Controls n=201	5 (2)	9 (4)	0.628
eGFR (ml/min/1.73^2) ‡ Donors n=231 Controls n=181	96 ± 15	93 ±15	**0.048**
Weight, kg Donors n=237 Controls n=193	74.8 ± 13.8	76.0 ± 13.5	0.383
Current or ex-smoker Donors n=246 Controls n=193	67 (33)	113 (46)	**0.007**
Normalised isotopic GFR (ml/min/1.73m^2) Donors n=115 Controls n=24	89 ± 13	89 ± 12	0.884

*Categorical variables are presented as n (valid %) and analysed using Chi squared tests. Continuous data are represented as mean ± standard deviation and analysed using independent samples *t*-tests. ACE; Angiotensin Converting Enzyme. ARB; Angiotensin receptor blocker. CKD; Chronic Kidney Disease. eGFR; Estimated Glomerular Filtration Rate.

Table 3.11. Baseline biochemical and office blood pressure characteristics of the whole cohort recruited. † First published in Price et al.[362]

Variable Sample size =n	**Controls**	**Donors**	***p-value*** *
Urea (mmol/L) Donors n=232 Controls n=184	5.1 ± 1.4	5.0 ± 1.3	0.666
Creatinine (µmol/L) Donors n=232 Controls n=182	72 ± 15	74 ± 14	0.290
Albumin (g/L) Donors n=217 Controls n=183	42 ± 5	43 ± 4	**0.006**
Corrected calcium (µmol/L) Donors n=217 Controls n=183	2.3 ± 0.1	2.3 ± 0.1	0.241
Phosphate (pmol/L) Donors n=198 Controls n=175	1.1 ± 0.2	1.1 ± 0.2	0.156
Magnesium (mmol/L) Donors n=123 Controls n=114	0.9 ± 0.1	0.9 ± 0.1	0.643
Uric acid (µmol/L) Donors n=121 Controls n=127	287 ± 66	299 ± 70	0.180
Urine albumin: creatinine ratio (mg/mmol) Donors n=126 Controls n=125	3.0 ± 6.4	2.8 ± 5.3	0.787
Seated office systolic BP (mmHg) Donors n=234 Controls n=195	125 ± 16	126 ± 14	0.640
Seated office diastolic BP (mmHg) Donors n=233 Controls n=194	77 ± 10	78 ± 9	0.196

* Continuous data are represented as mean ± standard deviation and analysed using independent samples *t*-tests.
†447 participants recruited into the study with valid data sets are represented

BPM; Beats per minute. BP; Blood Pressure, CI; Confidence interval.

Table 3.12. Baseline ambulatory blood pressure and haemodynamic characteristics of the whole cohort recruited. † First published in Price et al.[362]

Variable Sample size =n	Controls	Donors	*p-value* *
Ambulatory day systolic BP (mmHg) Donors n=174 Controls n=158	123 ± 10	124 ± 10	0.562
Ambulatory day diastolic BP (mmHg) Donors n=174 Controls n=158	78 ± 9	79 ± 8	0.365
Ambulatory day HR (bpm) Donors n=109 Controls n=124	73 ± 9	74 ± 11	0.496
Ambulatory night systolic BP (mmHg) Donors n=169 Controls n=156	111 ± 12	112 ± 11	0.740
Ambulatory night diastolic BP (mmHg) Donors n=169 Controls n=156	67 ± 8	67 ± 9	0.579
Central systolic BP (mmHg) Donors n=142 Controls n=148	110 ± 16	113 ± 13	**0.040**
Central diastolic BP (mmHg) Donors n=142 Controls n=148	75 ± 10	77 ± 8	0.064
Augmentation index, corrected for heart rate (%) Donors n=140 Controls n=148	20 ± 13	23 ± 15	0.103
Adjusted carotid-femoral pulse wave velocity (m/s) Donors n=200 Controls n=174	6.9 ± 1.3	7.0 ± 1.4	0.667

* Continuous data are represented as mean ± standard deviation and analysed using independent samples *t*-tests.
†447 participants recruited into the study with valid data sets are represented

BP; Blood Pressure, CI; Confidence interval. HR; Heart rate.

3.6 Discussion

This prospective study of ambulatory blood pressure monitoring and arterial haemodynamics in kidney donors provides important findings. There was no difference in office or ambulatory blood pressure in donors compared to controls at 12 months after nephrectomy. Carotid-femoral PWV also did not differ in these groups. Central systolic blood pressure increased slightly more in donors than controls and at 12 months was higher in the donor group. These results suggest that the risk of a significant rise in blood pressure at 12 months in kidney donors is small. This is in keeping with findings from the smaller sub-study CRIB-DONOR but is surprising in view of the high prevalence of hypertension in patients with CKD and similar levels of GFR.[241] This data suggests that a simple loss of nephron numbers does not invariably result in an elevated blood pressure and that other aspects of CKD such as inflammation and nephron dysfunction may be required for this key pathophysiological mediator to occur.

3.6.1 Changes in peripheral blood pressure after living kidney donation

Previous data have been contradictory. In a 2006 meta-analysis of 48 studies of office blood pressure in kidney donors, including a total of 5145 patients, there was an increase in systolic blood pressure of 6 (95% CI 2-11) mmHg and an increase in diastolic pressure of 4 (95% CI 1-7) mmHg in donors at 5 years.[233] More recently however, Kasiske et al. found no significant difference in over 300 participants between kidney donors and controls in office blood pressure at any time point up to 36 months.[235] There was also no difference in ambulatory blood pressure in 135 donors and 126 controls at 36 months.[235] Taken together, this data and the study of Kasiske et al. suggest that the risk of a clinically important change in blood pressure in the short term following kidney donation is low.[235] Longer term data are of course required.

3.6.2 Changes in central blood pressure after living kidney donation

Despite the absence of change in peripheral pressure, the mean change in central systolic pressure was greater in donors at 12 months compared to controls (+ 2.1 vs -1.2mmHg, p=0.030). While this small difference may be a chance result due to multiple comparisons it may be important. Central blood pressure is more strongly related to LV mass, CIMT and cardiovascular events than peripheral pressure.[371, 372] Given that the central blood pressure was not measured directly but was derived from brachial pressure using a generalized transfer function, these data need to be interpreted with caution.[373] The greater ambulatory heart rate in donors compared to controls at 12 months does raise the possibility that there may have been an increase in sympathetic neural activity which may have influenced central blood pressure.

3.6.3 Changes in carotid-femoral pulse wave velocity after living kidney donation

Previous studies examining arterial stiffness in kidney donors have been small, uncontrolled or lacking in longitudinal assessment. De-Seigneux et al. studied only 21 patients before and one year after nephrectomy and found no change in AIx or PWV.[208] Similarly Fesler et al. found no change in PWV at 12 months post nephrectomy in 45 donors.[248] A cross sectional study of 101 living kidney donors, however, found that PWV was 10% higher compared to healthy controls.[250] Nevertheless, a small effect on PWV in donors cannot be excluded, the study was not powered to detect a difference of less than 0.4m/s.

3.6.4 Changes in central blood pressure without corresponding changes in carotid-femoral pulse wave velocity

The small increases in AIx and central blood pressure were not accompanied by a similar change in carotid-femoral PWV. Discrepancies between changes in AIx and PWV have been found by other observers in a number of situations and remain incompletely explained.[374] Any increase in AIx suggests an increase in wave reflection which might explain the increase in

central blood pressure. As carotid-femoral PWV was unchanged it is possible that this increased reflection occurred due to changes in peripheral, rather than central large conduit arterial stiffness. This may have occurred as a consequence of ligation of one of the renal arteries causing amplification of the reflection site without a corresponding change in carotid-femoral PWV, although to date this has no supportive animal or human evidence.

3.6.5 Changes in biochemistry after living kidney donation

Most of the biochemical changes after donation are in accord with previous studies [235, 241] The increase in uric acid after donation is seen consistently in other studies and presumably reflects reduced clearance.[235, 241] Urate is associated with cardiovascular risk and may play a direct pathophysiological role.[375]

Our finding of lower phosphate levels in donors is perhaps surprising in view of the renal excretion of phosphate but is consistent with a large prospective study of bone metabolism in kidney donors.[255] This may be a result of an increase in FGF23 which has a pivotal role in phosphate homeostasis and has been associated with LVH.[376, 377] In the original CRIB-DONOR sub study an increase in LV mass in donors was observed with a corresponding increase in FGF23.[241] The lack of change in peripheral blood pressure in this larger study is consistent with the suggestion that any cardiovascular structural change in kidney donors is more likely to be due to circulating factors than haemodynamic causes.

3.6.6 Changes in weight and BMI after living kidney donation

Compared to controls, whose weight remained unchanged, kidney donors had a 1.7kg increase in weight over 12 months although there was no significant between-group difference in either weight or BMI. This may be an important finding as weight gain post donation has been shown to be associated with the development of hypertension and diabetes.[378] Donors are also more likely to develop metabolic syndrome even after adjustment for BMI.[379]

3.6.7 Participants with mild hypertension

Participants with mild hypertension at baseline were included in this study. This is based on UK living kidney donor guidelines allowing potential donors with mild-moderate hypertension taking one or two anti-hypertensive agents without target organ damage.[272] The British guidelines are also in keeping with recommendations from Australia, Canada, European Best Practice and KDIGO.[380] As more transplant centres are relaxing current guidelines for eligibility, extended criteria marginal living kidney donors such as those that are hypertensive are likely to be at the highest risk.[380-382] Consequently, those with hypertension that meet current kidney donor criteria were typical of the true donor population representing real world data. Furthermore, sensitivity analysis suggests that the presence of hypertension at baseline made no difference to the primary measures of ambulatory blood pressure or carotid-femoral PWV.

3.7 Limitations

The main limitation of this study is that the planned sample size was not reached and a substantial proportion of participants did not return for follow up at one year, which reduced the study power and introduces the potential for selection bias. Barriers to studies of living kidney donors have been reported by others.[234] They are often geographically remote from the transplant centre (in contrast to the recipient) and after donation are usually in full-time work. Barriers to ambulatory blood pressure monitoring in this study were in keeping with those previously observed, where one in five patients describe 24-hr monitoring as uncomfortable and nearly 70% were woken from sleep.[383, 384]

These limitations however, do not affect the internal validity of the results. There were only minor differences between participants who did and did not return for follow up so these results should be generalisable to the wider pool of potential kidney donors. Although not statistically different, the healthy controls were on average 2 years younger, more likely to be male and

more likely to have a history of hypertension. Whilst differences in morbidity at baseline between groups have been acknowledged, in any non-randomised study differences between groups may occur due to random chance and all participants recruited into the study met strict requirements for kidney donation.

There was a greater rate of smoking amongst donors which could be due in part to social deprivation based on geographical area or reflect health promoting behaviour in healthy controls. This however made no difference to the primary outcome measure during sensitivity analysis.

In addition, the large number of parameters measured beyond the pre-specified primary outcomes mean that there are issues of multiple testing necessitating caution in interpreting results as some differences may have arisen by chance.

Lack of ethnic diversity has been a notable problem in living kidney donor research.[2] Over 90% of this cohort were Caucasian and this does reflect the vast racial disparity currently facing transplantation.[385] Whilst data at 12 months are reassuring, longer term (5-10yrs+) and more diverse studies are required particularly in light of literature showing higher cardiovascular risk in the long term.[223] Marked pathogenic differences between donors and controls in non-invasive vascular measures may not be evident at the 12 month mark.

Finally, although our control group participants were carefully selected to adhere to living kidney donor criteria, those recruited included both those genetically related to recipients and those attending blood donation services. It is recognised that genetic relationships could have implications for both future risk of CKD and aortic stiffness can be considered to some extent an inheritable trait.[229, 386]

3.8 Conclusion

In summary, this multi-centre controlled longitudinal prospective study of haemodynamics in living kidney donors indicates that there is no change in ambulatory blood pressure or arterial stiffness at 12 months post nephrectomy despite changes in biochemistry. This has important implications for the future of living kidney donors but also provides valuable insight into the pathophysiology of hypertension and myocardial disease in CKD suggesting that an increase in blood pressure is not an inevitable consequence of an isolated reduction in GFR.

CHAPTER 4

CARDIOVASCULAR EFFECTS OF UNILATERAL NEPHRECTOMY IN LIVING KIDNEY DONORS AT 5 YEARS. THE CHRONIC RENAL IMPAIRMENT IN BIRMINGHAM (CRIB)-DONOR II STUDY

4.1 Extent of personal contribution

This study was a 5-year longitudinal, parallel group, blinded end point study funded through a personal British Heart Foundation Clinical Training Fellowship (FS/16/73/32314). Ethical approval was obtained by myself through the Integrated Research Application System and I presented the study to the West Midlands-Solihull Research Ethics Committee for final approval. All regulatory documents, audits and correspondence with the sponsor were my primary responsibility. I maintained the site file and dealt with and documented any protocol deviations and adverse events.

I recruited and consented all the participants at 5 years and arranged the 5-year follow up visits myself. The arterial stiffness measures (PWA/PWV), ultrasound for CIMT, blood pressure measurements and all blood sampling were taken by myself as part of that visit. CMR imaging safety checklist, scanning of participants and administration of gadolinium to participants was conducted by myself with the assistance of a radiographer. The Wellcome Trust Birmingham Clinical Research Facility centrifuged blood samples for storage of serum and plasma. I then later performed the ELISA for FGF23. CMR imaging analysis at baseline and 5 years were carried out myself including all mass and volumetric assessments, feature tracking, T1/T2 mapping, and assessment of aortic distensibility and LGE with the exception of a second observer for reproducibility.

Final data interpretation, statistical analysis and presentation of data was carried out by myself with the advice and guidance of a medical statistician. The first draft of the manuscript was written by myself and I was responsible for all edits and revisions. This chapter is based on the published first author original article where this data was first published[354]: Price AM, Moody WE, Stoll VM, Vijapurapu R, Hayer MK, Biasiolli L, Weston CJ, Webster R, Wesolowski R, McGee KC, Liu B, Baig S, Pickup LC, Radhakrishnan A, Law JP, Edwards NC, Steeds RP, Ferro CJ, Townend JN. Cardiovascular

effects of unilateral nephrectomy in living kidney donors at 5 years. Hypertension. 2021; 77: In press (April). DOI: https://doi.org/10.1161/HYPERTENSIONAHA.120.15398.

4.2 Abstract

The **C**hronic **R**enal **I**mpairment in **B**irmingham-DONOR II study (CRIB-DONOR II) was a 5-year longitudinal, parallel group, blinded end point study designed to investigate the associations of an isolated reduction in kidney function on cardiovascular structure and function at 5 years after nephrectomy.

Living kidney donors (n=50) and healthy controls (n=45) who had previously undergone extensive cardiovascular assessment at baseline and 12 months were recruited for follow up at 5 years. The primary end point, LV mass, was measured using CMR. Secondary end points, 24-hr ambulatory blood pressure, and PWV were measured using validated blood pressure monitors and the SphygmoCor device. Effect sizes were calculated as differences between change from baseline in the donor and control groups.

Baseline (pre-donation) eGFR in donors was 95 ± 15ml/min/1.73m^2, 65 ± 13ml/min/1.73m^2 at 12 months and 67 ± 14ml/min/1.73m^2 at 5 years compared to a -1 ±2 ml/min/1.73m^2 decline in controls per annum. Change in LV mass at 5 years compared to baseline was not different between donors and controls, [mean difference +0.40g (95% CI -4.68, 5.49), p=0.876], despite an initial increase in mass observed in donors compared to controls at 12 months. There was also no difference in either LV volumes, LV geometry or LV functional parameters on MRI.

Carotid-femoral PWV, which had increased in donors at 12 months, returned to levels no different from controls at 5 years, [mean difference -0.24 m/s (95% CI -0.69, 0.21), p=0.303]. Office and ambulatory blood pressures in donors did not differ significantly from controls at any time point. At 5 years, there were no significant differences in the prevalence of a detectable troponin or mean plasma NT pro-BNP. Uric acid remained persistently elevated in kidney donors compared to controls on interval assessment.

The reduction in GFR to levels of 60-70 ml/min/1.73m^2 (CKD stage 2 range) after kidney donation does not lead to deleterious changes in cardiovascular structure and function or cardiac biomarkers at 5 years. Factors associated with kidney disease other than an isolated fall in GFR are likely to explain the increased cardiovascular risk in patients with CKD.

4.3 Introduction

CKD is an independent risk factor for cardiovascular morbidity and mortality even after adjusting for comorbidities such as hypertension and diabetes.[15, 41] A graded inverse relationship between eGFR and cardiovascular risk is observed in epidemiological studies beginning at marginally reduced levels of kidney function, between 60 and 75ml/min/1.73m^2.[15, 387] The threshold of kidney function associated with increased cardiovascular risk is unclear, yet a 10 year follow up study of 8913 healthy participants found those with an eGFR as high as 76.6-89.4ml/min/1.73m^2 still had an increased risk of cardiovascular mortality [HR 2.48 (1.26-4.87)] after adjustment for common confounding factors.[388]

Evidence from echocardiography and CMR imaging studies suggest that not only is there an increased risk of cardiovascular morbidity and mortality at the early stages of CKD but there are also adverse changes to cardiac structure and function including elevated LV mass.[125, 353, 389]

The immediate effect of kidney donation is a 50% fall in eGFR but, following hyperfiltration in the remaining kidney, patients regain 60-70% of their baseline eGFR. [390, 391] To date, most clinical outcome studies from kidney donors have not demonstrated an increase in major cardiovascular events.[221] A recent 15-year retrospective study of living kidney donors however, reported an increase in cardiovascular mortality with a HR of 1.40 compared to healthy controls raising concern about the long term safety of kidney donation.[223] Furthermore, the CRIB-DONOR study (NCT01028703) highlighted potentially important short term adverse changes in cardiovascular structure and function.[241] Compared to controls, donors at 12 months after nephrectomy had an increase in LV mass, a deterioration in myocardial strain and arterial function without change in blood pressure.[241]

The CRIB-DONOR II study was designed to follow up the same cohort at 5 years to examine the medium term effects of kidney donation on cardiovascular structure, function and haemodynamics. It was considered important to find out whether the small adverse changes: myocardial mass, systolic function and arterial function continued to progress up to 5 years, remained unchanged or tended to resolve. Any evidence of progression would have implications for potential kidney donors and for subjects with early stage CKD.

4.4 Brief methods

4.4.1 Study design and patient population

CRIB-DONOR II (NCT02973607) was a 5-year longitudinal, parallel group, controlled, blinded end point study of healthy controls and living kidney donors[241] All participants who originally consented to take part in the CRIB-DONOR study (NCT01028703) were approached for follow up between May 2017 and May 2019.[241]

Patients in the original CRIB-DONOR study were recruited from March 2011 to August 2014. In this study 124 subjects (68 living kidney donors and 56 healthy controls) underwent extensive cardiovascular assessment at baseline (within 6 weeks of donation for living kidney donors) and 12 months after nephrectomy. For controls the second assessment was 12 months after the baseline assessment.

The aim of CRIB-DONOR II was to restudy the same cohort of patients at approximately 5 years from donation. Patients were eligible for inclusion into the study only if they participated in the original CRIB-DONOR study. Only subjects who were pregnant or lacked capacity for informed consent were excluded. This accepts that those patients followed up may have had medical events in the last 5 years which means they no longer meet the inclusion criteria of the original study.

4.4.2 Ethical and local approval

This study was supported by a British Heart Foundation Clinical Research Fellowship awarded to Dr Anna Price (FS/16/73/32314). Professor J Townend was the principal investigator. Ethical approval for the study was applied for using the Integrated Research Application System (IRAS ref: 214780). The study was presented to the West Midlands-Solihull Research Ethics Committee (ref: 17/WM/0048 and 214780) and granted approval from the Health Research Authority on 08/03/17. Site specific approval was granted by the University Hospitals Birmingham local Research and Development team who also acted as

the study sponsor (RRK5913). The study was routinely audited by the Trust in May 2019 in line with their responsibilities as a sponsor and it was satisfied that the study adhered to Good Medical Practice guidance. Due to the use of radiation an ARSAC certificate for the study was also granted (reference number RPC 290/1051/36062). All subjects gave informed consent to take part in accordance with the principles set out in the Declaration of Helsinki.

4.4.3 Study protocol

The study was designed to collect data approximately 5 years after the original date of enrolment and, as far as possible, to use the same methods, equipment and assays described in the CRIB-DONOR study.[241, 249] The methods and protocol have previously been published.[249] In brief, participants underwent CMR imaging (including sequences for aortic distensibility assessment), SphygmoCor measures of haemodynamics (PWA, carotid-femoral PWV and central blood pressures), office and ambulatory blood pressure assessment, CIMT imaging and blood and urine sampling.

See **Table 4.1** for an overview of the study protocol and methodology.

4.4.4 Definition of hypertension

The definition of hypertension was in accordance with the European Society of Hypertension ambulatory blood pressure monitoring guidelines.[269] The European Hypertension guidelines for 24-hr ambulatory blood pressure monitoring suggest the following thresholds for the diagnosis of hypertension; 24-hr >130/80mmHg or Day >135/85mmHg or Night >120/70mmHg.[269] Any result crossing any one of these thresholds (systolic or diastolic) was identified as hypertensive.

Table 4.1. Summary of study protocol and methodology.

Study protocol	Methodology	Measure
Clinical assessment	Demographics Office blood pressure Electrocardiogram Ambulatory blood pressure monitoring	Sitting and lying blood pressure 24-hr blood pressure
Biochemical tests and biomarkers	Blood and urine samples Enzyme linked immunosorbent assays Multiplex magnetic immunoassays	Estimated GFR Isotopic GFR FGF-23, α-Klotho, CRP, NT Pro BNP Selected cytokines
Non-invasive vascular assessment	SphygmoCor® Philips iE33 ultrasound machine	Pulse wave analysis Pulse wave velocity Carotid intima-media thickness
Cardiac magnetic resonance imaging	3 Tesla MRI scanner 0.15mmol/kg of gadolinium contrast Post processing software cvi42®	Ventricular mass and volumes analysis T1 mapping T2 mapping Late gadolinium enhancement Aortic distensibility

CRP; C-reactive protein. GFR; Glomerular filtration rate. NT pro BNP; N-terminal-pro B type natriuretic peptide

4.4.5 Primary and secondary end points

The primary end point was change in LV mass at 5 years compared to baseline. Exploratory secondary end points included changes in blood pressure, PWA, PWV, aortic distensibility, biomarkers and CIMT.

4.4.6 Composite blood pressure end point

The composite end point was part of a post hoc analysis of blood pressure. Time was defined at the date of the baseline visit to the date of the final follow up. A composite end point defining clinically significant increases in blood pressure was defined using 4 combined outcomes:

1. 24-hr systolic blood pressure increase of 10mmHg or more.
2. 24-hr diastolic blood pressure increase of 5mmHg or more.
3. 24-hr ambulatory blood pressure monitoring of 130/80mmHg or more.
4. Receipt of anti-hypertensive medication.

An event was identified as the time to occurrence of the first of the listed outcomes above. Subjects that were receiving anti-hypertensive treatment at baseline were excluded from this analysis.

4.4.7 Power calculations and sample size

Using the effect sizes and variances from the original CRIB-DONOR study (change in LV mass 7g, standard deviation of change 10g), recruiting 50 subjects in each group would provide 93% power to detect a difference in LV mass of 7g with an alpha value of 0.05.[241, 392] For 80% power, 34 subjects in each group were required.

4.4.8 Data reanalysed

To allow adequate comparison of the primary end point, LV mass and volume measurements at baseline and 5-year scans were reanalysed by a single observer blinded to both donor/control status and temporal order (cvi42® software version 5.3.4, Circle

Cardiovascular Imaging, Canada). In addition, 3D feature tracking for GCS, GLS and GRS, was performed as previously described with the baseline and 12 month data reanalysed (TomTec 2D not available for CRIB-DONOR II) to allow comparison.[241, 347] Aortic distensibility was also reanalysed for all time points using the latest software developed in Matlab version R2017a (MathWorks, US).

4.4.9 Statistical analysis

Statistical analysis was carried out using SPSS©, version 23 (IBM, Armonk, New York, US). Continuous variables were assessed graphically using histograms to determine normality. Non-parametric data was log10 transformed and assessed graphically. For continuous data, within-group change from baseline to 12 months and baseline to 5 years was analysed using paired samples *t* tests. Between-group difference was analysed using independent samples *t* tests to compare within-group change at 5 years between groups and generate the p value for the primary end point. Non-parametric data was analysed in a logged format then antilogged and displayed as multipliers. Categorical data is displayed as counts and percentages, between-group changes are displayed as relative risks and 95% CI, and analyses were performed using MedCal for windows, version 19.4 (MedCal Software, Ostend, Belgium).

Interactions between each variable and donor/control status were determined by general linear models. Multivariable model analysis was performed using linear regression and incorporating any significant interactions. An interval censored cox regression was used for analysis of the combined blood pressure end point using the icenReg package in R. Reproducibility was assessed using intraclass correlation coefficients.

Further analysis using all available data was carried out using generalized estimating equations. Generalized estimating equations were used to compare change in variables over time for cardiac, haemodynamic and biochemical effects. A linear or binary, unstructured model was chosen with the variable of interest as the dependent variable and both subject identification

and study time point as unique identifiers for each data point. Study time was selected as the within-subject factor and subgroup (donor/control) as a between-subject predictor. An interaction term between each group and study time point was included to assess change over all time points (p-value for interaction). Pairwise analysis was also generated as part of the generalized estimating equations giving comparisons between donors and controls at each corresponding time point.

4.5 Results

The results are presented as changes from baseline in **Tables 4.5-4.15** only from patients who had follow up investigations at 5 years (i.e. not the entire CRIB-DONOR cohort). The between-group difference at 5 years was then compared using an unpaired *t* test. The absolute values of all participants at each time point are given in the appendix in **Tables 4.A1-4.A6** and were analysed using a generalized estimating equations.

4.5.1 Study subjects

Records from all 124 subjects who took part in the original study were reviewed. Of these, 1 had died of bronchial carcinoma and 3 were not contactable; 120 were considered eligible for approach.

4.5.2 Follow up

A total of 67 living kidney donors and 53 healthy controls were approached; 50 kidney donors and 45 healthy controls agreed to participate, see **Figure 4.1.** One kidney donor and one healthy control declined a CMR study. Nine subjects did not undergo a baseline CMR study, therefore, there were 42 kidney donors and 42 controls with paired sets of end point data (baseline and 5-year CMR data). At follow up three patients had contraindications preventing them from undergoing 3 Tesla strength CMR and had 1.5 T scans (intrauterine device, shrapnel in situ and stapedectomy).

4.5.3 Subject characteristics

At follow up, the mean age was 54 ± 12 yrs. in living kidney donors and 50 ± 13 yrs. in controls, see **Table 4.2**. The majority in both groups were female and Caucasian. Over the follow up period there was an increase in rates of hypercholesterolemia in both living kidney donors (4% to 16%) and healthy controls (7% to 16%). One healthy control was diagnosed with diabetes as a result of the study and one was diagnosed with ischaemic heart disease. There was an increase from baseline in the prevalence of self-reported hypertension in living kidney donors (4% to 16%) with little change in controls (7% to 9%). There was a decline in current smoking in both groups but more current and ex-smokers amongst living kidney donors compared to controls. There were no significant differences in baseline demographics of those who attended follow up compared to those lost to follow up with the exception of family history of cardiovascular disease, see **Table 4.3**.

4.5.4 Medication use

Both groups reported increasing use of medication. At 5 years the proportion of donors and controls on anti-hypertensive medication was not different between groups. On review of all anti-hypertensive usage there was a net increase of +1 (+2%) in healthy controls vs. +3 (+6%) in living kidney donors for all drugs. The increasing use of medication was due to one healthy control requiring a second agent (addition of a beta blocker) and three living kidney donors commencing ACE inhibitors. With a 4% difference between-groups. Both groups had more subjects taking non-steroidal anti-inflammatories at 5 years compared to baseline.

4.5.5 Events

There were no deaths or major cardiovascular events in subjects during the study period. One living kidney donor was found to be in atrial fibrillation during the study visit. Two living kidney donors were found to be in first degree heart block. One of whom described a history of several episodes of syncope and was referred to a cardiologist.

Following the study visit one living kidney donor was diagnosed with bowel carcinoma as a result of an incidental finding of microcytic anaemia during the study. A further patient (living kidney donor) was admitted to hospital with a new diagnosis of fast atrial fibrillation having previously been in sinus rhythm during the study. For details of all incidental findings during the study, see **Table 4.4**.

4.5.6 Kidney function

In kidney donors, the mean eGFR was 95 ± 15 ml/min/1.73m^2 at baseline before donation, 65 ±13 ml/min/1.73m^2 at 12 months and 67 ± 14 ml/min/1.73m^2 at 5 years. Changes in iGFR (normalised to body surface area) in kidney donors were comparable: baseline 91 ± 12 ml/min/1.73m^2; 12 months 59 ± 11 ml/min/1.73m^2; 5 years 64 ± 11 ml/min/1.73m^2. In controls there was a mean -1 ± 2ml/min/1.73m^2 decline annually in eGFR: (baseline 99 ± 16 ml/min/1.73m^2; 12 months 96 ± 15 ml/min/1.73m^2; 5 years 94 ± 15 ml/min/1.73m^2).

4.5.7 Effects on LV mass, volumes and geometry

At 12 months a greater increase in LV mass was observed in donors in comparison to controls (+10g vs. +1g), similar to the pattern seen in the original CRIB-DONOR study. At 5 years, however, change in LV mass in kidney donors was no different to healthy controls [+0.40g (95% CI -4.68, 5.49), p=0.876], see **Table 4.5, Figure 4.2A and Table 4.A1**. Consequently, LV mass in donors had resolved to a level comparable to healthy controls at 5 years. There was also no significant between-group difference in LV or LA volumes indexed for body surface area, or LV geometry as evidenced by mass/volume ratio, see **Table 4.5**.

4.5.8 Effects on LV function

At 5 years, no significant between-group differences were demonstrated in either LV ejection fraction, peak GLS, peak GCS or peak GRS, see **Table 4.6, 4.7 and 4.A1**. Similarly there were no significant differences in any of the myocardial strain rate parameters (peak systolic, early diastolic or late diastolic), see **Table 4.6, 4.7 and 4.A2**.

4.5.9 Effects on blood pressure

The increase in self-reported hypertension in the living kidney donor group at 5 years was not consistent with a significant increase in mean office or ambulatory blood pressures compared to the control group. There were no significant between-group differences in office blood pressure or heart rate at 5 years, see **Table 4.8 and 4.A3**. Compared to baseline, mean office systolic blood pressure had fallen in both groups by 5 years. Both mean day and night ambulatory blood pressure readings increased from baseline in both groups at 5 years see **Table 4.9 and 4.A3.** Although mean day ambulatory readings were higher in donors compared to controls at 5 years the difference was not significant between groups: [day systolic blood pressure +1.91mmHg (95% CI-2.72, 6.54)] and [day diastolic blood pressure +1.59 (95% CI-1.99, 5.16)] see **Figure 4.2B and Table 4.9.**

A further sub analysis using a composite end point of clinically significant increases in blood pressure also showed no significant differences between the two groups. At 5 years a total of 21 (47%) controls and 28 (56%) donors had reached the composite end point. The HR for hypertension using the combined outcome in donors relative to controls was increased but not significant [HR, 1.38 (95% CI, 0.74, 2.60), p=0.313].

4.5.9 Effects on prevalence of diagnosed hypertension

Despite the lack of effect of kidney donation on any significant measure of blood pressure the diagnosis of hypertension was increased in donors. There was however no significant difference in hypertension prevalence according to European Society of Hypertension ambulatory blood pressure monitoring criteria, see **Table 4.9**.[269]

4.5.10 Effects on haemodynamics and arterial stiffness and structure

At 12 months, there was an increase from baseline in carotid-femoral PWV in kidney donors, which was not seen in controls, see **Figure 4.3A**. From 12 months to 5 years, PWV increased in both groups and by 5 years the between-group difference was not significant, see **Figure**

4.3A, Table 4.10 and 4.A4. A similar pattern was observed in the augmentation index corrected to 75 beats per minute (AI_{75}) in which there was a small increase in kidney donors at 12 months compared to a fall in healthy controls, see **Figure 4.3B**. AI_{75} at 5 years was not significantly different between kidney donors and controls, see **Table 4.10**. In both groups PWV and AI_{75} increased with time, see **Figure 4.3A and 4.3B**. Aortic distensibility in the proximal ascending and descending aorta decreased in both groups over time with no between-group difference, see **Table 4.11**. There was no significant change in CIMT over time, **Table 4.11.**

4.5.11 Biochemical effects

Biochemical data are given in **Table 4.12-4.15, 4.A5 and 4.A6.** No significant differences were seen in haemoglobin over time, see **Table 4.12**. Six donors developed detectable levels of ACR compared to baseline in comparison to one control, however, the difference between them was not significant, see **Table 4.12**. There was no significant difference in lipid profiles between the two groups or over time, see **Table 4.13**. At 12 months there was a greater prevalence of detectable troponin in donors compared to controls (+25% vs. 1%) and a greater increase in NT pro BNP in donors compared to controls (×1.69 vs. ×0.72) but by 5 years the effect size had dissipated so that levels were comparable to controls, see **Table 4.13**. All detectable troponin levels were low, only two donors (4.7%) had a troponin level >14 ng/L at 5 years.

A similar pattern was observed with FGF23, in which levels at 12 months were greater in donors but the difference between groups had narrowed by 5 years, see **Figure 4.4A**. In contrast to other biomarkers, FGF23 however remained significantly higher in donors than in controls [mean difference ×1.25 (95% CI 1.02, 1.54), p=0.020].

There was an increase in high sensitivity CRP, vitamin D and corrected calcium over time in both donors and controls with no significant difference between groups, see **Table 4.14 and**

Figure 4.4B. A small reduction at 5 years was seen in phosphate levels in donors compared to stable levels in controls, however, there was no between-group difference, see **Table 4.14**. Marked differences were seen in uric acid with increases continuing in donors beyond 12 months in contrast to a small reduction seen in controls, see **Table 4.14** and **Figure 4.5A.**

There was no significant change in PTH, renin or aldosterone between the groups at 5 years, see **Table 4.15 and Figure 4.5B.**

4.5.12 Factors influencing change in LV mass

A linear regression analysis was carried out to determine variables influencing change in LV mass from baseline to 5 years adjusted for both follow up time and donor/control status, see **Table 4.16**. There was no significant influence of sex or LV mass at baseline on change in LV mass at 5 years. Change in ambulatory systolic blood pressure, however, was significantly associated with change in LV mass. None of the other variables were significant when included in a multivariable model with change in day systolic blood pressure.

4.5.13 Relationships between GFR and LV mass

The relationship between GFR and LV mass in both groups is illustrated in **Figure 4.6**. Whilst the mean GFR declines in controls over 5 years compared to baseline values, there is a small increase seen in donors following nephrectomy, see **Table 4.12**. This is in contrast to the small rise in LV mass seen in the healthy control group and the fall in LV mass seen in donors after 12 months, see **Table 4.5**.

4.5.14 Reproducibility for primary end point

There was high reproducibility for LV mass assessment at 5 years. The interclass correlation coefficients (95% CI) for inter-study, intra-observer and inter-observer variability were: 0.99 (0.98 - 0.99), 0.99 (0.96 - 0.99) and 0.99 (0.97, 0.99) respectively, see **Table 4.17 and 4.18**. Bland-Altman plots of bias are illustrated in **Figure 4.7.** The least variability was seen in inter-study reproducibility followed by intra-observer and inter-observer reproducibility. In

addition there was high LV reproducibility for LV mass assessment across all time points [mean bias -1.15±2.6 (95% CI -6.24, 3.94), p=0.066] with an interclass correlation coefficient (95% CI) of 0.99 (0.97, 0.99).

4.5.15 Longitudinal sub analysis of all cases

All available data at all time points are shown in the appendix. In addition to the paired analysis in the main study, a generalized estimating equation analysis was performed using all cases and all time points available, see **Tables 4.A1-4.A6.** A significant p value for the interaction term between each group and study time point was seen for PWV, LV mass and PTH reflecting changes in the donor group at 12 months.

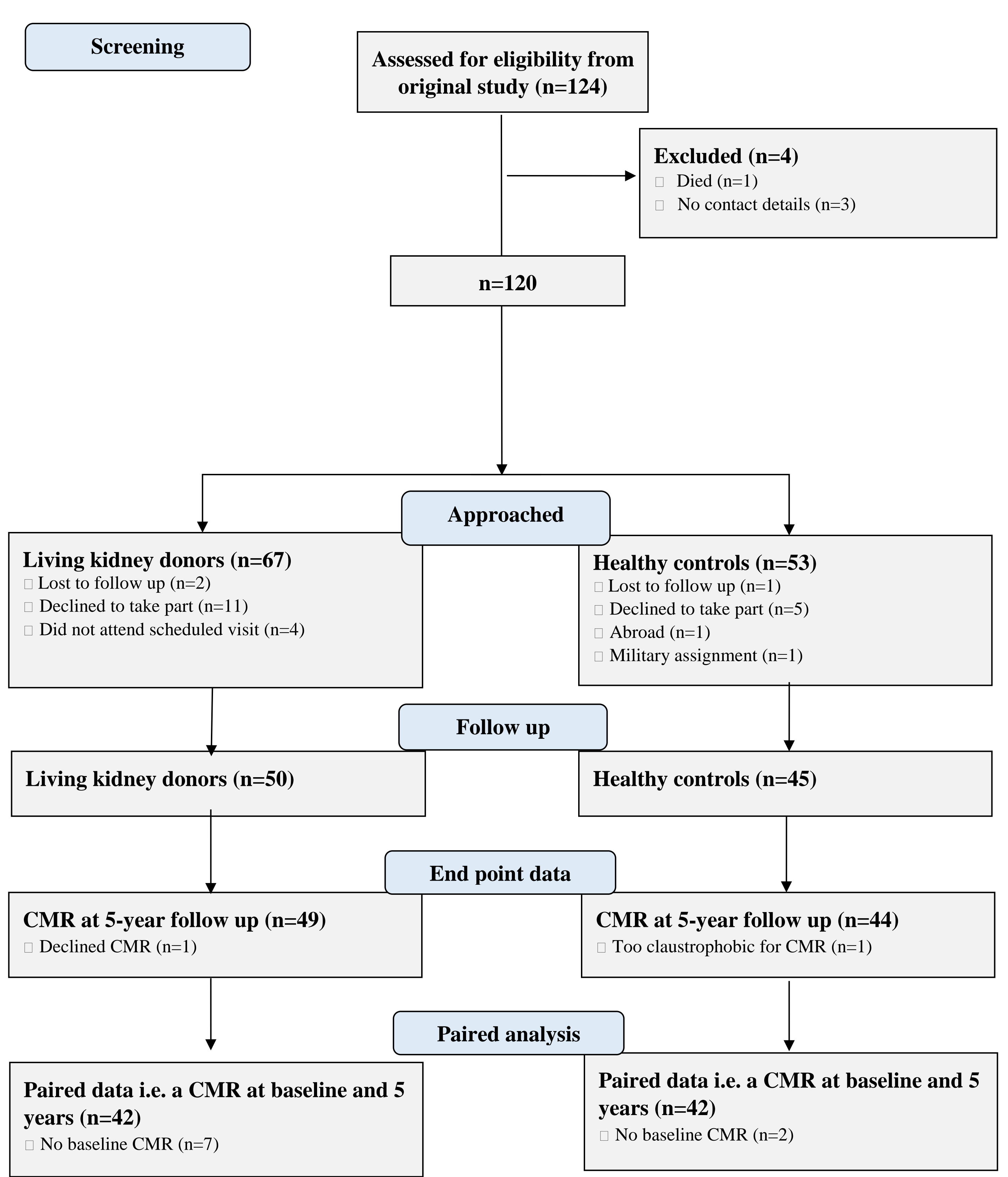


Figure 4.1. Flow diagram of recruitment. First published in Price et al.[354]

Table 4.2. Clinical demographics at baseline and 5 years. First published in Price et al.[354]

Variable	Healthy controls n=45				Living kidney donors n=50			
	N	Baseline visit	N	5 years	N	Baseline visit	N	5 years
Age (years)	45	44.33 ± 13.07	45	50.30 ± 12.91	50	47.96 ± 12.49	50	54.28 ± 12.31
Male sex	45	17 (38)	-	-	50	18 (36)	-	-
Race								
White	45	38 (84)	-	-	50	47 (94)	-	-
Asian	45	4 (9)	-	-	50	3 (6)	-	-
Black	45	3 (7)	-	-	50	0 (0)	-	-
Cardiovascular risk factors								
Hypercholesterolemia	43	3 (7)	45	7 (16)	49	2 (4)	50	8 (16)
Diabetes	43	0 (0)	45	1 (2)	49	0 (0)	50	0 (0)
Hypertension	43	3 (7)	45	4 (9)	49	2 (4)	50	8 (16)
Stroke/TIA	43	0 (0)	45	0 (0)	49	0 (0)	50	0 (0)
IHD	43	0 (0)	45	1 (2)	49	0 (0)	50	0 (0)
Family history								
Cardiovascular	43	10 (22)	45	10 (22)	48	17 (34)	50	17 (34)
Smoking history								
Current smoker	43	2 (4)	44	1 (2)	49	4 (8)	49	3 (6)
Ex-smoker	43	12 (27)	44	13 (29)	49	15 (30)	49	17* (34)
Anti-hypertensive usage								
ACEi	43	3 (7)	45	3 (7)	49	0 (0)	50	3 (6)
β blocker	43	0 (0)	45	1 (2)	49	1 (2)	50	1 (2)
Calcium channel	43	1 (2)	45	1 (2)	49	1 (2)	50	1 (2)
Other medication usage								
Statin	43	3 (7)	45	5 (11)	49	2 (4)	50	2 (4)
Aspirin	45	0 (0)	45	1 (2)	50	0 (0)	50	0 (0)
NSAIDs	45	1 (2)	45	3 (7)	50	1 (2)	50	5 (10)

Data are displayed as mean ± standard deviation or number of patients (percentage). ACEi; Angiotensin converting enzyme inhibitor, BMI; Body mass index. IHD; Ischaemic heart disease; N; Number of subjects. NSAIDS; Non-steroidal anti-inflammatories. TIA; Transient ischaemic event. *One subject started smoking during follow up period for a total of 3.5 years and then gave up.

Table 4.3. Baseline demographics in those that attended follow up and were lost to follow up at the 5-year study visit.

Variable	Follow up at 5 years n=95	Lost to follow up n=29	*p-value*
Donor	50 (53)	18 (62)	0.249
Age (years)	46 ± 13	43 ± 11	0.230
Male sex	35 (37)	15 (51)	0.113
Race			
White	85 (90)	2 (83)	0.288
Asian	7 (7)		
		3 (10)	
Black	3 (3)	2 (7)	
Cardiovascular risk factors			
Hypercholesterolemia	5 (5)	1 (3)	0.557
Diabetes	0 (0)	0 (0)	-
Hypertension	5 (5)	2 (7)	0.534
Stroke/TIA	0 (0)	0 (0)	-
IHD	0 (0)	0 (0)	-
Family history			
Cardiovascular	27 (28)	3 (10)	0.048
Smoking history			
Current smoker	6 (6)	5 (17)	0.126
Ex-smoker	27 (28)	7 (24)	0.466
Anti-hypertensive usage			
ACEi	3 (3)	1 (3)	0.671
β blocker	1 (1)	1 (3)	0.423
Calcium channel	2 (2)	0 (0)	0.564
Other medication usage			
Statin	5 (5)	1 (3)	0.557
Aspirin	0 (0)	0 (0)	-
NSAIDs	2 (2)	0 (0)	0.585

Data are displayed as mean ± SD or number of patients (percentage). Analysis was carried out using independent samples *t* tests for continuous data and fisher's exact tests or chi squared tests for categorical data. ACEi; Angiotensin converting enzyme inhibitor, BMI; Body mass index. IHD; Ischaemic heart disease. NSAIDS; Non-steroidal anti-inflammatories. SD; Standard deviation. TIA; Transient ischaemic event

Table 4.4. Incidental findings during the study. First published in Price et al.[354]

Group	n=sample size, incidental finding
Healthy controls	n=1 Hypertension requiring medication. n=1 Large pericardial cyst. n=1 Prolapsing mitral valve under surveillance. n=1 Abnormal liver function. n=1 Iron deficiency anaemia. n=1 Dilated aorta. n=1 Asymptomatic 1st degree heart block.
Living kidney donors	n=1 Borderline diabetes. n=2 Breast cysts requiring referral. n=1 Iron deficiency anaemia. n=1 Asymptomatic 1st degree heart block. n=1 1st degree block and syncope requiring loop recorder. n=1 Atrial fibrillation.

Table 4.5. Cardiovascular structural effects. First published in Price et al.[354]

Variable	Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value* †
LVM (g)					
Donor	112 ± 27	+10.19 (6.04, 14.34)	+3.37 (-0.70, 7.46)		
Control	112 ± 30	+1.19 (-1.77, 4.15)	+2.97 (-0.18, 6.14)	+0.40 (-4.68, 5.49)	0.876
LVMi (g/m^2)					
Donor	59 ± 9	+6.10 (3.75, 8.44)	+1.51 (-0.41, 3.44)		
Control	59 ± 12	+1.46 (0.00, 2.94)	+1.21 (-0.37, 2.80)	+0.30 (-2.16, 2.76)	0.809
LVEDVi (ml/m^2)					
Donor	64 ± 10	+2.10 (-0.15, 4.37)	-4.11 (-6.47, -1.75)		
Control	67 ± 11	+2.75 (0.27, 5.22)	-2.91 (-5.15, -0.67)	-1.20 (-4.40, 2,00)	0.457
LVESVi (ml/m^2)					
Donor	18 ± 6	+1.66 (0.23, 3.08)	-0.14 (-1.62, 1.32)		
Control	21 ± 7	+1.10 (-0.25, 2.46)	-0.85 (-2.56, 0.85)	+0.71 (-1.51, 2.92)	0.529
Mass/volume ratio (g/ml)					
Donor	0.92 ± 0.12	+0.06 (0.02, 0.11)	+0.09 (0.05, 0.14)		
Control	0.90 ± 0.15	-0.00 (-0.03, 0.02)	+0.06 (0.02, 0.10)	+0.03 (-0.02, 0.09)	0.230
Atrial volumes					
LAVi (ml/m^2)					
Donor	39 ± 8	+6.95 (4.24, 9.67)	-1.40 (-4.98, 2.16)		
Control	41 ± 11	+2.24 (-0.65, 5.13)	-7.18 (-11.77, -2.58)	+5.78 (0.00, 11.54)	0.050

Data are displayed as mean ± standard deviation at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference. CI; Confidence interval. LAVi; Left atrial indexed volume. LVEDVI; Left ventricular end diastolic volume. LVESVI; Left ventricular end systolic volume index. LVM; Left ventricular mass. LVMI; Left ventricular mass index.

*Within-group change and 95% CI were determined using paired samples *t* tests. Results are displayed as the mean change in values (95% CI) between baseline and 12 months and baseline and 5 years for each group.

† Between-group difference and 95% CI were determined using independent samples *t* tests for comparison of within-group change at 5 years between groups. Results are displayed as the mean difference in values (95% CI) between groups for within-group change. The p value is from the independent samples *t* tests.

Figure 4.2. Longitudinal change in LV mass and day systolic blood pressure in donors and controls.
Data plotted include data available at baseline and 5 years (i.e only for those who had both baseline and 5 year data). Black solid lines are means and standard errors for donors. Black diashed lines are means and standard errors for controls. Black squares indicate study visits. The p values are from inedpendent samples *t* tests of between-gruup difference for 5 year change for participants with paired data sets.

A. Left ventricular mass (g), n=84. First published in Price et al.[354]
B. Day systolic blood pressure (mmHg), n=60.

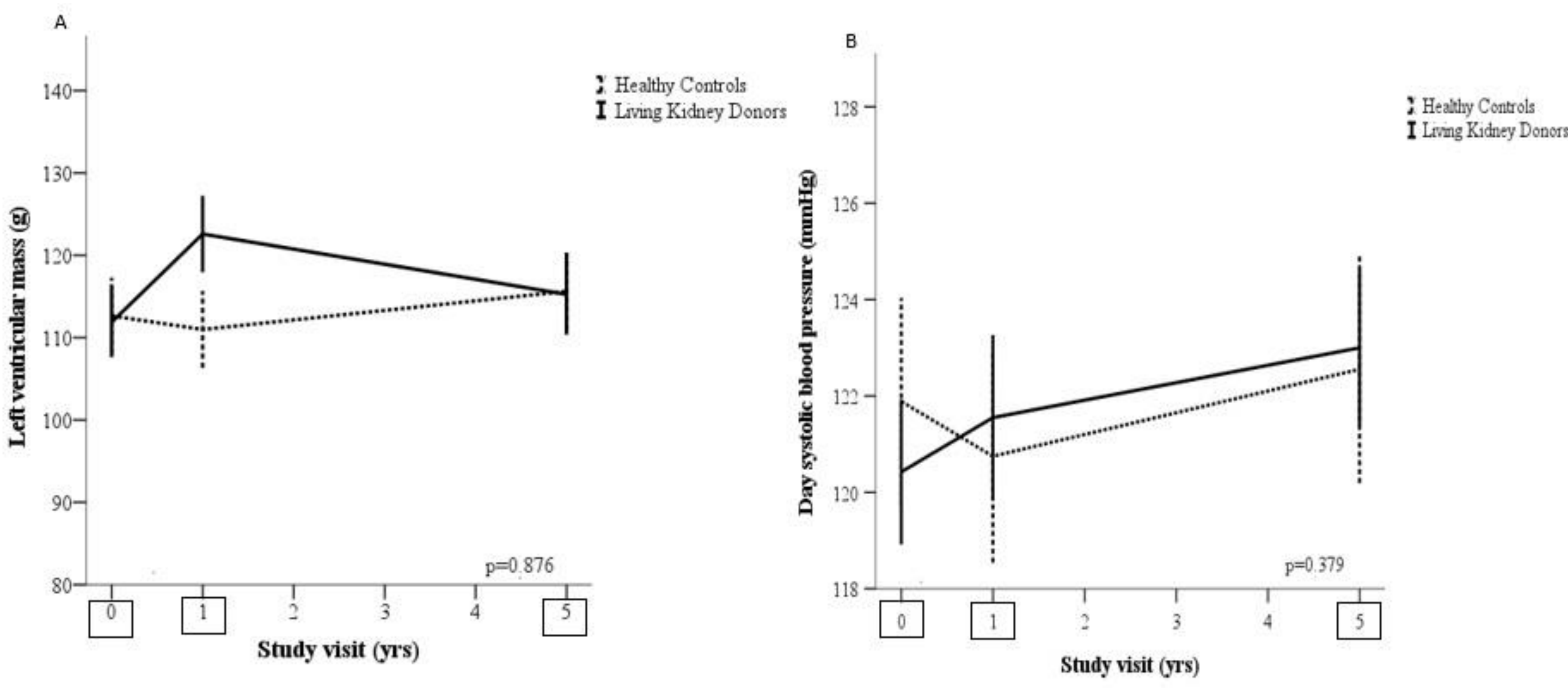


Table 4.6. Cardiovascular functional effects. First published in Price et al.[354]

Variable	Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value* †
LVEF (%)					
Donor	72 ± 6	-1.45 (-3.19, 0.28)	-1.49 (-3.42, 0.43)		
Control	69 ± 7	-0.46 (-2.24, 1.31)	-0.41 (-2.48, 1.66)	-1.08 (-3.87, 1.70)	0.443
Peak GLS %					
Donor	-14.8 ± 3.0	-0.31 (-1.56, 0.92)	-1.18 (-2.46, 0.09)		
Control	-15.1 ± 2.3	+0.16 (-0.96, 1.28)	+0.19 (-0.58, 0.96)	-1.37 (-2.82, 0.07)	0.063
GLS S'					
Donor	-0.73 ± 0.18	-0.10 (-0.20, -0.01)	-0.01 (-0.08, 0.05)	-0.09 (-0.19, 0.00)	0.054
Control	-0.76 ± 0.22	-0.00 (-0.09, 0.09)	+0.08 (0.01, 0.15)		
GLS E'					
Donor	0.70 ± 0.23	+0.05 (-0.07, 0.17)	+0.15 (0.04, 0.25)	+0.08 (-0.05, 0.21)	0.818
Control	0.80 ± 0.25	-0.04 (-0.14, 0.04)	+0.07 (-0.02, 0.16)		
GLS A'					
Donor	0.48 ± 0.17	+0.03 (-0.02, 0.09)	+0.08 (0.01, 0.15)	+0.05 (-0.03, 0.13)	0.247
Control	0.44 ± 0.15	+0.06 (0.00, 0.13)	+0.03 (-0.01, 0.08)		

Data are displayed as mean ± standard deviation at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference.
A'; Late diastolic peak. E'; Early diastolic peak. CI; Confidence intervals, GCS; Global circumferential strain, GLS; Global longitudinal strain, GRS; Global radial strain. LVEF; Left ventricular ejection fraction. S'; Peak systolic peak.

*Within-group change and 95% CI are from paired analyses.
† Between-group difference, 95% CI and p values are from unpaired analyses.

Table 4.7. Myocardial strain and strain rates. First published in Price et al.[354]

Variable	Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value* †
Peak GCS (%)					
Donor	-18.3 ± 2.2	-0.09 (-0.78, 0.59)	-0.63 (-1.32, 0.05)		
Control	-17.8 ± 2.3	-0.01 (-0.79, 0.76)	+0.14 (-0.45, 0.75)	-0.77 (-1.68, 0.11)	0.087
GCS S'					
Donor	-0.90 ± 0.14	-0.05 (-0.13, 0.02)	0.00 (-0.05, 0.06)		
Control	-0.89 ± 0.18	-0.02 (-0.09, 0.04)	0.06 (0.01, 0.11)	-0.06 (-0.13, 0.01)	0.118
GCS E'					
Donor	0.96 ± 0.22	-0.02 (-0.11, 0.05)	+0.10 (0.01, 0.18)		
Control	0.98 ± 0.23	-0.06 (-0.16, 0.03)	+0.11 (0.01, 0.21)	-0.01 (-0.14, 0.11)	0.820
GCS A'					
Donor	0.55 ± 0.19	+0.04 (-0.04, 0.14)	+0.07 (0.00, 0.14)		
Control	0.46 ± 0.15	+0.04 (-0.00, 0.10)	+0.07 (0.02, 0.11)	+0.00 (-0.07, 0.08)	0.845
Peak GRS (%)					
Donor	45.0 ± 11.8	-3.31 (-8.60, 1.97)	-0.48 (-4.59, -3.61)		
Control	43.6 ± 12.7	-0.33 (-4.56, 3.89)	-3.20 (-6.32, -0.09)	+2.72 (-2.30, 7.74)	0.337
GRS S'					
Donor	2.72 ± 1.01	-0.02 (-0.52, 0.47)	-0.24 (-0.62, 0.14)	+0.38 (-0.14, 0.90)	0.155
Control	2.70 ± 1.32	-0.05 (-0.50, 0.39)	-0.62 (-0.99, -0.24)		
GRS E'					
Donor	-2.83 ± 0.91	+0.40 (-0.03, 0.85)	+0.11 (-0.29, 0.52)	+0.15 (-0.30, 0.61)	0.506
Control	-2.85 ± 1.02	+0.20 (-0.19, 0.59)	-0.03 (-0.28, 0.21)		
GRS A'					
Donor	-0.77 ± 0.33	-0.09 (-0.26, 0.07)	-0.01 (-0.16, 0.13)	-0.03 (-0.22, 0.13)	0.621
Control	-0.66 ± 0.28	-0.07 (-0.16, 0.02)	+0.02 (-0.07, 0.13)		

Data are displayed as mean ± standard deviation at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference. A'; Late diastolic peak. E'; Early diastolic peak. CI; Confidence intervals, GCS; Global circumferential strain, GRS; Global radial strain. LVEF; S'; Peak systolic peak.
*Within-group change and 95% CI are from paired analyses. † Between-group difference, 95% CI and p values are from unpaired analyses.

Table 4.8. Office blood pressure effects. First published in Price et al.[354]

Variable	Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value* †
Office measures					
BMI (kg/m^2)					
Donor	26.5 ± 4.3	+0.16 (-0.33, 0.66)	+1.05 (0.20, 1.91)		
Control	25.9 ± 3.3	-0.12 (-0.56, 0.31)	+0.68 (0.03, 1.33)	+0.37 (-0.70, 1.45)	0.494
SBP (mmHg)					
Donor	125 ± 12	-0.54 (-3.59, 2.51)	-2.62 (-6.24, 0.99)		
Control	125 ± 13	-3.21 (-6.33, -0.09)	-4.46 (-8.73, -0.20)	+1.84 (-3.63, 7.32)	0.506
DBP (mmHg)					
Donor	75 ± 9	+2.06 (-0.01, 4.15)	+2.39 (0.07, 4.71)		
Control	76 ± 10	-0.12 (-2.68, 2.42)	-1.14 (-4.22, 1.93)	+3.53 (-0.20, 7.28)	0.063
HR (bpm)					
Donor	67 ± 10	-0.03 (-3.22, 3.14)	-0.62 (-4.08, 2.83)		
Control	66 ± 10	+1.02 (-1.64, 3.69)	+0.07 (-2.96, 3.10)	-0.69 (-5.29, 3.90)	0.765

Data are displayed as mean ± standard deviation at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference.

BMI; Body mass index. CI; Confidence interval. DBP; Diastolic blood pressure. HR; Heart rate. SBP; Systolic blood pressure.

*Within-group change and 95% CI are from paired analyses.

† Between-group difference, 95% CI and p values are from unpaired analyses.

Table 4.9. Ambulatory blood pressure effects. First published in Price et al.[354]

Variable	**Baseline**	**Within-group change * (baseline to 12 months)**	**Within-group change * (baseline to 5 years)**	**Between-group difference † (for 5 year change)**	***p-value* †**
Day SBP (mmHg)					
Donor	121 ± 9	+0.20 (-4.01, 4.42)	+2.57 (-0.63, 5.78)		
Control	122 ± 11	-1.25 (-3.48, 0.98)	+0.66 (-2.79, 4.12)	+1.91 (-2.72, 6.54)	0.413
Day DBP (mmHg)					
Donor	73 ± 7	+0.96 (-1.89, 3.82)	+5.03 (2.52, 7.54)		
Control	75 ± 9	+0.45 (-1.78, 2.70)	+3.44 (0.80, 6.08)	+1.59 (-1.99, 5.16)	0.379
Day HR (bpm)					
Donor	72 ± 9	+2.60 (-1.83, 7.04)	+2.43 (-0.55, 5.42)		
Control	73 ± 9	-1.41 (-4.50, 1.67)	-0.96 (-3.25, 1.32)	+3.39 (-0.39, 7.19)	0.078
Night SBP (mmHg)					
Donor	104 ± 9	+3.00 (-1.19, 7.19)	+6.64 (2.23, 11.04)		
Control	109 ± 11	-3.12 (-9.46, 3.21)	+1.65 (-3.15, 6.46)	+4.99 (-1.34, 11.31)	0.120
Night DBP (mmHg)					
Donor	60 ± 7	+1.71 (-1.68, 5.11)	+5.84 (2.65, 9.02)		
Control	64 ± 10	-0.87 (-5.57, 3.82)	+2.91 (-0.99, 6.82)	+2.93 (-1.94, 7.79)	0.233
Hypertension criteria on ABPM ‡§					
Donor	4 (8.2)	4 (9.8)	9 (20.0)	1.95 (0.65, 5.84)	0.245
Control	6 (14.0)	0 (0.0)	4 (10.3)		

Data are displayed as mean ± standard deviation at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference. ABPM; Ambulatory blood pressure monitoring. CI; Confidence interval. DBP; Diastolic blood pressure. HR; Heart rate. SBP; Systolic blood pressure.

*Within-group change and 95% CI are from paired analyses.

† Between-group difference, 95% CI and p values are from unpaired analyses.

‡ For categorical data, the baseline prevalence is presented and the within-group change is the incidence at 12 months and 5 years in those who did not have the condition at baseline. Prevalence and incidence are given as counts (%). The between-group difference is the relative risk (incidence for donors relative to controls) and 95% CI. The p value is from a fisher’s exact test.

§ The definition of hypertension was in accordance with the European Society of Hypertension ambulatory blood pressure monitoring guidance and in addition included those who had commenced anti-hypertensive medication.[269]

Figure 4.3. Longitudinal change in adjusted pulse wave velocity and augmentation index in donors and controls.

Data plotted include data available at baseline and 5 years (i.e only for those who had both baseline and 5 year data). Black solid lines are means and standard errors for donors. Black diashed lines are means and standard errors for controls. Black squares indicate study visits. The p values are from inedpendent samples *t* tests of between-gruup difference for year change for participants with paired data sets.

A. Adjusted pulse wave velocity (m/s), n=84. First published in Price et al.[354]
B. Augmentation index (Log10), n=85.

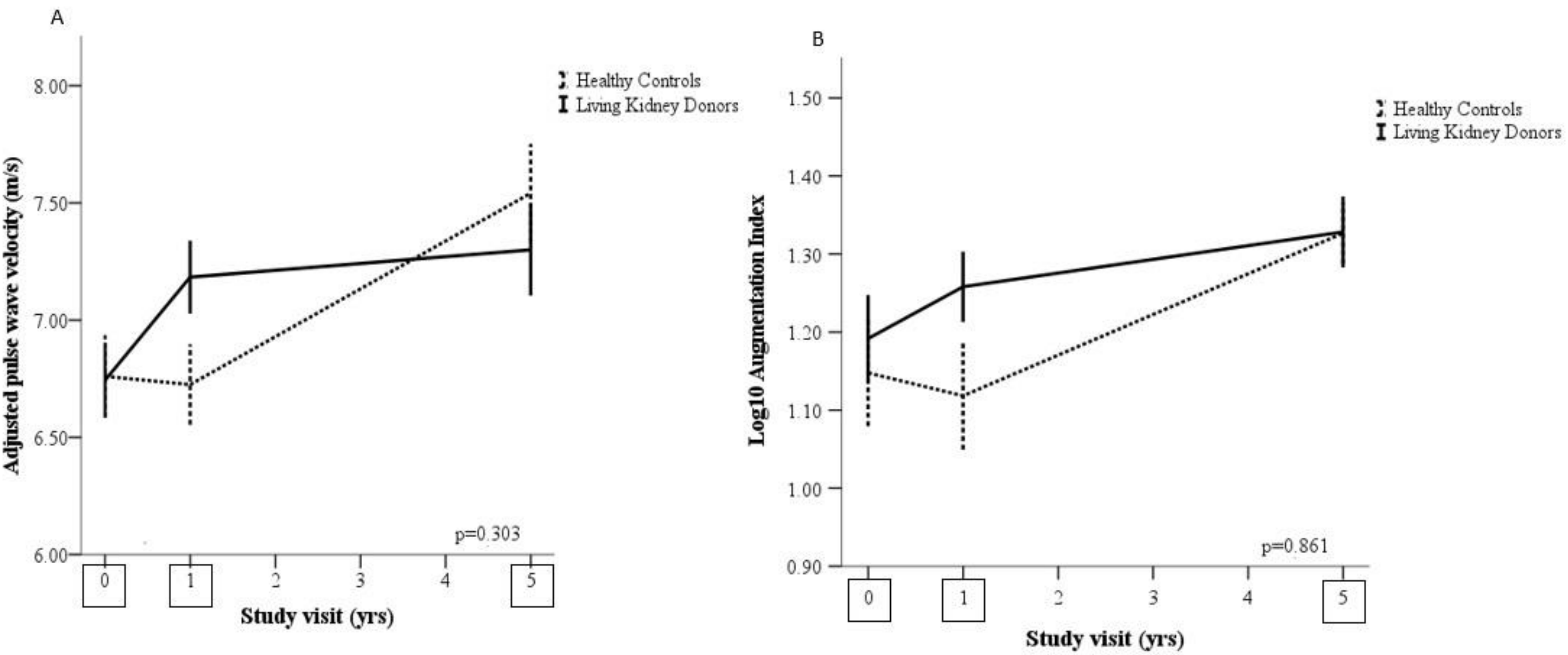


Table 4.10. Central blood pressure and haemodynamic effects. First published in Price et al.[354]

Variable	Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value*
Central SBP (mmHg)					
Donor	112 ± 11	+1.02 (-2.07, 4.13)	+3.03 (-0.42, 6.49)		
Control	111 ± 14	-0.42 (-3.03, 2.18)	+2.12 (-2.47, 6.72)	+0.91 (-4.70, 6.51)	0.748
Central DBP (mmHg)					
Donor	77 ± 9	+1.55 (-0.96, 4.07)	+1.48 (-1.43, 4.40)		
Control	76 ± 10	+0.35 (-2.27, 2.97)	+0.24 (-3.14, 3.63)	+1.24 (-3.14, 5.62)	0.576
AI_{75} ||					
Donor	15.84 [12.30, 19.95]	×1.17 (1.04, 1.31)	×1.69 (1.41, 2.04)		
Control	14.79 [10.00, 19.05]	×0.95 (0.66, 1.34)	×1.73 (1.34, 2.23)	×0.97 (0.72, 1.31)	0.861
Adj cfPWV (m/s)					
Donor	6.74 ± 1.04	+0.50 (0.30, 0.70)	+0.54 (0.26, 0.82)		
Control	6.76 ± 1.09	-0.03 (-0.23, 0.17)	+0.78 (0.40, 1.15)	-0.24 (-0.69, 0.21)	0.303

Data are displayed as mean ± standard deviation or geometric mean [95% CI] at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference.
AI_{75}; Augmentation index corrected for 75 beats per minute. Adj cfPWV; carotid-femoral pulse wave velocity adjusted for mean arterial pressure and heart rate. CI; Confidence interval. DBP; Diastolic blood pressure. SBP; Systolic blood pressure.

*Within-group change and 95% CI are from paired analyses.
† Between-group difference, 95% CI and p values are from unpaired analyses.
|| Non-parametric data was log10 transformed prior to analysis. Values for within-group change and between group differences are displayed as antilogged values with (95% CI). These values are multipliers. For example, for AI_{75} the within group change at 12 months is 1.17 in living kidney donors. The 12 month result on average is ×1.17 the baseline value but the 95% confidence interval indicates that the multiplier could be anywhere between 1.04 and 1.31. The multiplier for between group differences is 0.97 meaning the within-group 5 yr. change in donors is ×0.97 that of the change seen in controls. Therefore the between group difference is a ratio of the donor multiplier to control multiplier.

Table 4.11. Aortic distensibility and carotid intima-media thickness effects. First published in Price et al.[354]

Variable	Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value* †
Aortic distensibility					
Proximal ascending aorta ($\times 10^{-3}$ mm Hg^{-1}) ‖					
Donor	2.81 [2.18, 3.54]	×0.97 (0.85, 1.07)	×0.91 (0.75, 1.04)		
Control	3.01 [2.34, 3.89]	×1.00 (0.93, 1.04)	×0.91 (0.77, 1.04)	×1.00 (0.77, 1.23)	0.894
Proximal descending aorta (×10–3 mm Hg−1) ‖					
Donor	3.31 [2.81, 3.89]	×1.04 (0.95, 1.14)	×1.00 (0.85, 1.12)		
Control	3.46 [3.09, 3.89]	×1.00 (0.95, 1.04)	×0.93 (0.79, 1.09)	×1.04 (0.85, 1.31)	0.644
Arterial structure					
CIT (mm)					
Donor	0.59 ± 0.09	+0.01 (-0.00, 0.02)	+0.00 (-0.01, 0.03)	+0.02 (-0.00, 0.06)	
Control	0.59 ± 0.11	-0.00 (-0.01, 0.01)	-0.02 (-0.05, 0.00)		0.089

Data are displayed as mean ± standard deviation or geometric mean [95% CI] at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference.
CI; Confidence interval. CIT: Carotid intima-media thickness.

*Within-group change and 95% CI are from paired analyses.
† Between-group difference, 95% CI and p values are from unpaired analyses.
‖ Non-parametric data was log10 transformed prior to analysis. Values for within-group change and between group differences are displayed as antilogged values with (95% CI). These values are multipliers.

Table 4.12. Haematological and kidney function effects. First published in Price et al.[354]

Variable	Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value* †
Haemoglobin (g/dl)					
Donor	13.7 ± 1.2	-0.35 (-0.57, -0.13)	+0.00 (-0.28, 0.29)	-0.07 (-0.44, 0.31)	
Control	13.5 ± 1.2	+0.01 (-0.21, 0.24)	+0.07 (-0.16, 0.30)		0.730
Creatinine (µmol/L)					
Donor	73 ± 14	+27.97 (24.46, 31.49)	+23.14 (19.40, 26.87)		
Control	71 ± 13	+1.17 (-1.48, 3.84)	+0.86 (-1.70, 3.42)	+22.28 (17.65, 26.90)	<0.001
eGFR (ml/min/1.73m^2)					
Donor	95 ± 15	-29.20 (-32.09, -26.32)	-27.64 (-31.09, -24.18)		
Control	99 ± 16	-2.12 (-5.63, 1.37)	-4.90 (-8.02, -1.78)	-22.74 (-27.39, -18.07)	<0.001
ACR (≥3mg/mmol) ‡					
Donor	1 (2.0)	3 (6.4)	6 (12.2)		
Control	1 (2.2)	0 (0.0)	1 (2.3)	5.26 (0.66. 42.02)	0.083

Data are displayed as mean ± standard deviation at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference.
ACR; Albumin creatinine ratio. CI; Confidence interval. eGFR; estimated glomerular filtration rate.

*Within-group change and 95% CI are from paired analyses.
† Between-group difference, 95% CI and p values are from unpaired analyses.
‡ For categorical data the baseline prevalence is presented and the within group change is the incidence at 12 months and 5 years in those who did not have the condition at baseline. Prevalence and incidence are given as counts (percentage). The between-group difference is the relative risk (incidence for donors relative to controls) and 95% confidence intervals. The p value is from a fisher's exact test.

Table 4.13. Lipids profile and cardiovascular biomarker effects. First published in Price et al.[354]

Variable		Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value* †
Total Cholesterol (mmol/L)						
	Donor	5.4 ± 1.1	-0.01 (-0.27, 0.23)	-0.17 (-0.38. 0.02)		
	Control	5.0 ± 1.0	-0.00 (-0.21, 0.20)	-0.07 (-0.32, 0.17)	-0.10 (-0.42, 0.21)	0.513
LDL cholesterol (mmol/L)						
	Donor	3.1 ± 1.0	+0.07 (-0.10, 0.24)	-0.13 (-0.34, 0.07)		
	Control	2.9 ± 0.9	-0.09 (-0.30, 0.11)	-0.24 (-0.53, 0.03)	+0.11 (-0.23, 0.45)	0.522
Triglycerides (mmol/L) ‖						
	Donor	1.11 [0.97, 1.28]	×1.07 (0.97, 1.20)	×1.14 (1.00, 1.34)		
	Control	0.96 [0.85, 1.09]	×1.02 (0.93, 1.17)	×1.17 (1.00, 1.38)	×0.97 (0.79, 1.20)	0.812
FGF23 (RU/ml) ‖						
	Donor	72.44 [63.09, 83.17]	×1.23 (1.09, 1.41)	×1.02 (0.91, 1.17)		
	Control	75.85 [63.09, 89.12]	×1.04 (0.89, 1.28)	×0.81 (0.69, 0.97)	×1.25 (1.02, 1.54)	0.020
hsTNT ≥ 5(ng/L) ‡						
	Donor	3 (7.0)	10 (25.0)	28 (59.6)		
	Control	4 (10.0)	1 (3.1)	20 (48.8)	1.25 (0.84, 1.85)	0.212
NT pro BNP (pmol/L) ‖						
	Donor	1.17 [0.61, 2.18]	×1.69 (0.79, 3.71)	×0.60 (0.30, 1.14)		
	Control	1.21 [0.58, 2.51]	×0.72 (0.42, 1.23)	×0.38 (0.19, 0.75)	×1.54 (0.61, 3.98)	0.354

Data are displayed as mean ± standard deviation or geometric mean [95% CI] at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference. CI; Confidence interval. FGF23; Fibroblast growth factor-23. hsTNT; High sensitivity troponin T. NT pro BNP; N-terminal pro B-type natriuretic peptide. LDL; Low-density lipoprotein.
*Within-group change and 95% CI are from paired analyses. † Between-group difference, 95% CI and p values are from unpaired analyses.
‡ For categorical data the baseline prevalence is presented and within group change is the incidence at 12 months and 5 years in those who did not have the condition at baseline. Prevalence and incidence are given as counts (percentage). The between-group difference is the relative risk (incidence for donors relative to controls) and 95% confidence intervals. The p value is from a fisher's exact test.

|| Non-parametric data was log10 transformed prior to analysis. Values for within-group change and between group differences are displayed as antilogged values with (95% CI). These values are multipliers.

Table 4.14. Biochemical effects. First published in Price et al.[354]

Variable	Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value* †
Corrected calcium (mmol/L)					
Donor	2.20 ± 0.98	+0.01 (-0.01, 0.05)	+0.08 (0.05, 0.11)		
Control	2.19 ± 0.08	+0.02 (-0.01, 0.05)	+0.05 (0.03, 0.08)	+0.03 (-0.00, 0.06)	0.117
Phosphate (mmol/L)					
Donor	1.07 ± 0.16	-0.02 (-0.06, 0.01)	-0.05 (-0.10, -0.00)		
Control	1.09 ± 0.16	+0.04 (-0.06, 0.15)	+0.00 (-0.06, 0.08)	-0.05 (-0.14, 0.02)	0.146
Vitamin D (nmol/L)					
Donor	50 ± 27	+7.12 (-0.36, 14.61)	+5.20 (-4.55, 14.95)		
Control	53 ± 22	+0.62 (-6.90, 8.15)	+7.37 (-3.60, 18.35)	-2.17 (-16.66, 12.31)	0.765
Uric acid (µmol/L)					
Donor	267 ± 74	+55.52 (44.44, 66.59)	+68.08 (53.64, 82.53)		
Control	288 ± 54	-1.13 (-11.90, 9.62)	-9.51 (-27.31, 8.29)	+77.59 (55.25, 99.94)	**<0.001**
hsCRP (mg/L) ||					
Donor	0.87 [0.60, 1.23]	×1.65 (1.20, 2.23)	×2.18 (1.58, 3.01)		
Control	0.83 [0.61, 1.12]	×1.14 (0.91, 1.44)	×1.58 (1.24, 2.04)	×1.38 (0.93, 2.04)	0.122

Data are displayed as mean ± standard deviation or geometric mean [95% CI] at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference. CI; Confidence interval. hsCRP; High sensitivity C-reactive protein.

*Within-group change and 95% CI are from paired analyses.
† Between-group difference, 95% CI and p values are from unpaired analyses.
|| Non-parametric data was log10 transformed prior to analysis. Values for within-group change and between-group differences are displayed as antilogged values with (95% CI). These values are multipliers.

Table 4.15. Hormonal effects. First published in Price et al.[354]

Variable	Baseline	Within-group change * (baseline to 12 months)	Within-group change * (baseline to 5 years)	Between-group difference † (for 5 year change)	*p-value* †
Parathyroid hormone (pmol/L)					
Donor	4.4 ± 1.36	+1.31 (0.72, 1.89)	+0.78 (0.26, 1.31)		
Control	4.28 ± 1.19	+0.29 (-0.10, 0.69)	+0.45 (-0.03, 0.95)	+0.33 (-0.37, 1.03)	0.349
Renin (ng/L) ‖					
Donor	9.5 [8.5, 11]	×0.74 (0.61, 0.89)	×1.09 (0.91, 1.31)		
Control	9.8 [8.3, 12]	×1.09 (0.95, 1.26)	×1.14 (0.93, 1.41)	×0.95 (0.71, 1.27)	0.764
Aldosterone ‖					
Donor	107 [81, 141]	×1.14 (0.78, 1.65)	×1.80 (1.23, 2.64)		
Control	110 [89, 135]	×1.34 (1.03, 1.74)	×1.38 (0.97, 1.98)	×1.29 (0.77, 2.17)	0.313

Data are displayed as mean ± standard deviation or geometric mean [95% CI] at baseline for the whole cohort. Mean (95% CI) are displayed for within-group change and between-group difference.
CI; Confidence interval.

*Within-group change and 95% CI are from paired analyses.
† Between-group difference, 95% CI and p values are from unpaired analyses.
‖ Non-parametric data was log10 transformed prior to analysis. Values for within-group change and between-group differences are displayed as antilogged values with (95% CI). These values are multipliers.

Figure 4.4. Longitudinal change in biochemistry in donors and controls.

Data plotted include data available at baseline and 5 years (i.e only for those who had both baseline and 5 year data). Black solid lines are means and standard errors for donors. Black diashed lines are means and standard errors for controls. Black squares indicate study visits. The p values are from independent samples *t* tests of between-gruup difference for year change for participants with paired data sets.

A. Fibroblast growth factor-23 (Log10), n=83.
B. High sensitivity C- reactive protein (Log10).

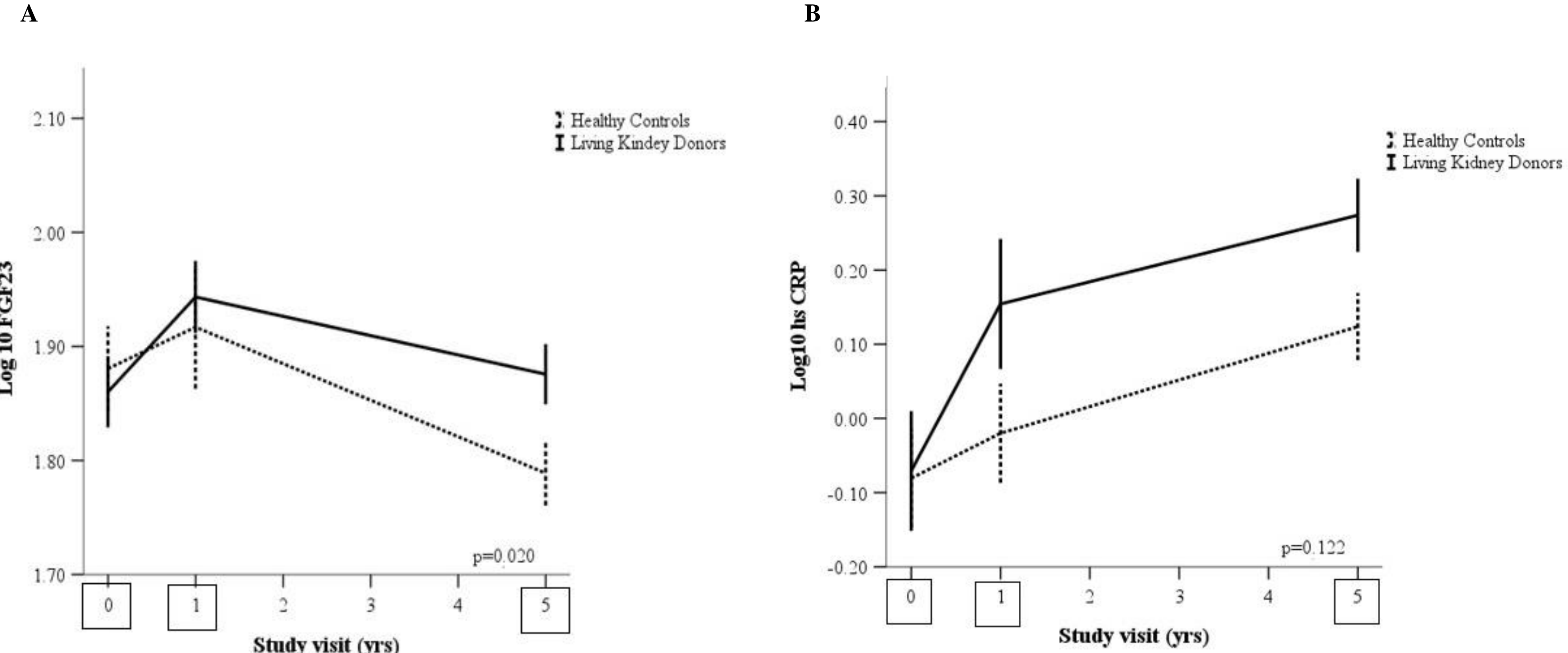


Figure 4.5. Longitudinal change in biochemistry in donors and controls.

Data plotted include data available at baseline and 5 years (i.e only for those who had both baseline and 5 year data). Black solid lines are means and standard errors for donors. Black diashed lines are means and standard errors for controls. Black squares indicate study visits. The p values are from independent samples *t* tests of between-gruup difference for year change for participants with paired data sets.

A. Uric acid (µmol/L), n=86.
B. Parathyroid hormone (pmol/L), n=55.

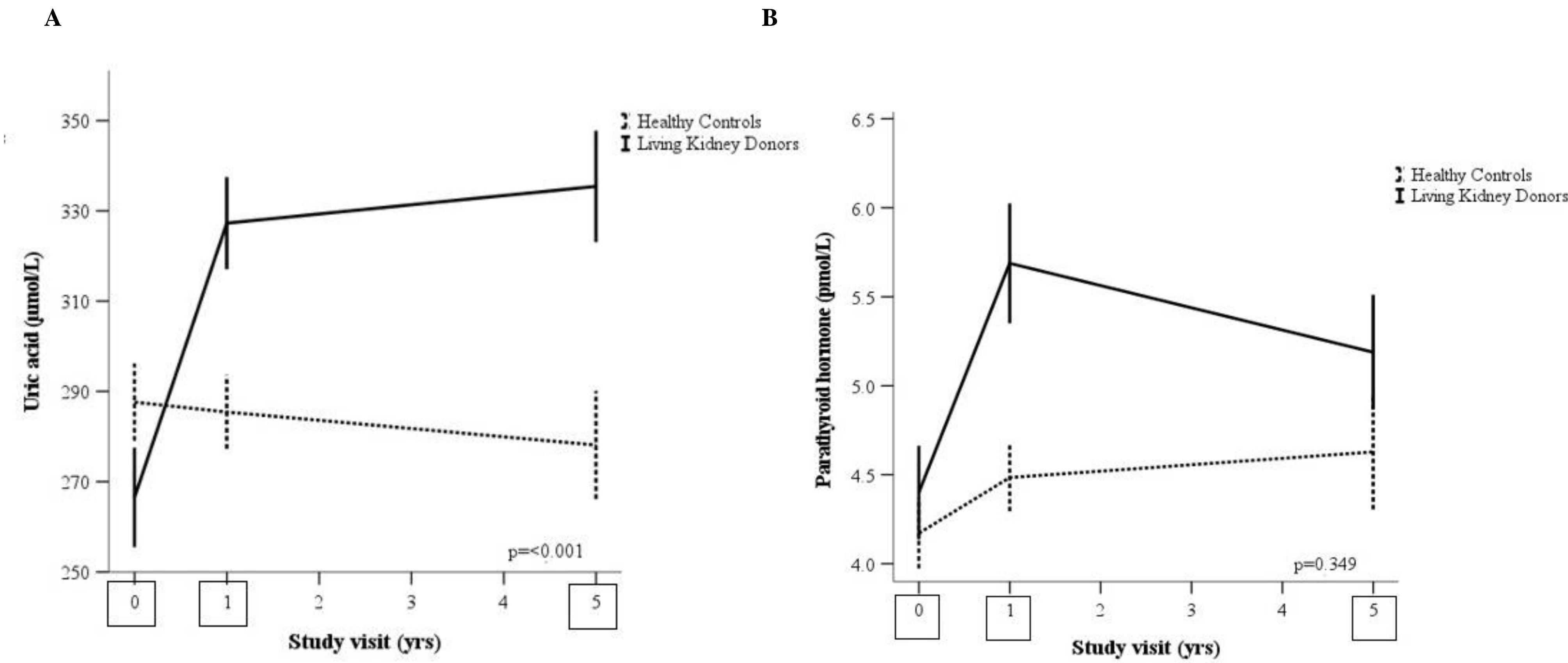


Table 4.16. Multiple linear regression model of variables influencing change in absolute LV mass (g) at 5 years in the whole cohort. First published in Price et al.[354]

	Multivariable analysis			Final model analysis		
	β	**CI**	***p-value***	**B**	**CI**	***p-value***
Donor	*	*	*	0.668†	-6.675, 5.339†	0.824
Follow up (mths.)	*	*	*	0.217	-0.147, 0.580	0.237
Age at baseline (yrs.)	*	*	*	0.212 ‡	-0.225, 0.648 ‡	0.335
Interaction between age and donor/control status	*	*	*	-0.484	-0.225, 0.648	0.065
Male sex	2.458	-2.675, 7.591	0.343			
Baseline LVM (g)	-0.018	-0.110, 0.073	0.689			
Change in eGFR (ml/min/1.73m^2)	-0.029	-0.265, 0.208	0.811			
Change in BMI (kg/m^2)	0.897	-0.050, 1.844	0.063			
Change in office MAP (mmHg)	0.148	-0.156, 0.451	0.336			
Change in day SBP (mmHg)	0.459	0.063, 0.855	**0.024**	0.459	0.063, 0.855	**0.024**
Change in day DBP (mmHg)	0.312	-0.197, 0.821	0.224			
Change in adjusted PWV (m/s)	1.057	-1.493, 3.608	0.411			
Change in uric acid (µmol/L)	0.037	-0.014, 0.087	0.154			
Change in PTH (pmol/L)	-0.502	-2.174, 1.709	0.649			

Change in absolute left ventricular mass (g) was the dependent variable in all analyses. A general linear model was used to test for the interaction between each variable and donor/control status. The only significant interaction was between age at baseline and donor/control status and this was therefore incorporated into all models. For each explanatory variable the values reported under multivariable analysis are from a linear regression model that also included follow up time, donor/control status, age at baseline and the interaction between age and donor/control

status. As the only significant variable in the multivariable analyses, change in day SBP was chosen for the final model, which also included follow up time, donor/control status, age at baseline and the interaction between age and donor/control status. None of the other variables were significant when added to this model.

* Indicates variables included in all multivariable analysis models.

†The estimated difference in means (donor minus control) is given for an individual of mean age (46 yrs.).
‡ Value for a control.

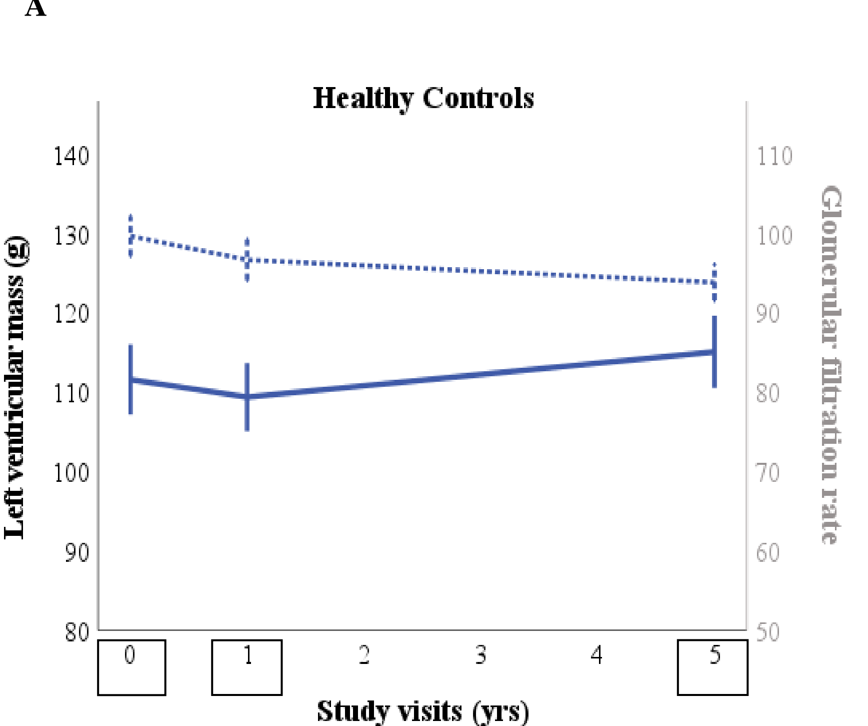
BMI; Body mass index. β; Unstandardized beta coefficient. CI; Confidence interval. eGFR; estimated glomerular filtration rate. DBP; Diastolic blood pressure. LVM; Left ventricular mass. MAP; mean arterial pressure. Mths: Months. PTH; Parathyroid hormone. PWV; Pulse wave velocity adjusted for MAP and HR. SBP: Systolic blood pressure. Yrs: Years. Coefficients are given per unit change e.g. per year for age.

Figure 4.6. Relationships between GFR and LV mass in living kidney donors and controls over time.

Solid lines are means with one standard error for left ventricular mass (g). Dashed lines are means with one standard error for estimated glomerular filtration (ml/min/1.73m^2). Black squares indicate study visits.

A. Healthy controls.
B. Living kidney donors.

A



B

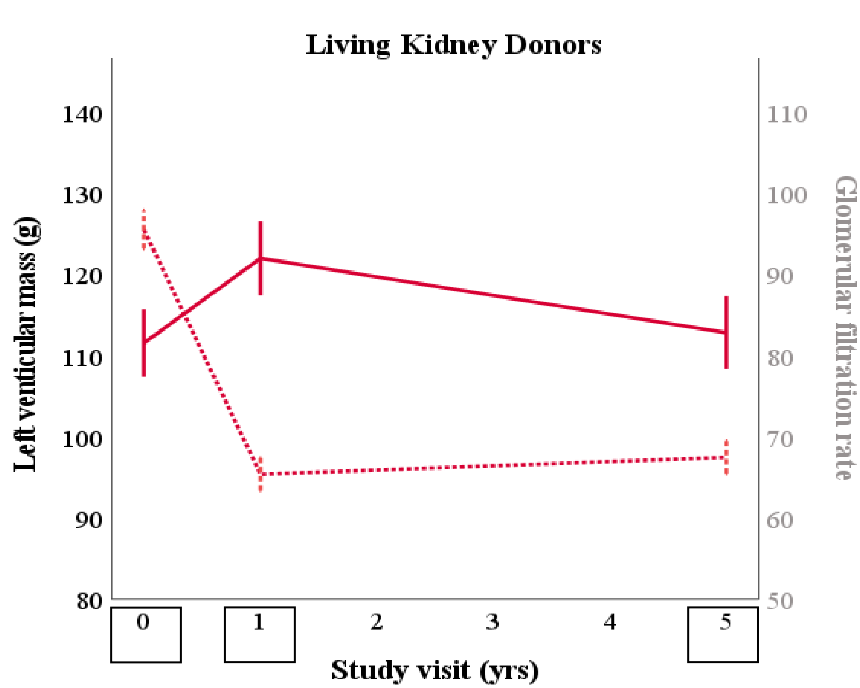


Table 4.17. Inter-study and Intra-observer reproducibility for LV mass and volumes.

A. Inter-study reproducibility.
B. Intra-observer reproducibility.

A Inter-study	Scan	Re-scan	Absolute bias	95% CI		*p-value**	ICC (95% CI) †
LVEDV (ml)	120 ± 24	123 ± 24	2.45 ± 3.4	9.1336	-4.2336	**0.049**	0.98 (0.92, 0.99)
LVEDVI (ml/m^2)	64 ± 8	65 ± 8	1.35 ± 1.9	5.1132	-2.4132	0.053	0.96 (0.80, 0.99)
LVESV (ml)	34 ± 10	35 ± 9	1.26 ± 3.2	7.63	-5.11	0.250	0.94 (0.78, 0.98)
LVESVI (ml/m^2)	18 ± 5	19 ± 4	0.77 ± 1.8	4.3372	-2.7972	0.215	0.89 (0.65, 0.97)
LVSV (ml)	86 ± 15	88 ± 17	1.77 ± 2.2	5.5408	-3.2008	0.130	0.98 (0.95, 0.99)
LVEF (%)	72 ± 4	71 ± 4	-0.80 ± 0.7	3.7864	-5.3864	0.303	0.84 (0.50, 0.95)
LVM (g)	118 ± 27	117 ± 28	-1.11 ± 2.3	3.3296	-5.5296	0.153	0.99 (0.98, 0.99)
LVMI (g/m^2)	63 ± 9	62 ± 9	-0.65 ± 1.2	1.8392	-3.1392	0.141	0.98 (0.95, 0.99

B Intra-observer	Analysis 1	Analysis 2	Absolute bias	95% CI		*p-value**	ICC (95% CI) †
LVEDV (ml)	118 ± 20	118 ± 20	0.41 ± 3.7	7.662	-6.842	0.712	0.98 (0.94, 0.99)
LVEDVI (ml/m^2)	61 ± 9	61 ± 9	0.23 ± 1.7	3.562	-3.102	0.687	0.98 (0.93, 0.99)
LVESV (ml)	35 ± 11	37 ± 12	1.32 ± 3.9	8.964	-6.324	0.320	0.94 (0.79, 0.98)
LVESVI (ml/m^2)	18 ± 5	19 ± 6	0.71 ± 2.0	4.63	-3.21	0.306	0.93 (0.75, 0.98)
LVSV (ml)	82 ± 10	82 ± 9	-0.88 ± 1.9	2.844	-4.604	0.193	0.97 (0.90, 0.99)
LVEF (%)	70 ± 5	69 ± 5	-0.93 ± 2.60	4.166	-6.026	0.287	0.87 (0.59, 0.96)
LVM (g)	113 ± 22	113 ± 22	-0.21 ± 3.13	5.9248	-6.3448	0.834	0.99 (0.96, 0.99)
LVMI (g/m^2)	58 ± 9	58 ± 9	-0.07 ± 1.62	3.1052	-3.2452	0.881	0.98 (0.94, 0.99)

Data are displayed as mean ± standard deviation with (95% confidence intervals).
CI; Confidence interval. ICC; Intra-class correlation coefficient. LVEDV; Left ventricular end diastolic volume. LVEDVI; Left ventricular end diastolic volume index. LVESV; Left ventricular end systolic volume LVESVI; Left ventricular end systolic volume index. LVSV; Left ventricular stroke volume. LVEF; Left ventricular ejection fraction. LVM; Left ventricular mass. LVMI; Left ventricular mass index.

*The p-values are derived from paired samples *t* tests comparing either each scan result (inter-study reproducibility) or each analysis (intra-observer reproducibility).
†A single measures intra-class correlation coefficient is given with 95% confidence intervals for absolute agreement.

Table 4.18. Inter-observer reproducibility for LV mass and volumes.

Inter-observer	Rater 1	Rater 2	Absolute bias	95% CI		*p-value**	ICC (95% CI) †
LVEDV (ml)	147 ± 39	144 ± 33	-3.96 ± 11.29	18.1684	-26.0884	0.296	0.95 (0.82, 0.98)
LVEDVI (ml/m^2)	72 ± 17	70 ± 14	-1.84 ± 5.53	8.9988	-12.6788	0.320	0.93 (0.78, 0.98)
LVESV (ml)	46 ± 18	44 ± 15	-2.03 ± 6.10	9.926	-13.986	0.319	0.93 (0.76, 0.98)
LVESVI (ml/m^2)	22 ± 8	21 ± 6	-0.92 ± 3.03	5.0188	-6.8588	0.363	0.91 (0.71, 0.97)
LVSV (ml)	101 ± 23	99 ± 20	-1.92 ± 8.66	15.0536	-18.8936	0.500	0.92 (0.74, 0.98)
LVEF (%)	69 ± 5	70 ± 4	0.27 ± 3.04	6.2284	-5.6884	0.778	0.81 (0.41, 0.95)
LVM (g)	137 ± 37	139 ± 39	1.28 ± 4.24	9.5904	-7.0304	0.363	0.99 (0.97, 0.99)
LVMI (g/m^2)	67 ± 15	67 ± 16	0.23 ± 1.98	4.1108	-3.6508	0.714	0.99 (0.97, 0.99)

Data are displayed as mean ± standard deviation with (95% confidence intervals).

CI; Confidence interval. ICC; Intra-class correlation coefficient. LVEDV; Left ventricular end diastolic volume. LVEDVI; Left ventricular end diastolic volume index. LVESV; Left ventricular end systolic volume LVESVI; Left ventricular end systolic volume index. LVSV; Left ventricular stroke volume. LVEF; Left ventricular ejection fraction. LVM; Left ventricular mass. LVMI; Left ventricular mass index.

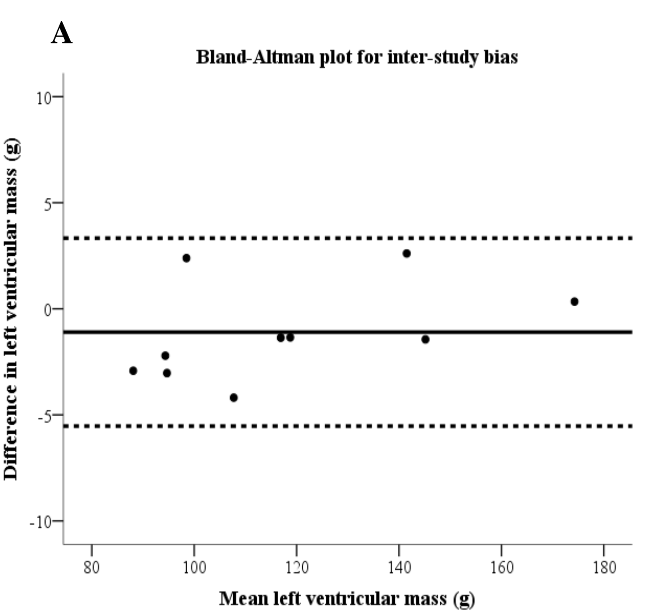
*The p-values are derived from paired samples *t* tests comparing each rater (inter-observer reproducibility).
†A single measures intra-class correlation coefficient is given with 95% confidence intervals for absolute agreement.

Figure 4.7. Bland-Altman plots for reproducibility of LV mass.

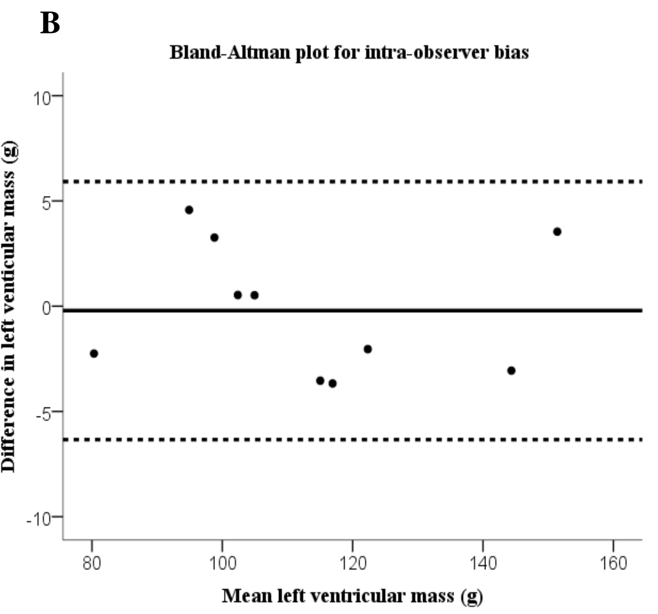
A. Bland-Altman plot for inter-study reproducibility
B. Bland-Altman plot for intra-observer reproducibility
C. Bland Altman plot for intra-observer reproducibility

Solid black line indicates mean absolute bias. Dashed lines indicate 95% limits of agreement.

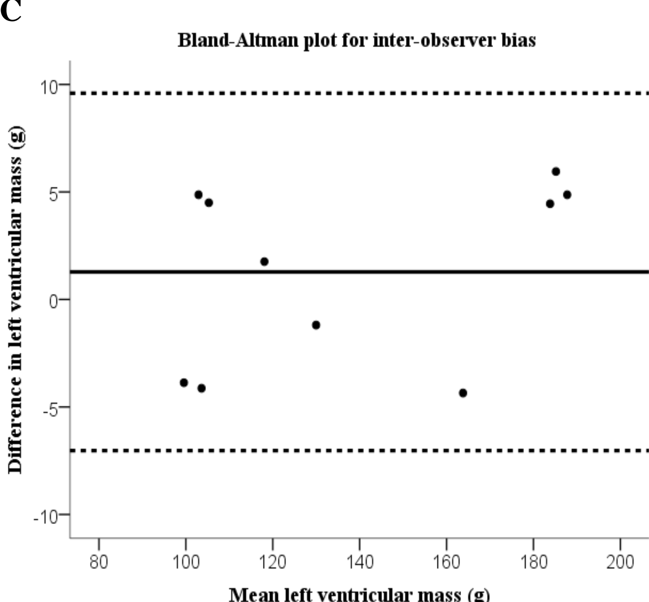
A



B



C



4.6 Discussion

This longitudinal study of living kidney donors examined cardiovascular structure and function, haemodynamics and biochemical parameters 5 years after nephrectomy. The main finding was that by 5 years, kidney donors did not differ significantly from control subjects in their LV mass, volumes or geometry. Change in office and ambulatory blood pressures, large arterial haemodynamics and biomarkers indicative of myocardial damage, inflammation and LV stretch were also not significantly different compared with controls. Of the biomarkers analysed, uric acid and FGF23 remained elevated compared to controls at 5 years. Only uric acid continued to increase in donors, diverging further from the control group.

In the first 12 months after donation, the reduction in eGFR was associated with increases in LV mass, PWV and troponin compared to controls, despite no difference in blood pressure. The differences previously reported in kidney donors at 12 months had not only failed to progress over the succeeding years but had either resolved or the control group had caught up over time so that the groups had become insignificantly different.

The determinants of change in LV mass at the 5 year visit were change in ambulatory systolic blood pressure, a strong predictor for increasing LV mass in the general population.[393] These results indicate that kidney donors show no detectable evidence of cardiovascular structural or functional change at 5 years from donation compared to a healthy control group. These data should be viewed as reassuring findings for those considering kidney donation and for clinicians involved in live donor transplant programs.

4.6.1 Longitudinal changes in LV mass after living kidney donation

In the first CRIB-DONOR study, there was a significant increase in LV mass in kidney donors compared to healthy controls at 12 months (+7±10g in donors vs. -3±8g in controls; p=<0.001).[241] These results were reinforced by a later small uncontrolled study of 23 kidney

donors from Germany, Altmann et al. found LV mass increased from 112±22g to 115±23 g ($p<0.001$) on CMR imaging at 12 months after nephrectomy.[242]

Whilst this is currently the only CMR study of donors at 5 years, these latest results are in contrast to the previous literature demonstrating an increase in LV mass at 12 months. These data suggest that changes previously seen at 12 months resolve over time and become insignificantly different to changes seen in healthy controls by 5 years. The reasons for these fluctuations in LV mass over time are unclear.

A contributing factor to the reduction in between-group differences at 5 years may have been the reduction over time in the differences in eGFR. In donors, while 12 month iGFR reduced by about 30ml/min/1.73m^2, by 5 years there was a mean increase from this nadir of 2ml/min/1.73m^2. In contrast, eGFR in healthy controls declined by -1 ml/min/1.73m^2 per year. In the first CRIB-DONOR study, there was a significant association between the increase in LV mass and change in iGFR ($\beta=-0.3$; $R2=0.19$; $P<0.001$).[241] Given this, and the strong associations of LV mass with reduced eGFR in community studies, a reduced difference in eGFR might be expected to be associated with a reduced difference in LV mass.[97, 100, 394] The relationship between eGFR and LV mass has also been demonstrated by LV mass regression following renal transplantation in which an improvement in eGFR was associated with a reduced risk of fatal and non-fatal cardiovascular events [HR 0.95 (0.91–0.99), p=0.03].[101] It should also be noted that other studies have demonstrated small increases in eGFR in kidney donors after nephrectomy and some report that eGFR may actually continue to increase over 15-17 years before reaching a plateau.[217, 395]

4.6.2 Effects of nephrectomy surgery on LV mass at 12 months

Other direct and indirect effects of the nephrectomy surgery on LV mass seem unlikely to explain the 12 month findings. Although donors experience a small acute reduction in

haemoglobin, a rise in erythropoietin and in CRP, most of these effects have resolved by 12 months.[396] The prevalence of late anaemia in kidney donors has been reported at only 11%.[397] Consistent with this, there was no difference in haemoglobin at 12 months in the cohort.[397] Erythropoietin, however, was not measured and has been associated with LVH.[398] Influences on LV mass at 12 months due to circulating and haemodynamic factors which either were not measured or were too small to be detected cannot be excluded.

Although acute post-operative pain may cause sympathetic nervous system activation, the resultant short term haemodynamic changes seem unlikely to explain changes in LV mass at 12 months after surgery.[399] Furthermore, laparoscopic nephrectomy seldom causes long term debilitating pain, particularly hand assisted laparoscopic nephrectomy which has been demonstrated to be less painful that open nephrectotmy.[400, 401] Hand assisted laparoscopic nephrectomy has been consistently used at our unit since 2004 and therefore a change in surgical technique during the course of this study cannot be used to account for the results at 12 months.

4.6.3 Reasons for a regression in LV mass

Other causes of a regression in LV mass from 12 months to 5 years may be explained by a change in either preload or afterload principally due to a reduction in blood pressure. Aggressive blood pressure control through early commencement of anti-hypertensive medication has been demonstrated to have a positive effect on LV mass reduction.[402] ACE inhibitors in particular have been shown to reduce LV mass in both those with hypertension and those with CKD.[403, 404] Although there was an increased use of ACE inhibitors in the donor group the difference between donors and controls was small and there were no corresponding differences demonstrated in mean blood pressure to account for the change in LV mass.

4.6.4 Phenotype of left ventricular hypertrophy

As the CMR abnormalities seen at 12 months had resolved by 5 years it raises the question as to whether the nature of the rise in LV mass could be a benign adaptive response to physiological stressors, similar to those changes seen in athletic hearts.[93] Cardiac changes in response to exercise can be rapid but ultimately are reversed when training stops.[93] The increase in LV mass at 12 months in donors may be a physiological response which is both adaptive and reversible at 5 years. This would be in contrast to the rise in LV mass associated with CKD which can be considered maladaptive.[112] Elevated LV mass in CKD may also be expected to be more permanent as previous studies have found it is associated with increased fibrosis, functional impairment and increased risk of arrhythmia.[130, 135] Therefore, perhaps the increase in LV mass seen in donors is of a different phenotype. Further study would be required to determine whether this is the case.

4.6.5 Surveillance bias in living kidney donors

The apparent finding of increased rates of self-reported hypertension in donors could be the result of surveillance bias.[2] The increase in self-reported hypertension in the living kidney donor group at 5 years was not consistent with the use of anti-hypertensives nor significant changes in office or ambulatory blood pressure. This perhaps suggests that donors have a greater awareness of their own blood pressure due to post donation annual surveillance but ultimately did not reach a threshold in which medication was commenced. This phenomenon of surveillance bias has been described repeatedly in living kidney donor studies as a result of several factors.[2] Firstly, it is well known that donors tend to have greater contact with primary care services as a result of annual surveillance.[2, 215, 222] Secondly, in addition there may be a higher rate of health seeking behaviour. An example of this was reported by Reese et al. who found higher diagnoses of non-melanoma skin cancer, arguably completely unrelated to donation and possibly a reflection of increased contact with primary care.[222]

Similarly there are several reports of high rates of white coat hypertension in donors with great discrepancies between office and ambulatory blood pressure unlikely to have been observed to the same degree in the general population.[373, 405] Finally, there is a lack of clarity on management of post donation hypertension leaving it to the discretion of the individual clinician.[380] Whilst there is a general consensus that donors should be followed up annually it falls short on any specific thresholds of blood pressure patients such treated at meaning some primary care providers may term a donor hypertensive at a lower threshold than others.[380, 406]

Finally, this study was not powered to detect small effects on blood pressure and, as the ambulatory blood pressure values in donors at 5 years were numerically slightly higher than those in controls, longer and larger studies of ambulatory blood pressure in kidney donors are still required.

4.6.6 GFR threshold for cardiovascular damage

It is possible that epidemiological studies have attributed increased cardiovascular risk to early stage CKD as a result of inadequate correction for traditional risk factors and that the reduction in eGFR in donors is insufficient to cause cardiovascular damage. The precise threshold at which cardiovascular damage and risk occurs is still a subject under study and this will be described in chapter 7. While epidemiological studies using calculated eGFR values suggest an effect beginning at 75ml/min/1.73m^2 other studies using cystatin C suggest this threshold is lower at around 66ml/min/1.73^2.[387] Despite this, adverse cardiovascular structural and functional effects in subjects with early stage CKD who have eGFR values similar to this cohort have been reported.[151] Furthermore, of the donors in our cohort, 36% still had a GFR of <60ml/min/1.73^2 at 5 years, under the threshold expected to see cardiovascular damage in CKD.

4.6.7 Lack of harm at 5 years

These results provide reassuring information for donors suggesting lack of cardiovascular harm at 5 years. Although GFR in donors may not decline dramatically in the long term, longer follow up studies are required to address the long term effects of a reduction in GFR on the cardiovascular system. Large scale registry studies with diverse patient groups, long term follow up (+10yrs) and carefully selected healthy controls are crucial to address this. Complimentary data from patho-physiological studies aiming to identify possible mediators and key intermediates are also needed.

4.7 Limitations

The major strength of this study is that it was a blinded end point analysis from a prospective longitudinal study of a donor cohort with an appropriately healthy control group allowing assessment of serial change. A high return rate for a longitudinal study was achieved with 79% from the original cohort.

Limitations include potential selection bias due to attrition as a result of the longitudinal design. Whilst attempts were made to minimise changes in techniques and methodology, upgrades to our imaging system meant that the MRI scanner used at 5 years was 3T rather than 1.5T. Signal-to-noise ratio and artefact increases with increasing field strength and can potentially affect scan quality, but the field strength itself is not deemed to have a significant influence on mass and volume quantification.[407] This cohort were predominantly Caucasian and therefore, the results cannot be generalisable to all kidney donors. It has previously been established that risk is highly likely to be race and age dependent.[2] Finally, due to the large number of variables analysed it is recognised that some significant differences are likely to occur by chance.

4.8 Conclusion

In summary, there is no evidence from this study to suggest kidney donation has an adverse effect on cardiovascular structure and function at 5 years over and above those of ageing in the general population. The greatest predictor of a change in LV mass in this cohort are in keeping with those well established in the general population, systolic blood pressure.[393]

4.9 Appendix

Table 4.A1. Cardiovascular structural and functional effects.

	Baseline	12 month	5 years	*p-value**
LV volumetrics				
LVEDVi (ml/m^2)				0.598
Donor	64 ± 10	66 ± 12	60 ± 11	
Control	67 ± 11	69 ± 15	63 ± 9	
p-value †	0.304	0.297	0.068	
LVESVi (ml/m^2)				0.836
Donor	18 ± 6	20 ± 7	17 ± 7	
Control	21 ± 7	23 ± 7	20 ± 5	
p-value †	**0.014**	0.052	**0.021**	
LVEF (%)				0.704
Donor	72 ± 6	71 ± 5	71 ± 7	
Control	69 ± 7	68 ± 5	69 ± 6	
p-value †	**0.006**	**0.016**	**0.020**	
LVM (g)				**<0.001**
Donor	112 ± 27	122 ± 29	113 ± 31	
Control	112 ± 30	112 ± 28	115 ± 30	
p-value †	0.653	0.157	0.707	
LVMi (g/m^2)				**<0.001**
Donor	59 ± 9	65 ± 11	60 ± 10	
Control	59 ± 12	60 ± 11	60 ± 11	
p-value †	0.591	0.098	0.639	
LV geometry				
Mass/volume ratio (g/ml)				
Donor	0.92 ± 0.12	0.99 ± 0.16	1.00 ± 0.18	**0.002**
Control	0.90 ± 0.15	0.87 ± 13	0.96 ± 0.19	
p-value †	0.319	**0.002**	0.206	
Atrial volumes				
LAVi (ml/m^2)				0.068
Donor	39 ± 8	44 ± 8	38 ± 11	
Control	41 ± 11	44 ± 9	36 ± 9	
p-value †	0.168	0.638	0.311	
LV function				
Peak GLS (%)				0.066
Donor	-14.8 ± 3.0	-15.3 ± 2.5	-16.2 ± 2.5	
Control	-15.1 ± 2.3	-15.0 ± 2.4	-14.9 ± 2.1	
p-value †	0.603	0.457	**0.007**	
Peak GCS (%)				0.052
Donor	-18.3 ± 2.2	-18.3 ± 2.3	-19.0 ± 2.5	
Control	-17.8 ± 2.3	-18.1 ± 2.4	-17.7 ± 2.2	
p-value †	0.240	0.313	**0.004**	
Peak GRS (%)				0.473
Donor	45.0 ± 11.8	43.6 ± 10.3	44.8 ± 11.9	
Control	43.6 ± 12.7	41.0 ± 9.6	40.2 ± 10.2	
p-value †	0.455	0.127	**0.030**	

Data are displayed as mean ± standard deviation.
*p-values are from generalized estimating equation models. p-values reported are that of the 'interaction' i.e. the interaction between study time point and subgroup. The interaction p-value compares the change over time between the donor and control groups.
† p-values are from generalized estimating equation models. p-values represent pairwise comparisons at the corresponding time point i.e. values at baseline in living kidney donors vs. values at baseline in healthy controls.
GCS; Global circumferential strain, GLS; Global longitudinal strain, GRS; Global radial strain, LAVi; Left atrial indexed volume. LVEDVI; Left ventricular end diastolic volume. LVESVI; Left ventricular end systolic

volume index. LVSV; Left ventricular stroke volume. LVEF; Left ventricular ejection fraction. LVM; Left ventricular mass. LVMI; Left ventricular mass index.

Table 4.A2. Myocardial strain rates.

	Baseline	12 month	5 years	*p-value**
GLS strain rates				
S’				**0.039**
Donor	0.73 ± 0.18	0.80 ± 0.16	0.77 ± 0.15	
Control	0.76 ± 0.22	0.72 ± 0.15	0.68 ± 0.11	
p-value †	0.542	**0.024**	**0.001**	
E’				0.211
Donor	0.70 ± 0.23	0.72 ± 0.20	0.87 ± 0.22	
Control	0.80 ± 0.24	0.72 ± 0.20	0.86 ± 0.25	
p-value †	0.069	0.920	0.970	
A’				0.509
Donor	0.48 ± 0.17	0.51 ± 0.15	0.58 ± 0.17	
Control	0.45 ± 0.15	0.44 ± 0.13	0.50 ± 0.14	
p-value †	0.304	**0.046**	**0.009**	
GCS strain rates				
S’				0.234
Donor	0.90 ± 0.14	0.94 ± 0.13	0.90 ± 0.14	
Control	0.89 ± 0.18	0.89 ± 0.16	0.82 ± 0.10	
p-value †	0.631	0.147	**0.002**	
E’				0.992
Donor	0.96 ± 0.22	0.92 ± 0.20	1.0 ± 0.28	
Control	0.98 ± 0.23	0.92 ± 0.21	1.0 ± 0.28	
p-value †	0.993	0.986	0.916	
A’				0.821
Donor	0.55 ± 0.19	0.56 ± 0.16	0.64 ± 0.19	
Control	0.46 ± 0.15	0.48 ± 0.14	0.55 ± 0.15	
p-value †	**0.018**	**0.015**	**0.010**	
GRS strain rates				
S’				0.238
Donor	2.72 ± 1.01	2.83 ± 1.00	2.57 ± 1.00	
Control	2.70 ± 1.32	2.45 ± 0.89	2.09 ± 0.65	
p-value †	0.787	0.069	**0.004**	
E’				0.664
Donor	2.83 ± 0.91	2.59 ± 0.86	2.98 ± 1.06	
Control	2.85 ± 1.02	2.50 ± 0.83	2.76 ± 1.03	
p-value †	0.858	0.641	0.275	
A’				0.650
Donor	0.77 ± 0.33	0.83 ± 0.28	0.78 ± 0.33	
Control	0.66 ± 0.28	0.66 ± 0.23	0.67 ± 0.24	
p-value †	0.167	**0.005**	0.064	

Data are displayed as mean ± standard deviation.
* p-values are from generalized estimating equation models. p-values reported are that of the ‘interaction’ i.e. the interaction between study time point and subgroup. The interaction p-value compares the change over time between the donor and control groups.
† p-values are from generalized estimating equation models. p-values represent pairwise comparisons at the corresponding time point i.e. values at baseline in living kidney donors vs. values at baseline in healthy controls.
A’; Late diastolic peak. E’; Early diastolic peak. GCS; Global circumferential strain, GLS; Global longitudinal strain, GRS; Global radial strain. S’; Peak systolic peak.

Table 4.A3. Office and ambulatory blood pressure effects.

	Baseline	12 month	5 years	*p-value**
Office measures				
BMI (kg/m^2)				0.702
Donor	26.5 ± 4.3	26.9 ± 4.2	27.5 ± 4.9	
Control	25.9 ± 3.3	25.8 ± 3.3	26.4 ± 3.9	
p-value †	0.402	0.275	0.248	
SBP (mmHg)				0.382
Donor	125 ± 12	125 ± 9	122 ± 12	
Control	125 ± 13	122 ± 11	122 ± 14	
p-value †	0.858	0.250	0.613	
DBP (mmHg)				0.124
Donor	75 ± 9	78 ± 8	78 ± 8	
Control	76 ± 10	76 ± 8	76 ± 8	
p-value †	0.490	0.473	0.175	
HR (bpm)				0.654
Donor	67 ± 10	67 ± 11	66 ± 8	
Control	66 ± 10	67 ± 11	67 ± 13	
p-value †	0.885	0.643	0.416	
Ambulatory BP				
Day SBP (mmHg)				0.542
Donor	121 ± 9	122 ± 10	124 ± 11	
Control	122 ± 11	121 ± 11	122 ± 10	
p-value †	0.882	0.586	0.411	
Day DBP (mmHg)				0.554
Donor	73 ± 7	77 ± 8	79 ± 9	
Control	75 ± 9	76 ± 10	78 ± 8	
p-value †	0.656	0.925	0.616	
Day HR (bpm)				0.104
Donor	72 ± 9	74 ± 11	74 ± 8	
Control	73 ± 9	73 ± 1	73 ± 9	
p-value †	0.225	0.725	0.509	
Night SBP (mmHg)				**0.044**
Donor	104 ± 9	109 ± 10	111 ± 11	
Control	109 ± 11	107 ± 9	111 ± 10	
p-value †	0.080	0.294	0.970	
Night DBP (mmHg)				0.246
Donor	60 ± 7	65 ± 8	68 ± 8	
Control	64 ± 10	64 ± 10	67 ± 7	
p-value †	0.237	0.666	0.780	
Hypertension diagnosis ‡				0.369
Donor	3 (8)	7 (21)	8 (16)	
Control	5 (18)	4 (16)	6 (14)	
p-value †	0.322	0.602	0.722	

Data are displayed as mean ± standard deviation, median [IQR] or number (percentage).
* p-values are from generalized estimating equation models. p-values reported are that of the 'interaction' i.e. the interaction between study time point and subgroup. The interaction p-value compares the change over time between the donor and control groups
† p-values are from generalized estimating equation models. p-values represent pairwise comparisons at the corresponding time point i.e. values at baseline in living kidney donors' vs values at baseline in healthy controls.
‡ The definition of hypertension was in accordance with the European Society of Hypertension ambulatory blood pressure monitoring guidelines.[269] BMI; Body mass index. DBP; Diastolic blood pressure. HR; Heart rate. SBP; Systolic blood pressure.

Table 4.A4. Central haemodynamic and vascular effects.

	Baseline	12 month	5 years	*p-value**
Arterial stiffness				
Central SBP (mmHg)				0.724
Donor	112 ± 11	113 ± 11	114 ± 12	
Control	111 ± 14	110 ± 14	112 ± 14	
p-value †	0.659	0.304	0.413	
Central DBP (mmHg)				0.705
Donor	77 ± 9	79 ± 8	78 ± 9	
Control	76 ± 10	76 ± 9	77 ± 8	
p-value †	0.837	0.318	0.397	
AI_{75} (%) ‡				0.275
Donor	18 [5-24]	20 [10-25]	27 [19-35]	
Control	16 [3-26]	14 [3.6-23]	25 [11-29]	
p-value †	0.437	**0.043**	0.381	
Adj cfPWV (m/s)				**0.001**
Donor	6.74 ± 1.04	7.17 ± 1.03	7.29 ± 1.27	
Control	6.76 ± 1.09	6.69 ± 0.9	7.37 ± 1.36	
p-value †	0.758	**0.016**	0.780	
Aortic distensibility				
Proximal ascending aorta ($\times 10^{-3}$ mm Hg^{-1}) ‡				0.346
Donor	2.97 [1.83-4.66]	2.68 [1.89-4.29]	3.10 [1.63-4.90]	
Control	3.79 [2.25-5.06]	3.39 [2.05-5.05]	3.15 [1.50-5.43]	
p-value †	0.949	0.501	0.907	
Proximal descending aorta ($\times 10^{-3}$ mm Hg^{-1}) ‡				0.504
Donor	3.34 [2.77-4.14]	3.54 [2.48-4.55]	3.13 [2.34-4.74]	
Control	3.59 [2.72-4.52]	3.48 [2.77-4.22]	3.63 [1.80-5.31]	
p-value †	0.350	0.817	0.725	
Arterial structure				
CIT (mm)				0.101
Donor	0.59 ± 0.09	0.60 ± 0.09	0.59 ± 0.10	
Control	0.59 ± 0.11	0.58 ± 0.09	0.55 ± 0.07	
p-value †	0.849	0.433	**0.032**	

Data are displayed as mean ± standard deviation or median [IQR].

*p-values are from generalized estimating equation models. p-values reported are that of the 'interaction' i.e. the interaction between study time point and subgroup. The interaction p-value compares the change over time between the donor and control groups

†p-values are from generalized estimating equation models. p-values represent pairwise comparisons at the corresponding time point i.e. values at baseline in living kidney donors' vs values at baseline in healthy controls (under the baseline column).

‡ Log10 transformed prior to analysis.

AI_{75}; Augmentation index corrected for a heart rate of 75. Adj cfPWV; Adjusted carotid-femoral pulse wave velocity. CIT; Carotid intima-media thickness. DBP; Diastolic blood pressure. HR; Heart rate. SBP; Systolic blood pressure.

Table 4.A5. Haematological and biochemical effects.

	Baseline	12 month	5 years	*p-value**
Haemoglobin (g/dl)				**0.041**
Donor	13.7 ± 1.2	13.3 ± 1.1	13.7 ± 1.8	
Control	13.5 ± 1.2	13.4 ± 1.3	13.6 ± 1.2	
p-value †	0.364	0.536	0.561	
Creatinine (µmol/L)				**<0.001**
Donor	73 ± 14	101 ± 19	96 ± 21	
Control	71 ± 13	71 ± 13	72 ± 12	
p-value †	0.671	**<0.001**	**<0.001**	
eGFR (ml/min/1.73m^2)				**<0.001**
Donor	95 ± 15	65 ± 13	67 ± 14	
Control	99 ± 16	96 ± 15	94 ± 15	
p-value †	0.178	**<0.001**	**<0.001**	
ACR (≥3mg/mmol)				0.442
Donor	1 (2.0)	4 (8.0)	7 (14)	
Control	1 (2.2)	1 (2.2)	2 (4)	
p-value †	0.905	0.250	0.105	
Corrected calcium (mmol/L)				0.106
Donor	2.20 ± 0.98	2.23 ± 0.08	2.30 ± 0.08	
Control	2.19 ± 0.08	2.21 ± 0.07	2.25 ± 0.09	
p-value †	0.330	0.298	**0.006**	
Phosphate (mmol/L)				0.130
Donor	1.07 ± 0.16	1.06 ± 0.15	1.02 ± 0.18	
Control	1.09 ± 0.16	1.14 ± 0.26	1.13 ± 0.18	
p-value †	0.502	0.106	**0.010**	
Parathyroid hormone (pmol/L)				**0.002**
Donor	4.4 ± 1.36	5.55 ± 1.64	5.58 ± 3.38	
Control	4.28 ± 1.19	4.38 ± 1.22	4.57 ± 1.53	
p-value †	0.389	**<0.001**	0.065	
Vitamin D (nmol/L)				0.150
Donor	50 ± 27	62 ± 32	59 ± 30	
Control	53 ± 22	56 ± 24	63 ± 27	
p-value †	0.717	0.337	0.499	

Data is displayed as mean ± standard deviation or number (percentage).
*p-values are from generalized estimating equation models. p-values reported are that of the ‘interaction’ i.e. the interaction between study time point and subgroup. The interaction p-value compares the change over time between the donor and control groups
† p-values are from generalized estimating equation models. p-values represent pairwise comparisons at the corresponding time point i.e. values at baseline in living kidney donors’ vs values at baseline in healthy controls (under the baseline column).
ACR; Albumin creatinine ratio. eGFR; estimated glomerular filtration rate.

Table 4.A6. Biochemical and biomarker effects.

	Baseline	12 month	5 years	*p-value**
FGF23 (RU/ml)				0.099
Donor	70 [56-85]	81 [68-107]	74 [58-105]	
Control	67 [54-90]	65 [51-95]	59 [47-75]	
p-value †	0.893	0.267	0.054	
Urate (µmol/L)				**<0.001**
Donor	267 ± 74	326 ± 66	335 ± 83	
Control	288 ± 54	287 ± 51	276 ± 73	
p-value †	0.159	**0.002**	**<0.001**	
hsCRP (mg/L) ‡				0.105
Donor	1.0 [0.4-1.9]	1.7 [0.7-3.0]	1.3 [0.9-3.2]	
Control	0.8 [0.4-1.8]	1.0 [0.5-1.8]	0.9 [0.9-1.9]	
p-value †	0.979	0.114	**0.029**	
Total Cholesterol (mmol/L)				0.739
Donor	5.4 ± 1.1	5.4 ± 1.0	5.2 ± 0.8	
Control	5.0 ± 1.0	4.9 ± 1.0	5.0 ± 0.9	
p-value †	0.131	0.109	0.171	
LDL cholesterol (mmol/L)				0.423
Donor	3.1 ± 1.0	3.1 ± 0.9	2.9 ± 0.8	
Control	2.9 ± 0.9	2.8 ± 0.9	2.8 ± 0.8	
p-value †	0.498	0.105	0.288	
Triglycerides (mmol/L) ‡				0.406
Donor	1.1 [0.8-1.4]	1.2 [0.8-1.9]	1.3 [0.9-1.7	
Control	1.0 [0.7-1.3]	0.9 [0.6-1.5]	1.3 [0.8-1.7]	
p-value †	0.184	0.160	0.758	
Aldosterone (pmol/L) ‡				0.149
Donor	145 [68-209]	126 [81-188]	225 [139-347]	
Control	105 [65-188]	159 [73-275]	182 [110-270]	
p-value †	0.900	0.416	0.106	
Renin (ng/L) ‡				0.016
Donor	9.4 [7.1-13.1]	7.6 [4.8-11.0]	11.5 [6.1-14.8]	
Control	9.2 [7.3-13.2]	10.6 [7.0-16]	11.4 [7.0-19.9]	
p-value †	0.421	0.001	0.432	
hsTNT ≥ 5(ng/L)				0.071
Donor	3 (7)	13 (30)	31 (65)	
Control	4 (10)	1 (3)	22 (49)	
p-value †	0.585	**<0.001**	0.111	
hsTNT ≥ 14(ng/L)				
Donor	0 (0)	0 (0)	2 (4)	NA
Control	0 (0)	0 (0)	0 (0)	
p-value †	NA	NA	NA	
NT pro BNP (pmol/L) ‡				0.178
Donor	0.3 [0.3-7.0]	0.3 [0.3-22.2]	0.6 [0.4-1.1]	
Control	0.3 [0.3-17]	0.3 [0.3-3.8]	0.5 [0.2-0.9]	
p-value †	0.932	0.086	**0.043**	

Data are displayed as mean ± standard deviation or median [IQR] or number (percentage).
*p-values are from generalized estimating equation models. p-values reported are that of the 'interaction' i.e. the interaction between study time point and subgroup. The interaction p-value compares the change over time between the donor and control groups
† p-values are from generalized estimating equation models. p-values represent pairwise comparisons at the corresponding time point i.e. values at baseline in living kidney donors' vs values at baseline in healthy controls (under the baseline column).
‡ Log10 transformed prior to analysis.

FGF23; Fibroblast growth factor-23. hsCRP; High sensitivity C-reactive protein. NT Pro BNP; N-terminal pro B-type natriuretic peptide. LDL; Low density lipoprotein.

CHAPTER 5

MYOCARDIAL CHARACTERISATION AND INFLAMMATORY BIOMARKERS IN LIVING KIDNEY DONORS AT 5 YEARS, A CROSS SECTIONAL STUDY. THE CHRONIC RENAL IMPAIRMENT IN BIRMINGHAM (CRIB)-DONOR II STUDY

5.1 Extent of personal contribution

This study was an extension to the CRIB-DONOR II study. Ethical approval from the Research Ethics Committee/Health Research Authority and study sponsor was obtained by myself including the amendment to allow recruitment of a further cohort of patients with chronic kidney disease. I was responsible for all regulatory documents and maintenance of the site file. Recruitment and consent of all the participants were carried out by myself with the occasional assistance from a research nurse for the selected CKD cohort.

The study visit was carried out myself including CMR imaging scanning and blood sampling. For living kidney donors and healthy controls serum and plasma samples were prepared for storage by the Wellcome Trust Birmingham Clinical Research facility. All serum and plasma samples obtained from the CKD cohort were centrifuged and aliquoted for storage by myself.

I carried out all the CMR analysis including T1/T2 mapping, LGE quantification and calculation of ECV. The only notable exception being the reproducibility assessment for inter-observer variability which required a second observer. Laboratory analysis of α-klotho and all the multiplex immunoassays were carried out by myself including the subsequent data analysis of the output and calculation of the blood concentration.

All age-sex matching of participants for final analysis were carried out by myself. I also conducted all data interpretation, statistical analysis and written presentation of the results with the advice of a medical statistician.

5. 2 Abstract

CMR T1 mapping is a non-invasive measure of diffuse interstitial cardiac fibrosis. Elevated T1 times have also been observed in those with CKD. Native T1 times and post contrast derived ECV are predictive of cardiovascular events and mortality in non-ischaemic cardiomyopathies. This study aimed to establish whether living kidney donors had elevated T1 times and ECV compared to 1:1 age and sex matched healthy controls and secondly, whether donors had elevated biomarker profiles compared to both controls and early stage CKD.

A cross sectional blinded study of 1:1 age and sex matched living kidney donors 5 years after nephrectomy (n=26) with healthy controls (n=26). Participants underwent multi-parametric CMR imaging including LGE, T1 and T2 mapping. Mean age was 50 ± 13 years and 54% were male with no difference in race, body mass index or office blood pressure between groups. There was no significant difference in global native T1 times in the mid LV slice [mean difference -6ms (95% CI -29, 18) p=0.627)] or basal slice [mean difference -12ms (95% CI -34, 9) p=0.259]. Mean global ECV was also not significantly different between donors and controls. In a further cross-sectional comparison, the biomarker profiles (serum multiplex magnetic immunoassays) of donors and controls were compared to a cohort with early stage CKD (n=26). CKD participants had higher mean levels of monocyte chemoattractant protein 1 (500pg/ml) compared to both controls (387pg/ml) and donors (389pg/ml). This finding was independent of both age and eGFR.

Kidney donors do not have CMR imaging evidence of myocardial fibrosis compared to healthy controls. Biomarkers of myocardial fibrosis and inflammation in donors were comparable to a matched healthy control group whilst increases in some biomarkers were evident in the CKD cohort.

5. 3 Introduction

Since the introduction of haemodialysis in the 1940s there has been widespread reporting of the cardiovascular structural and functional changes associated with ESRD including; elevated LV mass, sudden cardiac death and changes in cardiac function.[87, 130, 408, 409] Endomyocardial biopsy studies have shown that subjects with uraemic cardiomyopathy have severe myocardial fibrosis with cardiomyocyte hypertrophy and disarray.[104, 105] Furthermore, this key intermediary phenotype has been associated with functional correlates and poor outcomes including LV chamber stiffness, systolic and diastolic dysfunction and life threatening arrhythmias.[410-412] The use of advanced CMR has determined that not only is focal myocardial replacement scarring present (as evidenced by LGE) in around 25% of ESRD subjects there is also a recurrent finding of elevated T1 times consistent with diffuse interstitial fibrosis in this cohort.[91, 130, 138] Native T1 times have also been found to correlate with histological fibrosis in those with heart failure.[413]

It is less clear at what point in the natural history of CKD these cardiovascular changes begin and what mechanisms may be responsible for these changes. In a CMR study of early stage non-diabetic CKD (mean GFR 50 ±22 ml/min/1.73 m^2) both native T1 times and ECV were elevated compared to a sex matched healthy control group, suggestive of diffuse interstitial fibrosis.[353] In addition, these differences were observed without significant corresponding changes in LV mass and volumes.[353] These small sub-clinical changes in myocardial tissue characterisation demonstrable on CMR at the earliest stages of CKD may represent the precursor to the uraemic cardiomyopathy seen in ESRD.[389]

Furthermore, changes in both ECV and T1 have important prognostic implications.[414] Native T1 times are an independent predictor of adverse outcomes such as cardiac death, heart failure and stroke (independent of LGE and LV ejection fraction) in those with atrial fibrillation and heart failure and therefore may be a more sensitive early predictor of

myocardial disease.[415] In a meta-analysis of 1524 participants a higher ECV was also associated with higher rates of cardiovascular mortality [HR 1.79 (95% CI 1.24 to 2.58)] and cardiovascular events [HR 1.11 (95% CI 1.08-1.15)].[414]

In the previous chapter it was established that by 5 years, kidney donors have no increase in LV mass, differences in LV geometry, increase in blood pressure or differences in arterial stiffness parameters including aortic distensibility compared to controls studied over the same time period.[354] It remains possible, however, that more subtle sub-clinical changes may have occurred including changes in myocardial tissue comprising fibrosis, oedema and systemic inflammation related to a reduced eGFR which has not yet manifest in cardiovascular structural and functional change.

This study aimed to establish whether living kidney donors had elevated T1 times and ECV consistent with cardiac fibrosis compared to healthy controls. In addition, this study also aimed to determine whether biochemical profiles, particularly cardiovascular biomarkers in donors differed significantly from both controls and early stage CKD.

5.4 Brief methods

5.4.1 Study design and participants

Living kidney donors and healthy controls were recruited as part of the CRIB-DONOR II (NCT02973607) study from May 2017-May 2019 as previously described. In addition, early stage CKD participants were also recruited as part of the CRIB-DONOR II from January 2020-March 2020.

5.4.2 Statement of ethics

Ethical approval for the recruitment of an early stage CKD group was obtained from the West Midlands Solihull Research Ethics Committee (REC 17/WM/0048) via a major amendment to the original CRIB-DONOR II study application. The amendment was approved by the Health Research Council in February 2018.

5.4.3 Inclusion and exclusion criteria

All participants were required to meet living kidney donor criteria at entry into the study as previously described.[249] The CKD cohort recruited also needed to meet living kidney donor criteria (with the exception of GFR) with selected additional requirements. These included the exclusion of those taking immune modulating drugs and immune mediated systemic illness such as vasculitis or systemic lupus erythematous. For full inclusion and exclusion criteria see **Table 5.1**.

5.4.4 Outcome measures

Primary measures:

1. Mean difference in native T1 and T2 times between donors and controls.
2. Mean difference in ECV between donors and controls.

Secondary exploratory measures included a comparison of both cardiovascular and renal biomarkers to those with early stage CKD.

5.4.5 Participant matching and rationale

In total, 48 living kidney donors and 42 healthy controls underwent 3T CMR with native T1 mapping, see **Figure 5.1**. Of these, 44 donors and 34 controls received gadolinium contrast. Due to the reported influence of both sex and age on native T1, times participants were age and sex matched for analysis of imaging parameters. [416] The small sample size meant a 1:1 case to control ratio was considered optimal.

5.4.6 Basic demographics and clinical assessment

All participants had basic demographics recorded at the time of the study visit which including office blood pressure, height, weight, and a non-fasted venous blood sample. Kidney function was determined for all participants using the CKD-EPI 2009 equation for calculation of eGFR.[417]

5.4.7 Reproducibility

Intra-observer variability for native T1 and ECV was assessed in 10 selected studies analysed twice by the same observer. Inter-observer variability was assessed in 10 randomly selected studies by a second observer blinded to patient identifying data.

5.4.8 Quality of MOLLI sequences

To ensure quality and consistency of T1 times throughout the study, quality assurance tests were periodically carried out using the Eurospin T05 (Diagnostic, Sonar, Livingston, Scotland) phantom object. This consisted of multiple vials containing agar at different T1 values. Scanning of the phantom used the same MOLLI sampling schemes as those used in the study 5(5)3 and 4(1)3(1)2 with a simulated heart rate. In addition, basic TSE sequence were also used to account for temperature fluctuation and any degradation of the phantom.

5.4.9 Statistical analysis

Statistical analysis was carried out using SPSS©, version 23 (IBM, Armonk, New York, US). Continuous variables were assessed for normality graphically using histograms. Parametric

data are displayed as mean and standard deviation and where there were two groups they were compared using independent samples *t* tests. For data that was not normally distributed the data are displayed as median and interquartile range. Non-parametric data was logged and assessed again graphically prior to analysis. If data are normally distributed, groups were compared by independent samples *t* tests in the logged format. In analysis of more than two groups an ANOVA was used with post hoc Tukey test for subgroup comparison. Non-parametric data was logged prior to assessment. A p value of <0.05 was considered significant. No adjustments were made for multiple comparisons. A listwise deletion approach was taken to ensure matched data was compared where one of the participants had an incomplete sets of sequences. Reproducibility for T1 mapping, ECV and analysis of the T1 phantom data was assessed using interclass coefficients and Bland-Altman plots of bias.

5.5 Results

5.5.1 Study subjects

Of the whole cohort, 26 healthy controls and 26 living kidney donors were 1:1 age and sex matched for comparison, see **Figure 5.1**. Of the 33 participants with early stage CKD recruited, 7 were excluded leaving 26 for analysis. Reasons for exclusion included: n=2 acute use of steroids; n=4 uncontrolled blood pressure and n=1 withdrew consent for venepuncture. The CKD cohort was not age and sex matched for biomarker comparisons due to a small sample size.

5.5.2 Clinical characteristics

There was no significant difference in age, sex, BMI or race, between the three groups, see **Table 5.2**. The CKD group had significantly lower mean eGFR than the control group [-17 ml/min/1.73^2 (95% CI -27,-6), p=<0.02] although there was no significant difference between the mean eGFR of CKD and donors [-9 ml/min/1.73^2 (95% CI -2, 19), p=0.104].

Participants with CKD had higher rates of both hypertension and hypercholesterolemia than either donors or controls. There was also higher use of ACE inhibitors/angiotensin receptor blockers and calcium channel blockers in those with CKD. The CKD cohort had higher rates of proteinuria with 50% having an ACR of 30-299 mg/mmol compared to 0 in the donor and control group. The CKD group also had a higher resting office heart rate than either donors or controls, see **Table 5.2**.

5.5.3 Renal aetiology

Of those with CKD, the aetiology of renal disease was varied; IgA nephropathy 4 (15), focal glomerular sclerosis 4 (15), membranous nephropathy 3 (12), Adult polycystic kidneys/Alport syndrome 7 (27), congenital abnormalities 3 (12) and other 5 (19).

5.5.4 Normal reference ranges for T1 time and extracellular volume

Normal native T1 times and ECV using the protocol and scanner described are detailed in **Table 5.3**.

5.5.5 Late gadolinium enhancement in matched donors and controls

RV insertion point LGE was demonstrated in both groups; 2 living kidney donors and 1 healthy control. No patients had LGE suggestive of focal replacement fibrosis.

5.5.6 Native T2 and T2 mapping in matched donors and controls

Twenty four matched healthy controls and living kidney donors underwent 3T CMR with complete T1 and T2 mapping sequences, see **Figure 5.1**. There was no significant difference in LV or RV volumes, geometry or mass between donors and controls. There were no differences in T2 times across the myocardium regardless of slice position or region within the septum, see **Table 5.4.** Native T1 time was numerically higher in the septal, global and regional area of interest in donors compared to controls however, at the most the mean T1 time was only +12.7ms higher in donors and did not reach statistical significance, see **Table 5.4**.

Regional differences throughout the myocardium were observed. Basal T1 times were higher than T1 times in the mid ventricular slice but this was only significant in donors. In healthy controls, global T1 values were marginally higher in the base than the mid ventricular slice [mean difference +0.4ms (95% CI -9,-10), p=0.925], similarly in the septum, T1 values were slightly higher in the basal slice compared to the mid slice [mean difference +4ms (95% CI -8,-16), p=0.490]. The differences, however, were not significant. In living kidney donors, global values were higher in the base [mean difference +7ms (95% CI 0.0, 4), p=0.05] with a more pronounced difference seen in septal values [mean difference +8ms (95% 0.8, 15), p=0.030].

5.5.7 Extracellular volume in matched donors and controls

Of the 26 matched donors and controls, 22 received paired post contrast imaging and calculation of ECV, see **Figure 5.1**. Septal and global ECV differences were small and non-significant between groups. There was also no regional discrepancy between basal and mid LV slices unlike that observed in native T1 times, **Table 5.5.**

5.5.8 Sensitivity analysis of all donors and controls

A sensitivity analysis was conducted in which all participants were analysed. In total, 48 donors and 42 controls had 3T T1 and T2 mapping at 5 years. Neither global native T1 time [+13.02 (-2.37, 28.42), p=0.096] nor T2 time [+0.00 (-0.95, 0.96), p=0.992] was significantly different in kidney donors compared to controls in the mid ventricular slice. In the 44 kidney donors and 34 controls who consented to contrast, there was also no significant difference in mean ECV [-0.11 (-0.95, 0.95), p=0.826] in the mid ventricular slice. LGE at the RV insertion points was seen in 4 living kidney donors (% of LV mass, 0.87± 0.15%) and in one control. There was no LV myocardial LGE.

5.5.9 T1 image quality and reproducibility

Of 680 segments, 159 were excluded due to either observed motion artefact on raw images, LGE or banding artefact allowing analysis of 77% of all segments to be analysed. T1 interclass correlation coefficients (95% confidence intervals) for intra-observer and inter-observer variability were: 0.97 (0.88 – 0.99) and 0.98 (0.92 – 0.99), respectively. ECV interclass correlation for intra-observer and inter-observer variability was 0.98 (0.91-0.99) and 0.99 (0.73-0.99) respectively, **see Table 5.6 and Figure 5.3A and 5.3B**. Analysis of the phantom data over the course of the study accurately captured expected T1 values. Reproducibility was high for 5(5)3 and 4(1)3(1)2 MOLLI to be 98.8 ± 0.3% and 98.4 ± 0.6%, respectively.

5.5.10 Cardiac and renal biomarkers

Biomarkers for progression of renal disease, cardiac disease and inflammatory cytokines were compared between matched donors, controls and early stage CKD, **Table 5.7**.

Monocyte chemoattractant protein 1 (MCP-1) was significantly higher in the CKD cohort compared to both controls [+112pg/ml (95% CI 6.7, 219), p=0.034] and compared to donors [+111pg/ml (95% 5.3, 217), p=0.037]. There was no significant difference between MCP-1 in donors compared to controls, p=0.999, see **Figure 5.3A**. Using a general linear model and univariate analysis, with the group as a fixed effect and continuous variables such as age and eGFR as confounding variables, MCP-1 was significantly greater in the CKD group independent of both age and eGFR (p=0.010).

Atrial natriuretic peptide was significantly higher in the CKD cohort compared to controls (8227 pg/ml vs 4478 pg/ml), p=0.002; however, donors were neither statistically different to the CKD or control group with levels mid-way between the two groups, see **Figure 5.3B**.

Matrix metallopeptidase 9 (MMP-9) was significantly different between the three groups; although, on post hoc comparison there was no significant inter-group difference suggesting the difference was likely to be underpowered.

IL-10 was lower than the minimal limit of detection in many cases however it was more likely to be high enough to be detected in the CKD cohort compared to donors and controls (92% vs 27%, vs 23%).

Table 5.1. Inclusion and exclusion criteria for early stage CKD participants.

Inclusion	Exclusion
>18 years of age and <80 years of age eGFR KDIGO stage 1-3	Pregnant Systemic immune mediated disease i.e. lupus/sarcoidosis Uncontrolled hypertension or requiring more than two anti-hypertensives LV dysfunction Diabetes Atrial fibrillation Cardiovascular or pulmonary disease Use of biologics or immune mediated drugs including steroids

KDIGO; Kidney Disease Improving Global Outcomes. LV; Left ventricular.

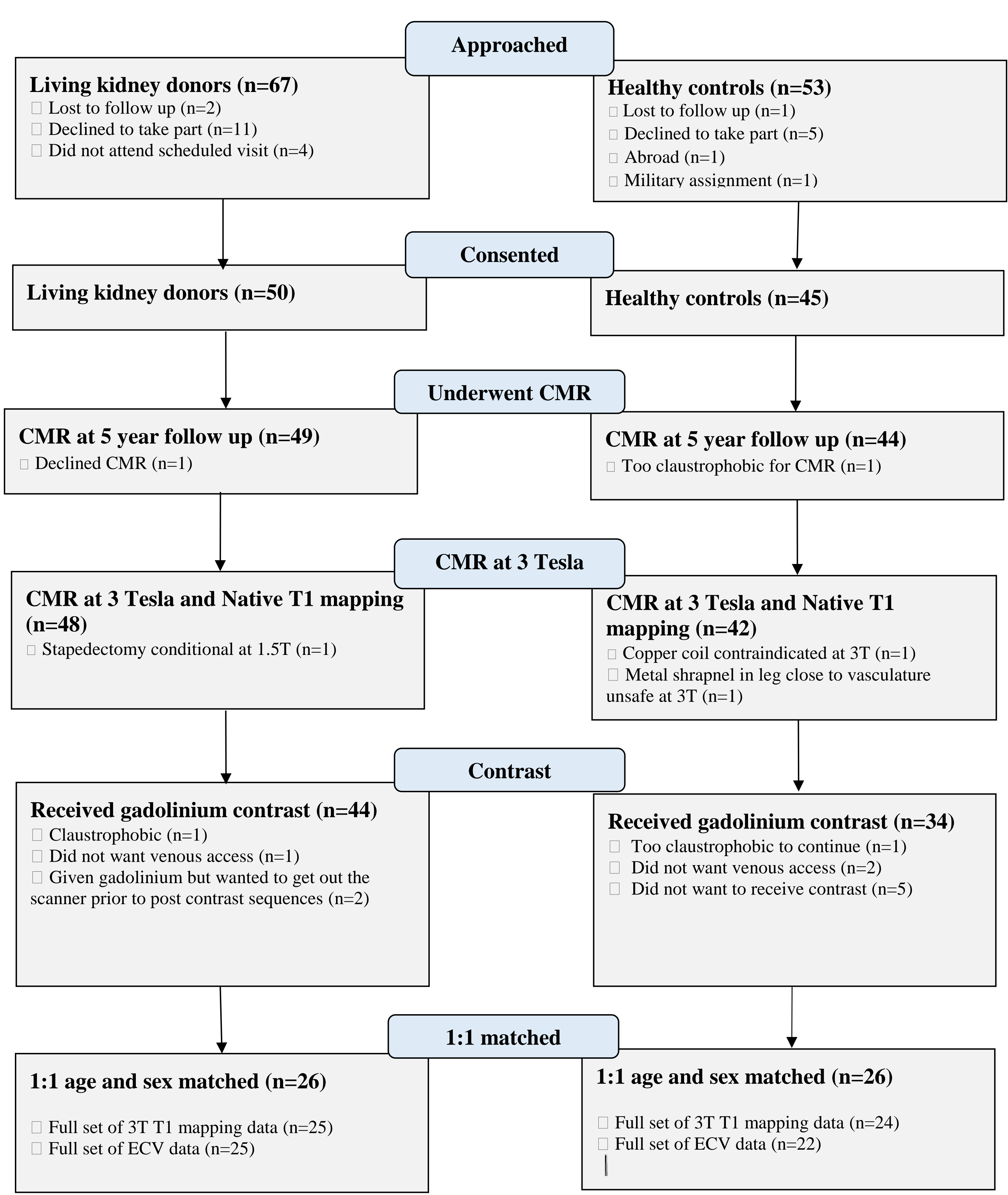


Figure 5.1. Flow chart of participants undergoing CMR and those receiving contrast. CMR; cardiac magnetic resonance. ECV; Extracellular volume.

Table 5.2. Baseline demographics and clinical characteristics of all patients.

	Healthy controls n=26	Living kidney donors n=26	Early stage chronic kidney disease n=26
Demographics			
Age (years)	50 ± 12	50 ± 13	46 ± 15
Male sex	14 (54)	14 (54)	15 (58)
BMI (kg.m^2)	26 ± 3	28 ± 5	28 ± 6
Race			
White	22 (85)	24 (92)	18 (69)
Asian	3 (12)	2 (8)	7 (27)
Black	1 (3)	0 (0)	1 (4)
Other	0 (0)	0 (0)	0 (0)
Cardiovascular risk factors			
Hypercholesterolemia	4 (15)	1 (4)	3 (12)
Hypertension	3 (12) † ‡	2 (8)	9 (35)
Anti-hypertensive usage			
ACE/ARB	1 (4) † ‡	1 (4)	20 (77)
B blocker	1 (0)	1 (4)	0 (0)
Calcium channel	0 (0) † ‡	0 (0)	3 (12)
Thiazide	0 (0)	0 (0)	1 (4)
Renal function			
eGFR EPI (ml/min/1.73^2)	93 ± 15 *†	68 ± 15	76 ± 22
KDIGO stage eGFR ml/min/1.73^2	†		
1a >105	7 (27)	1 (4)	3 (11.5)
1b 90-104	8 (31)	5 (19)	5 (19.2)
2a 75-89	7 (27)	11 (42)	5 (19.2)
2b 60-74	4 (15)	8 (31)	7 (26.9)
3a 45-59	0 (0)	1 (4)	5 (19.2)
3b 30-44	0 (0)	0 (0)	1 (3.8)
ACR (mg/mmol)	† ‡		
1a <10	25 (100)	24 (92)	11 (42)
1b 10-29	0 (0)	2 (8)	1 (4)
2 30-299	0 (0)	0 (0)	13 (50)
3 300-1999	0 (0)	0 (0)	1 (4)
Office blood pressure			
SBP (mmHg)	121 ± 14†	124 ± 15	133 ± 14
DBP (mmHg)	81 ± 10	82 ± 11	84 ± 10
HR (bpm)	73 ± 11†‡	69 ± 8	82 ± 13

Data are presented as mean ± standard deviation. For categorical variables, data are presented as number (percentage). ACE; Angiotensin converting enzyme. ACR; Albumin creatinine ratio. ARB; Angiotensin receptor blocker APKD; Adult polycystic kidney disease. BMI; Body mass index. BSA; Body surface area. DBP; Diastolic blood pressure. eGFR; Estimated glomerular filtration rate. HR; Heart rate. KDIGO; Kidney disease improving global outcomes. SBP; Systolic blood pressure.

* p value <0.05 Controls vs. Donors. † p value<0.05 Controls vs. CKD. ‡ p value <0.05 CKD vs. Donor

Table 5.3. Normal local reference values for T1 and extracellular volume.

	Sample size	**Mean± SD**	**Mean difference (95% CI)**
Basal slice			
Global T1 (ms)	42	1206 ± 28	(1197, 1214)
Global T2 (ms)	42	39 ± 2	(38, 39)
Global ECV (%)	34	25 ± 2	(24, 26)
Mid slice			
Global T1 (ms)	42	1201 ± 36	(1190, 1212)
Global T2 (ms)	42	40 ± 2	(39, 41)
Global ECV (%)	34	25 ± 2	(24, 26)

Data are presented as mean ± standard deviation. The 95% (lower confidence interval, upper confidence interval) is determined using a one sample *t* test.

ECV; Extracellular volume. SD; standard deviation

Table 5.4. Native T1 and T2 mapping in matched living kidney donors and controls.

	Healthy controls=24	**Living kidney donors=24**	**Mean difference (95% CI)**	***p-value***
T2 mapping				
HLA				
Septal ROI (ms)	39.62 ±1.82	40.54 ±3.22	-0.9 (-2.4,0.59)	0.229
Basal left ventricular slice				
Septal ROI (ms)	38.22 ±1.96	38.42 ±2.88	-0.2 (-1.6,1.2)	0.771
Septal mean (ms)	38.09 ± 2.11	38.29 ±2.27	-0.3 (-1.5,0.9)	0.633
Global mean (ms)	38.48 ±1.32	38.40 ±2.15	0.0 (-0.9,1.1)	0.880
Mid left ventricular slice				
Septal ROI (ms)	40.80 ±2.82	40.29 ±3.27	0.5 (-1.2, 1.7)	0.568
Septal mean (ms)	40.32 ±2.49	40.03 ±2.67	0.3 (-1.2,1.7)	0.698
Global mean (ms)	39.84 ±1.8	39.83 ±2.63	0.0 (-1.3,1.3)	0.985
Native T1 mapping				
HLA				
Septal ROI (ms)	1226 ± 28	1231 ±26	-4.9 (-20,10)	0.526
Basal left ventricular slice				
Septal ROI (ms)	1216 ±28	1225 ±34	-9.1 (-27,9)	0.314
Septal mean (ms)	1216 ±24	1228 ±34	-12.7 (-30,4)	0.140
Global mean (ms)	1202 ±30	1214 ±43	-12.2 (-34,9)	0.259
Pre-contrast blood time (ms)	1894 ±106	1895 ±66	-1.3(-52,50)	0.091
Mid left ventricular slice				
Septal ROI (ms)	1214 ±38	1224 ±35	-10 (-30,13)	0.355
Septal mean (ms)	1211 ±37	1220 ±36	-9 (-30,13)	0.407
Global mean (ms)	1201 ±41	1207 ±40	-6 (-29,18)	0.627
Pre-contrast blood time (ms)	1899 ±100	1868 ± 152	31 (-43,106)	0.405

Data are presented as mean ± standard deviation. The mean difference is determined using independent samples *t* tests and is given with 95% (lower confidence interval, upper confidence interval).

CI: Confidence interval. HLA: Horizontal long axis. ROI; region of interest.

Table 5.5. Extracellular volume in matched living kidney donors and controls.

	Healthy controls=22	Living kidney donors=22	Mean difference (95% CI)	*p-value*
Basal left ventricular slice				
Septal basal ECV (%)	25 ± 2	25 ± 3	-0.18 (-1.68, 1.32)	0.809
Global base ECV (%)	24 ± 2	25 ± 3	-0.40 (-1.87, 1.07)	0.585
Mid left ventricular slice				
Septal mid ECV (%)	25 ± 2	25 ± 2	-0.25 (-1.70, 1.19)	0.726
Global mid ECV (%)	25 ± 2	25 ± 2	-0.04 (-1.55, 1.45)	0.950

Data are presented as mean ± standard deviation. The mean difference is determined using independent samples *t* tests and is given with 95% (lower confidence interval, upper confidence interval).

ECV; Extracellular volume.

Table 5.6. Intra-study and Inter-observer reproducibility for T1 mapping and extracellular volume (basal slice).

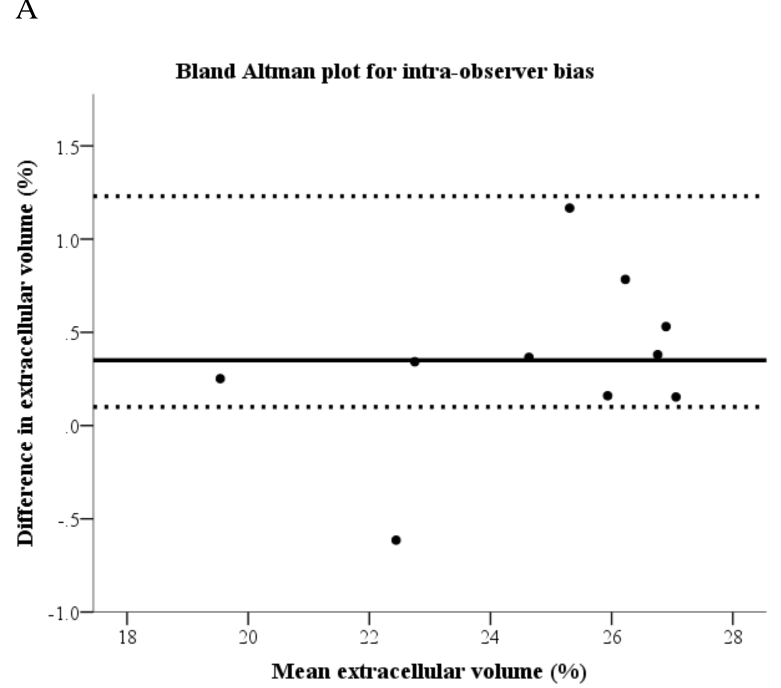
	Intra-observer		Inter-observer	
	Mean absolute bias	**ICC***	**Mean absolute bias**	**ICC***
T1				
Global T1	0.79 ± 18	0.97 (0.88-0.99)	-7.96 ± 9.62	0.98 (0.92, 0.99)
Septal T1	3.88 ± 8.77	0.97 (0.89, 0.99)	-6.44 ± 12.67	0.93 (0.75, 0.98)
ECV				
Global ECV	0.35 ± 0.45	0.98 (0.91-0.99)	-0.38 ± 0.28	0.99 (0.73-0.99)
Septal ECV	0.14 ±0.47	0.97 (0.91, 0.99)	-0.55 ± 9.46	0.94 (0.37, 0.98)

Data are displayed as mean ± standard deviation with (95% confidence intervals).
CI; Confidence interval. ICC; Intra-class correlation coefficient.
* A single measures intra-class correlation coefficient is given with 95% confidence intervals for absolute agreement

Figure 5.2. Bland-Altman plots for reproducibility of global extracellular volume.

A. Bland-Altman plot for intra-observer bias.
B. Bland-Altman plot for inter-observer bias.

A



B

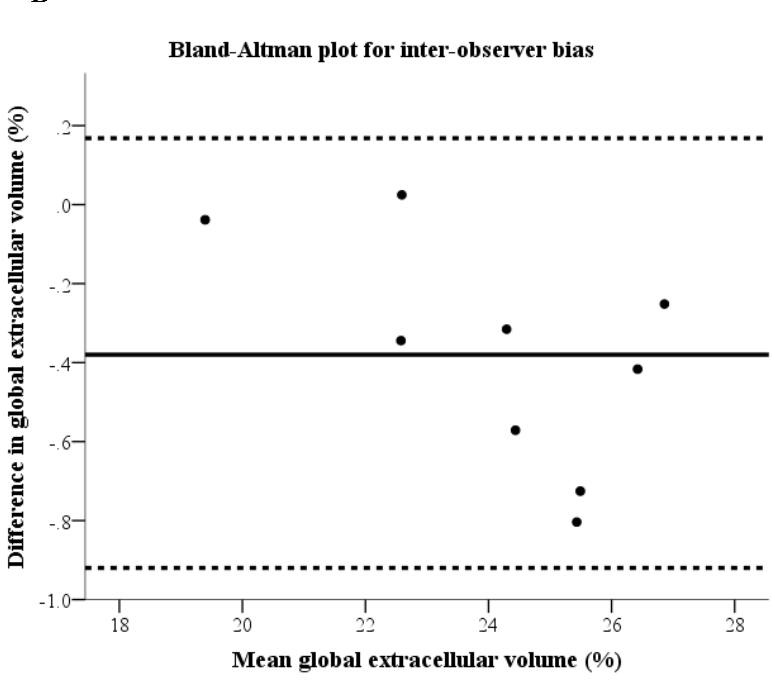


Table 5.7. Cardiovascular and renal biomarkers in living kidney donors, healthy controls and early stage CKD.

	Healthy controls n=26	**Living kidney donors n=26**	**Early stage chronic kidney disease n=26**	***p-Value***
Pro-inflammatory cytokines and chemokine				
MCP-1 †‡	387±133	389 ±138	500 ± 200	**0.018**
IL6	1.69 [0.66-2.14]	1.81 [1.24-3.07]	1.07 [0.87-1.86]	0.191
TNF-α	2.48 [1.28-4.32]	3.95 [2.58-6.20]	3.36 [2.41-4.28]	0.063
IL-8	12 [6.86-25.1]	11 [7.98-23.2]	11.64 [9.73-15.90]	0.765
Anti-inflammatory cytokines				
IL10 †‡	6 (23)	7 (27)	24 (92)	**<0.001**
IL-RA	467 [380-672]	638 [377-985]	610 [474-1024]	0.899
Involved in angiogenesis				
Angiopoetin-2	1011 [841-1448]	1280 [978-1594]	1338 [1020-1977]	0.897
VEGF	66.36 [32.82-109.63]	50.51 [26.93-11.62]	50 [17-82]	0.162
Cardiac biomarkers				
Leptin	6525 [2805-11356]	6375 [3109-13235]	9649 [3423-22520]	0.248
ST2	16697 [10286-19894]	14306 [8881-26607]	15446 [11335-20081]	0.974
ANP †	4778 [3190-7433]	6050 [4108-8217]	8227 [478011300]	**0.003**
MMP-9	20381 [8872-34581]	14525 [9990-22562]	26852 [15163-48569]	**0.047**
α-Klotho	706 [560-1219]	571 [504-863]		0.116
Renal biomarkers				
NGAL	12723 ±3340	13587 ±3321	12127 ± 2663	0.245
KIM-1	13 (50)	13 (50)	10 (38.5)	0.138
Galectin-3	1152 [1016-1432]	1161 [1029-1460]	1304 [1017-1521]	0.861
Uromodulin	115328 [77755-160937]	74565 [59812-105282]	97708 [55832-141775]	0.116

Data are displayed as mean ± standard deviation if normally distributed otherwise median [interquartile range]. For biomarkers that were not recordable, participants with a recordable level are indicated with as a number with (percentage). All biomarkers are given as pg/ml. The p value is determined from a one way ANOVA analysis with post hoc Tukey analysis for group comparisons.

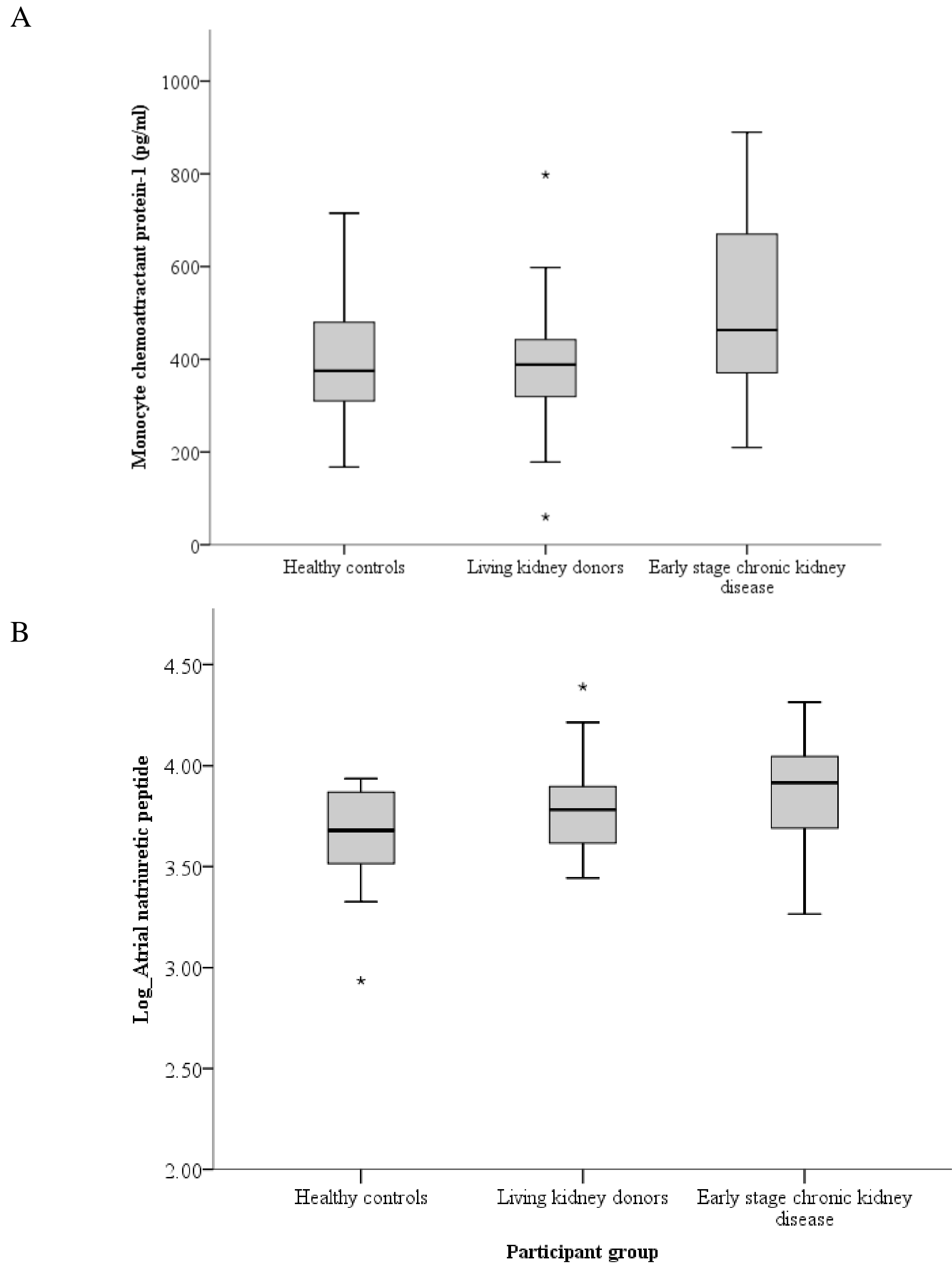
ANP; Atrial natriuretic peptide.IL-RA; Interleukin 1 receptor antagonist. IL10; Interleukin 10. IL-8; Interleukin 8. IL-6; Interleukin 6. MMP-9; Matrix metallopeptidase 9. MCP-1; KIM; Kidney injury molecule. Monocyte chemoattractant protein 1. NGAL; Neutrophil gelatinase-associated lipocalin. sST2; Soluble ST2. TNF-α; Tumour necrosis factor alpha. VEGF; Vascular endothelial growth factor.

* p value <0.05 Controls vs. Donors. † p value<0.05 Controls vs. CKD. ‡ p value <0.05 CKD vs. Donors

Figure 5.3. Comparison of monocyte chemoattractant protein 1 and atrial natriuretic peptide in donors, controls and early stage CKD.

The grey box represents the interquartile range with the thick black line representative of the median. The whiskers are 1.5x the interquartile range with outliers shown on the graph as stars.

A. Box plot comparison of MCP-1 levels between healthy controls, living kidney donors and early stage chronic kidney disease.
B. Box plot comparison of ANP levels (logged) between healthy controls, living kidney donors and early stage chronic kidney disease.



5.6 Discussion

This cross sectional study examined the myocardial characterisation and inflammatory biomarker profiles of kidney donors at a single time point approximately 5 years following nephrectomy and compared the results to those of an age and sex matched healthy control group. Myocardial T2 times were comparable in donors and controls thus providing no evidence that donors have increased myocardial water content despite their reduced GFR and potential for sodium retention. Native T1 time was not different in donors compared to controls providing no evidence of the early signs of myocardial diffuse interstitial fibrosis demonstrated in previous studies of ESRD.[130] There was no significant detectable difference in ECV in donors or controls suggesting no adverse effect on myocardial tissue. Regional discrepancies in T1 times were observed in which basal T1 times were significantly higher than the mid-level in donors but this was not a significant effect in controls indicating some potential differences in regional myocardial stress in donors that is not occurring in the control group.

On comparison of cardiovascular, renal and inflammatory biomarkers between donors, controls and early stage CKD, MCP-1 was elevated in subjects with CKD compared to both donors and controls. This suggests early increases in inflammatory cytokines. This rise was also independent of age and eGFR suggesting it may be the presence of CKD or the disease process itself which is important rather than the level of GFR. IL-10, an important anti-inflammatory cytokine, was also more likely to be detectable in the CKD cohort compared to controls and donors suggesting an early imbalance of inflammatory cytokines in CKD that has not been demonstrated in controls and donors. Reassuringly, there were no significant differences in biomarkers in donors compared to controls indicating donors have both cardiovascular and biochemical profiles more akin to controls than CKD patients with an equivalent eGFR.

5.6.1 Late gadolinium enhancement in living kidney donors

The only contrast enhanced CMR study of living kidney donors is a small cross-sectional study comparing 15 living kidney donors at least 5 years after nephrectomy and 15 age and sex matched controls.[251] This study examined focal replacement fibrosis in donors but not controls and did not perform T1 or T2 mapping and thus provided no information on myocardial diffuse interstitial fibrosis or water content. Inferolateral scarring shown by LGE was found in one donor.[251] These results extend this observation with no evidence of focal LV scarring in donors; the presence of RV insertion point scar in a small number of donors is probably of little significance and is often considered physiological and benign.[418] The same pattern was also seen in one participant in the control group. These findings are important as in subjects with CKD, the proportion with scar as evidenced by LGE can be as high as 25-30%.[91] This suggests that donors are not developing the same focal fibrosis patterns seen in CKD.

5.6.2 Normal T1 and ECV values for healthy controls

Normal values in the healthy control group using this scanner and sampling scheme are in keeping with previous literature. T1 times at a higher field strength of 3 tesla can be expected to be around 100ms greater than values seen at 1.5 T. [419] Other studies using the shortened 5(3)5 sampling scheme acquiring 8 images have also demonstrated native T1 times comparable to these results (T1 times; Kawel et al, 1286 ± 59ms[420] and ECV; Kellman et al $25.4 \pm 2.5\%$.[421]).

5.6.3 Native T1 in living kidney donors

Native T1 mapping and calculation of ECV appear highly sensitive to the presence of diffuse interstitial fibrosis.[321, 413] Elevated T1 times have been found by a number of independent investigators to be elevated in those with both ESRD and early stage CKD compared to a healthy control group.[130, 135, 353] Although histological correlation is lacking, specifically in CKD, quantification of diffuse fibrosis on endocardial biopsies in other cardiac pathologies have shown

significant associations with T1 times so that this imaging biomarker is widely accepted as an index of myocardial fibrosis.[132, 413] The key CMR findings in this study showed no significant differences in T1 times in donors compared to a matched control group.

5.6.4 Regional differences in T1 time

T1 times in the basal slice were greater compared to the mid slice in donors. There are several possible reasons for this. Firstly, it could indicate an asymmetric pattern of disease in which the basal slice is disproportionally affected as a response to increased afterload. Asymmetric and preferential septal remodelling is seen in other cardiac pathology such as Fabry disease and Hypertrophic cardiomyopathy.[422, 423] Isolated septal hypertrophy, or sigmoid septum, is present in approximately 10% of the general population and associated with older age and higher systolic blood pressure.[424] The basal septum is subject to the highest degree of wall stress of the myocardium and thus it is conceivable that the earliest changes could be seen within the base.[425] Although, as stated in the previous chapter, there is no observed increase in blood pressure in the donor group this may reflect small increases in arterial stiffness or peripheral resistance contributing to afterload which have not been detected by conventional measures.

Secondly, it could be due to variation in T1 and T2 from both the base to the apex and from segment to segment as a result of the effects of partial volume and the curvature of the LV.[426, 427] The partial volume effect at the base has been widely reported to reduce reproducibility and consequently the mid LV slices are often recommended in assessment of diffuse fibrosis.[428] Thus, basal results may be expected to be more spurious. The latter is perhaps more likely as it would also be expected that if T1 times were truly increased they would likely be associated with functional correlates, such as strain, as seen in patients with ESRD.[130] In this study T1 times were elevated in donors in the context of better systolic function than the control group

suggesting this is not the case, or at least T1 is not associated with adverse functional correlates in donors.[130]

5.6.5 Extracellular volume in living kidney donors

Despite discrepancies in native T1 mapping, ECV was no different in donors compared to controls in either the mid or the basal slice. ECV represents excessive collagen deposition and is considered a more reliable measure of fibrosis.[115] It also represents a ratio of T1 signals so, unlike post contrast times which are highly variable, it is more reproducible between field strengths.[115] Therefore, ECV provides the most reassurance of no differences in myocardial characterisation in donors compared to controls.

5.6.6 Myocardial characterisation in early stage CKD

In the only previous study of ECV in early stage CKD, Edwards et al. reported an ECV of 28 ± 4 % in early stage CKD compared to 25 ± 4% in age and sex matched controls although participants were on average seven years older with a mean eGFR lower than the donor cohort at 50 ± 22 ml/min/1.73^2.[353] Although ECV is less variable at different field strengths, age and sex differences are well described in ECV and consequently direct comparisons between studies are difficult.[320, 416] This study would, however, suggest ECV results of both the controls and donors are comparable to the healthy control group studied by Edwards et al., yet lower than those with early stage CKD.

In a study of native T1 time Chen et al. reviewed over 200 participants with CKD compared to age and gender matched controls.[429] T1 time and central PWV were found to be significantly higher than the control group although approximately half of participants had diabetes and all of the CKD cohort had an eGFR lower than 60 ml/min/1.73^2 which again means that direct comparison to this study is difficult.[429]

5.6.7 Cardiac and renal biomarkers in living kidney donors

Previous studies of living kidney donors have suggested increased levels of fibrotic and inflammatory markers such as CRP and elevated procollagen II N-terminal propeptide levels.[241, 251] In this study there were no significant differences in the selected cardiovascular, renal and inflammatory biomarkers between controls and donors 5 years after nephrectomy. The CKD cohort, however, had higher levels of both MCP-1 and detectable IL-10 despite being slightly younger with a higher GFR than donors. MCP-1 has been strongly linked to ischaemic heart disease, increased risk of death and inflammation.[430] Furthermore, the higher levels of MCP-1 seen in the CKD cohort were independent of eGFR suggesting that filtration may not cause increasing levels and there may be upregulated production in CKD as a result of a wider inflammatory process.[430] The corresponding prevalence of detectable IL-10 levels indicates that this may be the case. IL10 has been demonstrated to activate fibroblasts and promote collagen deposition causing a stiff LV and resultant diastolic dysfunction.[431] Circulating levels of IL10 have also been associated with adverse cardiovascular events in the general population.[432]

5.7 Limitations

This is the first study that has used T1 and T2 mapping techniques to perform myocardial tissue characterisation in living kidney donors compared to a matched healthy control group. Furthermore, recent concerns over use of intravenous gadolinium have also limited its use in research of healthy controls and those without cardiac pathology making such studies valuable comparators.[433]

Despite this, it is accepted that the sample size is small and it is vulnerable to both type 1 and 2 statistical errors. There were also multiple comparisons during statistical analysis which were

uncorrected so some significant findings may be due to chance. Due to its cross-sectional design at a single time point, causation is also not possible.

Whilst 1:1 matching of participants limited some confounding variables it does not account for all differences and it is recognised that it would have been preferable that the early CKD cohort were also matched if the sample size had been large enough.

5.8 Conclusion

There is little evidence of adverse effects on myocardial tissue in living kidney donors compared to controls. It is reassuring for kidney donors and also has important implications for pathophysiology in early stage CKD. Reduced GFR alone does not appear to result in detectable myocardial tissue disease at least in the medium term. The increases in inflammatory markers and fibrotic markers in those with early stage CKD suggest early inflammatory processes in CKD may be a contributing factor.

CHAPTER 6

FURTHER ANALYSIS

6.1 Introduction

It is recognised that the underlying molecular mechanisms of cardiovascular disease in CKD are poorly understood.[434, 435] Progressive deterioration in physiological function has led to CKD being termed a 'clinical model of premature ageing'.[178, 435] This process is in part due to a-klotho deficiency, deoxyribonucleic acid (DNA) damage and oxidative stress, inflammation, cellular senescence and shortening of telomeres.[178] Accelerated biological ageing is strongly associated with adverse effects on the cardiovascular system including arterial stiffness, atherosclerosis and vascular calcification.[436] In addition, molecular markers of ageing have been associated with poor cardiovascular outcomes, for example telomere length is inversely associated with cardiovascular disease in the general population.[437]

Compared to age matched controls, those on haemodialysis have demonstrated accelerated telomere shortening and pro inflammatory cytokine overproduction suggesting premature senescence of peripheral blood mononuclear cells (PBMC).[438] Telomerase, an enzyme important in maintaining and repairing telomeres, is also lower in those on haemodialysis.[439] This is not isolated to those with advanced renal disease. In a study of 120 participants with stages 2-5 CKD, significant differences have been observed in telomerase activity in advancing stages of CKD.[434] Furthermore, telomerase activity was negatively associated with eGFR and eGFR was an independent predictor for higher telomerase activity.[434]

Telomere length has been previously investigated in living kidney donors but usually for the purposes of predicting graft function in recipents.[440] More recently, however, DNA damage has been studied in donors. In 60 donors examined pre and post donation (3 year follow up) donors exhibited increased markers or oxidative DNA damage associated with a decline in antioxidant paraoxonase activity.[441] In a further study by the same authors, 55 living kidney donors were

compared to age and sex matched controls. Plasma malondialdehyde, a measure of oxidative stress was higher in donors whilst uperoxide dismutase and glutathione peroxidase (measures of antioxidants) were significantly lower.[441]

6.2 Further methodology

6.2 1 Peripheral blood mononuclear cell storage

During the clinical visit, a total of 18mls of venous blood was collected from each subject during venepuncture into green topped lithium heparin vacutainers. The blood samples were placed on a blood specimen rotator within the laboratory at room temperature whilst the patient was undergoing other components of the study visit. Isolation of PBMCs was undertaken within 8 hours from sample collection. Preparation of PBMCs was undertaken in sterile conditions within a class 2 biological safety cabinet. All surfaces and equipment were cleaned with 70% ethanol prior to use.

Whole blood was diluted (in a 1:1 dilution) with RPMI-1640 medium Sigma-Alrich® (supplemented with L-glutamine and penicillin streptomycin) and inverted to ensure the mixture was thoroughly mixed. The blood-RPMI-1640 mixture was then carefully layered over 6ml of Ficoll-Plaque™ PLUS (GE healthcare Bio-Sciences, Uppsala, Sweden), with a maximum of 12ml of the diluted blood for every 6ml Ficoll-Plaque™ PLUS. The layered blood samples were then centrifuged in a bench top centrifuge (Eppendorf 5804R, Eppendorf®, Hamburg, Germany) at 400g for 30 minutes at room temperature (21°c) with both the acceleration and deceleration brake set to zero.

Following centrifugation, mononuclear cells visible as a buffy coat layer above the Ficoll-Plaque™ PLUS, were extracted carefully using a Pasteur pipette without disturbing the red blood cell layer at the bottom and transferred to a universal container. The universal container was then

filled to the top with magnetic assisted cell sorting buffer (MACS; Phosphate buffered saline, EDTA, bovine serum albumin and 0.09% azide; Miltenyi Biotec, Bergisch Gladbach, Germany), and centrifuged at 300g for 10 min at room temperature (21ºC) with both the acceleration and deceleration brake fully on in order to wash the PBMCs. The supernatant was carefully poured off, leaving a cell pellet at the bottom of the universal container, which underwent a second wash in MACS buffer.

Following the second wash, the cell pellet was re-suspended in 10ml of MACS buffer. Using a haemocytometer, 10µl of the cell suspension was viewed using an x10 magnification and cells were counted manually. The cell suspension was then split into aliquots of approximately $5x10^6$ PBMCs which were centrifuged at 300g for 10 min at room temperature (21ºC) with the brake fully on to form a pellet. The supernatant was carefully removed and the pellets resuspended in 1ml of freezing solution (10% Dimethyl sulfoxide, Sigma-Aldrich ®, 90% foetal bovine serum), giving a concentration of approximately $5x10^6$ PBMCs/ml and transferred to labelled cryovials. The cryovials were then placed in a CoolCell® LX (BioCision, California, US) to control the rate of cooling to -1°c per minute and frozen at -80°c until required.

6.2.2 DNA extraction

A DNeasy blood and tissue kits with spin columns (Lot 163049320 Qiagen, Germany) were used for DNA extraction. DNA was extracted from PBMCs at room temperature. Preparation of all reagents was conducted according to manufacturer's instructions. DNA was quantified using the NanoDrop™ spectrophotometer prior to storage at -80°C.

6.3 Analysis so far

As part of the CRIB-DONOR II study all participants' consented to further blood sampling for both the isolation of PBMCs and extraction and analysis of DNA in addition to the inflammatory

cytokine panel already undertaken. PBMCs were isolated after the study visit for each participant (n=50 kidney donors, n= 45 healthy controls and n=33 early stage CKD) and stored at -80°C for future analysis. In addition, DNA was extracted from PBMCs at the end of recruitment.

6.4 Further plans

Continuation of this work was halted as a result of laboratory closure during the COVID-19 pandemic, however, it is hoped the following work can be resumed once laboratories are running again.

i. Analysis of telomere length (qPCR assay kit, ScienCell™, California Cat#8918) and measures of telomerase activity (telemetric repeat amplification protocol assay) for cross sectional comparison between donors, controls and early stage CKD.
ii. Exploration of relationships between measures of myocardial fibrosis (T1 time) and arterial stiffness (PWV) with telomere length and telomerase activity in donors and controls.
iii. Determine whether there are associations between 5 year telomere length/telomerase activity in donors with both final GFR and post-operative hyperfiltration GFR.

CHAPTER 7

FUTURE DIRECTION

7.1 Summary of key findings

Work in chapter 3 demonstrates that when compared to a healthy control group, donors did not have a significant increase in either peripheral ambulatory blood pressure or PWV at 12 months after nephrectomy. A small, but significant rise in central systolic blood pressure in donors was observed compared to controls in the absence of any significant changes in central diastolic pressure. These results indicate that neither systolic nor diastolic peripheral blood pressure increases significantly in donors compared to a healthy control group and there are no significant changes to arterial stiffness associated with nephrectomy in the medium term.

Chapter 4 examined serial change in LV volumes and mass, blood pressure and measures of arterial stiffness at 5 years in living kidney donors compared to a healthy control group. At 5 years, changes in LV mass, volumes and geometry were not significantly different to those seen in healthy controls. In addition, there was no significant difference in change in LV systolic function as measured by ejection fraction and changes in myocardial strain parameters between the two groups at 5 years. The increases in LV mass previously reported in living kidney donors at 12 months, were not sustained at 5 years and resolved to levels comparable to healthy controls. Furthermore, a similar pattern was seen in markers of arterial stiffness. Changes in PWV and AIx were not significantly different in donors compared to controls. FGF23 and uric acid were the only biomarkers significantly greater at 5 years in donors compared to controls. The remaining biomarkers previously reported to be significantly greater in donors than in controls at 12 months (e.g. detectable troponin, PTH and renin) had lessened to become insignificantly different from controls by 5 years. The only significant predictor of a change in LV mass was ambulatory systolic blood pressure, suggesting that nephrectomy and the resultant decline in GFR were not independently associated with changes in cardiovascular structural and functional

change at 5 years in donors. These results indicate that the cardiovascular and haemodynamic changes in kidney donors previously reported do not progress and by 5 years are not significantly different from controls in this cohort. Perhaps surprisingly, loss of a kidney and reduction of about 30% in GFR does not appear to result in changes over and above those naturally seen with advancing age.

Work in chapter 5 compared LGE, T1 time, T2 time and ECV in living kidney donors with 1:1 age and sex matched healthy controls using multi-parametric contrast enhanced CMR imaging. There were no significant differences in septal or global T1 time/ECV in donors compared to controls suggesting that kidney donation does not result in imaging findings consistent with increased myocardial fibrosis at 5 years. RV insertion point LGE was observed in both groups but there was no pathological LV wall LGE suggestive of focal fibrosis. In a separate further analysis of serum biomarkers, donors and controls were compared to a cohort with early stage CKD. Participants with early stage CKD had greater levels of MCP-1 and a higher prevalence of detectable levels of IL-10 compared to both donors and controls. Furthermore, MCP-1 was significantly greater in the CKD group independent of both age and eGFR. There were no significant differences in other measured cardiac/renal biomarkers or inflammatory cytokines in donors compared to controls. These results indicate that there are no significant detectable myocardial tissue differences occurring in living kidney donors compared to controls. In addition, I found no evidence of an inflammatory phenotype in kidney donors with the selected inflammatory cytokines and biomarker profiles related to cardiac and renal disease. Donors were more akin to healthy controls than those with early stage CKD despite having a comparable eGFR. This implies that factors associated with CKD other than simply the reduction in GFR

may have a role in mediating cardiovascular disease. This potentially important finding will be discussed further in section 7.3.

The strengths of these studies were:

1. The careful selection of a healthy control group (who met living kidney donor criteria) was maintained in all of the studies. This is often neglected from large scale living kidney donor registry studies leaving them susceptible to selection bias. Using a comparable group allows comparison of donors with controls who can be considered as equally ‘healthy’ as donors. Furthermore, following up the same control cohort prevents erroneous conclusions being drawn due to changes as a result of the ageing process.
2. The multi-centre UK wide approach used in the EARNEST study increased generalisability and reduced the risk of systemic bias from a single centre or observer.
3. In the CRIB-DONOR II study there were high rates of inter-study, intra-observer and inter-observer reproducibility for LV mass and volumes without systemic bias. All CMR analysis and biochemical assays were analysed with the observer blinded to both group and temporal order to avoid unconscious observer bias.
4. Quality and consistency of T1 and T2 mapping were maintained throughout the cross-sectional study. The sequences and all scanning parameters remained consistent (including timing of the haematocrit sample and intravenous gadolinium administration) throughout. In addition, regular phantom scanning was undertaken to ensure consistency and reproducibility of the scanner itself. Furthermore, there was high intra-observer and inter-observer reproducibility for T1 and ECV.

This work complements the work of Kasiske et al., the only other research group who have prospectively examined the long term biochemical and haemodynamic effects of both donors and a healthy control group.[235, 255] In 2015 Kasiske et al. reported the results of a prospective 3 year follow up study of donors and found no significant difference in office blood pressure at any time point or significant difference in urinary ACR between groups.[235] In addition they also reported higher levels of uric acid in living kidney donors.[235] Further analysis of the cohort was published in 2016 demonstrating that donors had significantly elevated levels of FGF23 compared to controls.[255] Despite this the work of Kasiske et al. overlooked the importance of ambulatory blood pressure monitoring and did not record this at baseline.[235] Results from the CRIB-DONOR II study extend this work indicating that not only do these findings persist at 5 years but in addition there was no significant difference in measures of arterial stiffness or ambulatory blood pressure.

During the final year of this thesis, Kasiske et al. published a nine year follow up study of the same cohort in 2020.[442] Perhaps in recognition of shortcomings in the design of their previous studies they began to examine additional haemodynamic parameters (carotid-femoral PWV and ambulatory blood pressure monitoring) between 3 and 9 years of follow up.[442] Although they lacked baseline values there were no significant differences observed in these parameters between donors and controls, results highly consistent with my work.[442]

Apart from the 1 and 5 year CRIB-DONOR studies, there is only one other published CMR study of kidney donors.[242] In contrast to my findings at 5 years Altmann et al. discovered a mean 3g rise in LV mass in 23 living kidney donors at 12 months.[242] This study however lacked a suitable comparator group failing to account for changes in the general population over time.[242] In addition blood pressure was not adequately considered, with a reliance only on office blood

pressure measures neglecting ambulatory recordings despite known discrepancies in donors between office and ambulatory blood pressure.[242, 373] It is not clear why changes in 12 months were not observed at 5 years. This may be a result of a difference in circulatory factors at 12 months that were not measured/not detectable, or changes in blood pressure that were too small to detect resulting in statistical errors.

To conclude, the studies in this thesis found no evidence to suggest that there are adverse cardiovascular structural or functional effects of living kidney donation at 5 years over and above the changes seen over time in a healthy control group. Important questions are raised about the causes of cardiovascular disease in early stage CKD at similar GFR values. Whilst these data provides reassurance for living kidney donors there are some remaining important clinical and experimental considerations to contemplate before embarking on future studies outlined in the subsequent sections.

7.2 Remaining unanswered experimental questions

7.2.1 What is the GFR 'threshold' for cardiovascular damage to occur?

One alternative explanation for the results is that the decline in renal function following nephrectomy was not sufficient enough to have an effect on the heart or that the resultant GFR at 5 years after compensatory hyperfiltration was not low enough to initiate any detectable cardiovascular damage.

Existing data from the largest, most well-known and frequently cited studies demonstrate that independent associations between all-cause and cardiovascular mortality are most robust for those with an eGFR of <60ml/min/1.73m^2.[13, 15] The landmark study by Go et al. used administrative health care data to reveal a significant association between increased risk of both death and hospitalisation rates with a declining GFR from 60ml/min/1.73m^2 and below.[13] Higher threshold values were not examined. In a collaborative meta-analysis of 13 studies, Astor et al. also found an increasing risk of both all-cause mortality and ESRD with declining GFR.[443] Both of these studies illustrate a graded increasing risk with declining GFR and were important in pioneering further research into the risks of those with pre-dialysis stages of CKD. Nevertheless, they both use categories of >60 ml/min/1.73m^2 or >45 ml/min/1.73m^2, respectively as a reference range for analysis making it impossible to look at thresholds. This approach runs the risk of making the assumption that all those with a GFR >60 ml/min/1.73m^2 denote the same risk. Despite the fact that the relationships between GFR and risk may be a compelling linear relationship at <60 ml/min/1.73m^2 it does not help us define an upper threshold.[444] A reanalysis of these studies with reference categories of >70 or even >80 ml/min/1.73m^2 would give us a greater insight into this relationship.

Shortcomings in current GFR measurements

A further complicating factor affecting our understanding of a ‘threshold’ at which cardiovascular damage occurs is methodological issues related to choosing appropriate eGFR equations. These issues have blighted comparisons between studies and could account for the discrepancies historically seen between studies when attempting to define an upper threshold.

Whilst the creatinine based Modification of Diet in Renal Disease (MDRD) equation has a high degree of accuracy and precision when compared to measured GFR in those with an eGFR of <60 ml/min/1.73m^2 there is considerable variation in those with an eGFR >60 ml/min/1·73 m^2.[445, 446] Furthermore, there is a greater degree of bias when using the MDRD equation for those of different races and ages which could lead to underestimation and missclassification.[445] The CKD-EPI equation is a creatinine based equation developed in 2009 with greater precision and accuracy at higher GFRs compared to MDRD.[447] In a meta-analysis of 1,130,472 participants the threshold at which cardiovascular mortality was significantly elevated began at a higher level when calculated using the CKD-EPI equation (77 vs. 68 ml/min/1.73 m^2).[448]

Whilst the use of CKD-EPI creatinine equation adjusted for age, sex and ethnicity is most frequently used, creatinine has known limitations often in cases of lower muscle mass.[10, 449] The addition of Cystatin C into the equation (an alternative filtration marker) tends to outperform creatinine only based equations.[450] In a meta-analysis of 11 cohorts from the general population and 5 CKD cohorts, the CKD-EPI equation with cystatin (eGFR creat-cys) or cystatin alone (eGFR-Cys) based estimates of eGFR are better predictors of all-cause mortality and cardiovascular mortality. [451] The point at which mortality risk was elevated compared to the reference point was as high 88 ml/min/1.73m^2 for eGFR-Cys, compared to 59 ml/min/1.73m^2 for eGFR-creat and 83 ml/min/1.73m^2 for the combination equation, eGFR creat-cys.[451]

The threshold GFR at which cardiovascular damage or events increase is also partly complicated by reports of a J or U shaped curve when the highest GFRs are studied.[15, 32, 444] This paradoxical relationship reported by some studies also complicates the idea of any specific threshold in which cardiovascular risk occurs. In studies using cystatin alone or the eGFR-cys equation however, the J shape almost disappears to reveal a linear relationship.[387, 450]

What is the GFR 'threshold' for cardiovascular structural and functional change to occur?

It must also be considered that risk of death and cardiovascular events are not the same as cardiovascular structural/functional change which, if causative, should occur before hard outcomes. In a large echocardiography study of over 4000 participants, Matsushita et al. found increasing trends of elevated LV mass with declining eGFR and a deterioration in systolic function in those with an eGFR as high as 74ml/min/1.73m^2 although there was no significant change in LV mass until the lower stages of GFR.[452] The Hoorn study (general population ~2400 participants) however concluded that a decline from 90 to 60 ml/min/1.73m^2 equated to a 8.3g/m^2 increase in LV mass.[453]

Conclusion

In conclusion it is recognised that many previous epidemiological studies of cardiovascular risk either use specific cohorts, analyse risk in broad terms (60-30 or <30 ml/min/1.73m^2 etc.) or use all values of greater than 60 ml/min/1.73m^2 as the reference.[388] By lumping all 'high' or 'normal' GFRs together we risk inappropriately attributing cardiovascular risk to a broad group, failing to differentiate and neglecting the true nature of the relationship between the two. [388, 454] In addition, flaws with chosen filtration markers for estimating GFR and imprecision with estimating equations at the highest levels of GFR have also contributed to uncertainty over a threshold and whether the association is actually linear or J shaped at the highest GFRs.

When considering both comparative studies between GFR equations and more recently studies using cystatin the evidence strongly suggests that cardiovascular risk is likely to begin to rise at a GFR as high as 89 ml/min/1.73m^2 and furthermore that the relationship is likely to be linear. [450, 451, 455]

Future study ideas

For future studies of subjects with renal impairment and cardiovascular clinical outcome measures there are firstly some general considerations:

1. GFR should be analysed as a continuous variable rather than dichotomous GFR categories.[456]
2. If measured (rather than estimated) GFR is not feasible cystatin c should be included.
3. All results should be interrogated with a sensitivity analysis using different estimating equations if step two cannot be achieved.

THE CRIB-DONOR II study has shed light on the paucity of data on those with a borderline low GFR which requires a refocus of efforts towards those with early CKD where therapeutic agents and preventative strategies are likely to be most beneficial. It also, raises the question of whether cardiac structural and functional changes may occur in those with CKD with a preserved GFR. If, as a result of this study, we conclude that GFR (in isolation) may not be the driving factor for the development of cardiovascular change, will those with CKD yet minimally reduced renal function have any early cardiac structural changes?

The next step would be a large multi-centre study of those with confirmed CKD (biopsy or imaging) as per the KDIGO definition. Only those with the highest GFRs (>60 ml/min/1.73m^2) would be recruited (stage 1 and 2). Based on the results of the Hoorne study of the general population, a 30mlmin/1.73m^2 decline in GFR was associated with an 8.3g/m^2 reduction in LV

mass therefore changes in LV mass, if any, are expected to be small.[453] Consequently, for adequate power a large sample size and a multi-centre approach will be required. Using the mean LV mass in living kidney donors of 59 ± 9g/m^2 and a native T1 time of 1214 ± 37ms at 5 years for an 80% power over 1000 participants would be required to detect a relationship.[354]

A measured GFR would be used to determine eligibility criteria and all participants would be required to be non-diabetic and without any previous cardiovascular history to eliminate the influence of traditional risk factors.

All participants would undergo multi-parametric imaging with contrast at one time point to assess whether there is graded cardiovascular and structural change within the group in those with a GFR >60 ml/min/1.73m^2.

Cardiovascular structural and functional end points would include:

1. LV mass: using CMR.
2. GLS: using feature tracking techniques from CMR images.
3. Native T1 time: using MOLLI sequences during CMR imaging.
4. ECV: after administration of gadolinium during CMR imaging.

In addition, all participants would require ambulatory blood pressure monitoring to account for the effect of systolic blood pressure on LV mass and ensure any graded association between GFR and LV mass is independent of blood pressure. A sensitivity analysis would also assess whether the effect persists in those taking anti-hypertensive agents as opposed to those who are not and is independent of ACR. A sub analysis would compare those with ‘preserved renal function’ >90 ml/min/1.73m^2 to healthy age, sex and GFR matched controls.

7.2.2 Would a significant effect have been observed in living kidney donors if there was a longer follow up?

Accumulative risk and 'exposure time'

When drawing conclusions from the CRIB-DONOR II results the time frame of follow up needs to be put into context. Five years is a short duration in the lifetime of a living kidney donor. Study visits at 12 months and 5 years cannot account for any added cumulative risk of cardiovascular disease that a nephrectomy might bring over the very long term nor consider 'disease free' life lived.[457] The duration of a sustained, reduced GFR may have an accumulative adverse effect on the heart which cannot be replicated in kidney donation at 5 years. Given that CKD is frequently asymptomatic and often goes undiagnosed in the early stages (~5% in the general population) it is conceivable that a significant 'exposure time' is required for cardiovascular changes to be detectable in CKD.[458] It may then be the case that 5 years is too short for detectable change to be seen in donors. There is some evidence to support this view. In the Norwegian study of over 1900 donors and the only study which found an increase in cardiovascular mortality, participants were followed up from 1963 to 2008, with a median follow up time of over 15 years. [223] In contrast, previous studies demonstrating no increased cardiovascular mortality have typically been at a much shorter follow up time of between 5.8-7.8 years.[220-222]

Longitudinal studies in CKD

Even in those with CKD, 5 years may not be sufficient for change to occur.[459] In a longitudinal echocardiography study of patients with stage 2-3 CKD there was no significant difference in LV mass at 5 years compared to healthy controls.[459] This indicates that significant changes in cardiovascular structure and function may take some time to develop and may require prolonged exposure to a reduced renal function.[459] The authors concluded that progression of cardiovascular structural change was much slower than anticipated.[459]

Despite this, other longitudinal studies of patients with CKD have demonstrated a change in cardiovascular structure and function in relatively short time periods.[460] In a prospective study of 300 participants with stage 3-5 CKD, significant changes were seen on echocardiography over just 12 months.[460] Cardiovascular structural and functional changes were, however, most significant in the more advanced stages of CKD with greater progression in LV mass in stage 4 and 5 compared to stage 3a [odds ratio 3.02 (95% CI 1.39 to 6.58)].[460] This suggests progression is more rapid in the more advanced stages but changes are still evident over 12 months.

Changes in GFR in donors over time

If we consider that the most significant cardiovascular structural and functional changes in CKD are seen in the more advanced stages of renal disease, is it more pertinent to wait longer for follow up in order for GFR to decline further in donors? Although GFR might be expected to decline in donors with age, small increases in GFR in kidney donors have also been reported and GFR may actually continue to increase over 15-17 years before reaching a plateau.[217, 395] Matas et al. examined the trajectory of eGFR in over 1000 donors and found eGFR tended to increase steadily after donation (maximum follow up +25 years) after donation before eventually plateuing.[395] The mean change from 6 weeks to 5 years, 5-10yrs, 10-20yrs and >20 years was +1.12ml, +0.24 and +0.07 and -0.19ml/min/1.73m^2 respectively.[395] As a consequence, ESRD in donors was much more likely to be related to acute sudden events and the development of new disease processes than post donation decline.[395] In a similar longitudinal study of 255 living kidney donors who underwent serial measures of GFR using iohexol (mean 12 year follow up) Ibrahim et al. found there was on average a +0.20ml/min/1.73m^2 increase in GFR per annum.[217] Although in part Ibrahim et al attributed this to survival bias, Lenihan et al. found donors undergo sustained adaptive hyperfiltration as a result of both increased renal blood flow and hypertrophied glomeruli.[217,

[391] This is consistent with findings of the CRIB-DONOR II study in which a mean +2ml/min/1.73m^2 increase in GFR was observed at 5 years compared to 12 months and renal function was relatively stable. In addition, there were no significant differences in demographics between those who attended follow up and were lost to follow up. Consequently, the natural history of 'renal dysfunction' in donors is in direct contrast to that in CKD which is expected to decline over time.

Conclusion

In conclusion, although longer term follow up (>5 years) is required, any changes in cardiovascular structure and function in the long term are unlikely to be attributed to any further dramatic declines in GFR. The acute nature of a GFR drop in donors and uncertainty over long term consequences warrants both short and long term follow up rather than one at the expense of another.[241, 242]

Future study ideas

A proposed study would be to incorporate cardiovascular assessment into routine annual post donation surveillance for all living kidney donors in the UK. It is perhaps surprising that routine measurement of simple cardiovascular variables such as ambulatory blood pressure have not been collected in previous years. One result is that despite kidney donation having been part of routine clinical practice for > 30 years, we are still unable to provide donors with accurate estimates of the effects of donation on blood pressure or indeed cardiovascular risk. The UK is well positioned to achieve this. Firstly the UK has one of the leading rates of living kidney donation worldwide and secondly the infrastructure of the NHS means basic data on all living kidney donation is already collated by the NHS Blood and Transplant in the UK living kidney donor registry.[211]

To achieve uniformity across sites the techniques below have been specifically chosen to reduce training required and thus inter-observer variability. This will also be associated with

a reduction in cost and therefore is more feasible within a NHS setting. In addition end points are chosen which do not require a prolonged study visit for the participant.

Cardiovascular structural and functional end points would include:

1. Ambulatory blood pressure, AIx and PWV: 24-hr monitoring using the Mobil-O-Graph®.[461] This system also measures PWV and AIx.[461]
2. PWV: Finger to toe PWV using the pOpmètre® (photodiode sensors placed on the finger and toe) which takes less than 30 seconds and has been validated in adults.[462]
3. Cystatin C: measured using blood taken for routine urea and electrolyte testing. This would allow alternative GFR equations to be calculated without a measured GFR.
4. LV mass and measures of myocardial deformation: using speckle-tracking echocardiography.

7.2.3 Do living kidney donors require a ‘second hit’ in order to develop cardiovascular damage?

Data from this thesis has indicated that there is no evidence of cardiovascular structural or functional changes in donors compared to controls at 5 years, however, the CRIB-DONOR II study aimed to compare those who matched 2012 British Transplantation Society living kidney donor criteria, resulting in a fairly homogeneous study group. These results cannot be generalised to expanded criteria donors. It remains unclear whether the addition of a second risk factor (e.g. hypertension, impaired glucose tolerance, obesity) to those who have undergone nephrectomy may have an additive effect on cardiovascular structure and function.

Insight from animal studies

Animal studies suggest the requirement for a second risk factor could lead to exponential changes in cardiovascular structure and function. Animal studies of CKD and hypertension frequently involve salt loading to induce a hypertensive response, potentially indicating it is not solely nephrectomy exerting an effect on the heart.[463]

The DOCA (deoxycorticosterone acetate)-salt rat model is of particular interest. Rats are given DOCA, a synthetic mineralocorticoid pellet under the skin and only saline to drink inducing a mineralocorticoid hypertension, mimicking excess aldosterone.[464] DOCA rats then undergo nephrectomy and develop severe cardiac fibrosis and remodelling.[465] When 'placebo' rats with two kidneys are compared to rats who have undergone uninephrectomy only, there is no significant difference in mean arterial pressure or heart weight. [466] It is only following the addition of DOCA to those who have undergone uninephrectomy that there is an observed increase in blood pressure and heart weight compared to DOCA rats with two kidneys which suggests that those with one kidney are vulnerable but require a 'second hit' to develop cardiovascular structural and functional change.[466] Only then do rats display salt associated hypertension.[463]

A shift in eligibility criteria

Identifying whether a second hit induces cardiovascular disease has important implications. Expanded criteria donors typically include those usually excluded such as those who are overweight or obese, hypertensive, have known vascular disease or impaired glucose tolerance.[467] Whilst the selection of expanded criteria deceased donors only requires concern over graft function in the recipient, expanding criteria for living kidney donation requires careful consideration of the ongoing risk to donors.[468] Data on long term outcomes for expanded criteria donors are sparse however; the increasing rates of expansion criteria in the last decade mean short term risks are being reported.[469]

Conclusion

Whilst the outcomes for recipients receiving a kidney from expanded criteria donors have been successful it is important that future studies of donors remain inclusive to these groups. The added effects of traditional cardiovascular risk factors in the context of nephrectomy will need to be prospectively examined. There is currently no existing study examining the

additive effects of hypertension/impaired glucose tolerance or obesity on cardiovascular structure and function after living kidney donation. Furthermore, the long term cardiovascular mortality and morbidity risks of expanded criteria donors are very limited with most current studies only having a median follow up of 5 years.[467, 469]

Future study ideas

To address whether expanded criteria has an additional adverse on cardiovascular structure and function further study is required. To investigate whether hypertension acts as a 'second hit' in donors a multi-group cross sectional design with the following groups:

1. Expanded criteria hypertensive living kidney donors (n=30-35).
2. Standard criteria normotensive donors (n=30-35).
3. Hypertensive 1:1 age and sex matched controls. Those with secondary causes of hypertension and CKD would be excluded. Participants would need to meet expanded criteria for living kidney donation (n=30-35).

Using the mean LV mass in living kidney donors of 59±9g/m^2 at 5 years for an 80% power for three groups with a minimum detectable difference of 7g/m^2 it is estimated that 26 are required in each group.[354] When considering drop out an estimated sample size would be 30-35 participants per group.

Cardiovascular structural and functional outcome measures would include:

1. Ambulatory blood pressure, AIx and PWV: 24-hr monitoring using the Mobil-O-Graph®.[461]
2. LV mass: using CMR.
3. GLS: using feature tracking techniques from CMR images.
4. Native T1 time: using MOLLI sequences during CMR imaging.
5. T2 mapping: using three single shot T2 weighted CMR images

Participants would be studied at baseline (6 weeks prior to living kidney donation), 1 year and 5 years. To achieve an adequate sample size and considering the relatively few expanded criteria donors proceeding to donation this study would need to be multi-centre but is likely to require recruitment throughout Europe and the US as a collaborative project.

7.2.4 Are changes in the myocardium of donors at 12 months (i.e. elevated LV mass) due to the same disease processes demonstrated in CKD?

At 12 months it was demonstrated that LV mass is elevated in the donor group. The reasons for this are so far unclear. Previous post mortem studies and more recent cardiac imaging have indicated that the increase in LV mass in CKD is likely to be the result of increasing interstitial fibrosis.[112, 130] Despite this, not all increases in LV mass are pathological and it cannot be assumed that the increase in LV mass shown at 12 months in donors represents the same disease process that has been demonstrated previously in ESRD.

Adaptive vs maladaptive change

Insight into the histological features of LV mass come from studies of athletes.[470] An athletic heart would be expected to have proportional increases in chambers and normal or even improved cardiac function.[470] In comparison, pathological remodelling consists of deteriorating cardiac function, cardiac fibrosis and evidence of myocyte death.[470] More recently, CMR techniques have been used to differentiate between adaptive changes and pathological remodelling in athletes.[471] Using advanced CMR techniques (including native and post contrast T1 mapping) McDiarmid et al. found that amongst 30 athletes increases in LV mass were secondary to increased cellular volume.[471] ECV actually declined in athletes and there was an inverse relationship with $VO_{2\,max.}$[471] This study indicates that ECV may have a role in differentiating between benign and pathological remodelling.[471]

Conclusion

ECV may be an important differentiator between adaptive and pathological remodelling. There are currently no studies investigating serial T1 mapping and ECV quantification in living kidney donors after donation. The question that remains is whether the increase in LV mass observed at 12 months is associated with a rise in ECV or secondary to the cellular increases seen in adaptive remodelling.

Future study ideas

To address this, living kidney donors would undergo serial CMR at baseline (6 weeks prior to nephrectomy), 12 months and 5 years.

Cardiovascular structural and functional outcome measures would include:

1. LV mass: using CMR.
2. LGE
3. Native T1 time: using MOLLI sequences during CMR imaging.
4. T2 mapping: using three single shot T2 weighted CMR images

The combination of T1, T2, ECV and LV mass would be used to determine serial cardiovascular structural change but also serial change in myocardial tissue characterisation.

7.3 Remaining unanswered clinical questions

This thesis suggests that it is not the consequences of reduced GFR in isolation nor the accumulation of any by-products which amass solely due to a reduced GFR following nephrectomy which lead to cardiovascular disease, at least in the medium term. This raises the question of what pathological differences there are between living kidney donors and early CKD and which differences may have cardiovascular consequences.

Key differences between living kidney donors and early stage CKD are:

1. The lack of active intrinsic renal pathology.
2. Proteinuria is rarely severe.
3. Renal function doesn't continue to decline.
4. The lack of any systemic disease process i.e. diabetes or vasculitis.
5. Hypertension is less common.
6. Absence of markers of inflammation.
7. Absence of activation of the renin-angiotensin aldosterone system.

In chapter 5 levels of both MCP-1 and detectable IL-10 were greater in those with early stage CKD compared to 1:1 age and sex matched healthy controls and living kidney donors. This work indicates that the biomarker profile of living kidney donors and controls were more similar to each other than that of the early stage CKD group despite having comparable GFRs.

Furthermore whilst CKD is known to be associated with inflammation it remains to be seen whether CKD generates an inflammatory process from damaged or hypoxic renal parenchyma or results as a consequence of it.[472, 473] With the majority of circulating cytokines residing from monocytes and the kidney being a highly vascularised organ receiving a quarter of blood volume it is clear it is a vulnerable target.[472] Yet this process doesn't appear to be

occurring in donors, perhaps suggesting that the simplistic view of reduced clearance of inflammatory mediators is probably not a factor.[474] There is some evidence to support this, Roubicek et al. found that those with ESRD had increased messenger ribonucleic acid expression for pro inflammatory cytokines in adipose tissue suggesting production is a factor.[474] The trigger for the production of inflammatory mediators is a subject of intense research particularly as inflammation is not only linked to poor renal outcomes but has strong relationships with cardiovascular disease.[181]

7.3.1 Does the underlying aetiology of early stage CKD have systemic consequences on cardiovascular structure and function?

Renal aetiology and systemic disease

There is emerging evidence that the underlying aetiology of renal disease is important in cardiovascular risk. In 2018, O'Shaughnessy et al. used a US national ESRD renal registry database to select participants with eight different underlying causes of ESRD.[475] Using a composite end point which included myocardial infarction, ischaemic stroke or cardiovascular/cerebrovascular death, the authors found event rates varied significantly depending on the underlying aeitiology.[475] After adjustment for confounding factors, IgA nephropathy had the lowest adjusted HR for cardiovascular mortality compared to lupus [HR 1.86 (95% CI 1.71–2.03)], adult polycystic kidney disease (APKD) (HR 1.29 [95% CI 1.19–1.39] and membranous nephropathy (HR 1.67 [95% CI 1.52–1.83].[475] A higher risk may be related to frequent use of immunosuppression and steroids which themselves induce metabolic syndrome, the likelihood of developing nephrotic syndrome or a pathway that leads to direct vascular injury.[475] This suggests that primary glomerulonephritis (GN), secondary GN and non-GN CKD should be considered separately. Cardiovascular outcome studies seldom differentiate the underlying aetiology of renal disease with a wide range of conditions examined under the homogenous umbrella term of CKD.

Conclusion

Evidence suggests that there is considerable heterogeneity across renal aetiologies and that some underlying causes of disease may lead to higher cardiovascular risk than others. The question then arises as to whether subtypes of renal disease also display cardiovascular structural and functional changes that differ from one another and whether there is an identifiable common denominator?

Future study ideas

One way to answer this question, is rather than examining early CKD as a single entity is to design a cross-sectional study comparing structural and functional cardiovascular differences between aetiologies and sub types. In order to exclude the influence of traditional cardiovascular risk factors non-diabetic stage 2 CKD participants would be recruited from the following subgroups:

1. Primary glomerular specific disease (n=50-60).
2. Adult polycystic kidney disease (n=50-60).
3. Small vessel vasculitis (n=50-60).

Groups would be 1:1 matched according to age and sex. A full list of inclusion and exclusion criteria is show in **Table 7.1**.

Using a mean LV mass of 65±12g/m^2 from a previous CMR study from our group in those with CKD stage 2, it is estimated that 46 participants would be required for 80% power to detect a mean difference of 7g/m^2.[476] Therefore when taking into consideration drop out approximately 50-60 participants would be required in each group.

Participants would undergo contrast enhanced CMR including LGE, T1, T2 mapping and functional correlates using feature tracking. Blood and urine samples would also be taken to

examine inflammatory biomarkers and oxidative stress using multiplex magnetic immunoassays. In addition patients would undergo ambulatory blood pressure monitoring to allow any findings to be corrected for blood pressure (a potential confounder). This approach would help to further risk stratify those with early stage CKD, explore potential inflammatory mediators and identify common pathways. Participants would then be asked to return for a repeat study at three year intervals.

Table 7.1. Suggested inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Confirmed renal diagnosis (ie.renal biopsy, family history or imaging)	Diabetes or Pre-diabetes
Stage 2 CKD	Heart failure
Must have two GFR measures at least three months apart	Angina
Sustained remission for at least 12 months	Acute coronary syndrome
	Peripheral vascular disease
	Stroke or Transient ischaemic attack
	Renal transplantation
	Immunosuppressive therapy
	Tolvaptan therapy
	Uncontrolled hypertension

7.3.2 Could proteinuria have effects on cardiovascular structure and function in early stage CKD independently of GFR?

Cardiovascular risk and proteinuria

Proteinuria, a marker of renal injury, is rarely elevated in living kidney donors and remains a key differentiator between donors and those with early stage CKD.[2] In the CRIB-DONOR II study although there was an increase in proteinuria in donors it was not significantly different from the control group. Proteinuria however, often occurs early in the course of CKD prior to any measurable decline in GFR.[477, 478] Furthermore, epidemiology data suggests that the relationship between rising proteinuria and all-cause and cardiovascular mortality is consistent and compelling.[44] The underlying mechanisms, nevertheless, are not well understood.[479]

Conclusion

It is unclear whether cardiovascular disease precipitates microalbuminuria or albuminuria triggers increases in cardiovascular disease as both are associated with a common factor.[479] Current evidence suggests a common pathology such as chronic inflammation or endothelial dysfunction is likely. [479]

Future study ideas

A proposed study would include recruitment of early stage CKD participants with a GFR of >90ml/min/1.73m^2 with a single aetiology. The reason for choosing one aetiology would be to limit pathological differences between them and variation in medication. Those with APKD would be an ideal cohort, firstly because in the early stages many participants are healthy with few comorbidities. Secondly, the aetiology and management of APKD is uniform as opposed to those with glomerulonephritis. Thirdly, they frequently have a preserved renal function in the early stages and decline in GFR is slow. Finally, although those with APKD develop proteinuria the development of nephrotic syndrome is relatively unusual.

Proteinuria would be measured using ACR. Cardiovascular end points would include:

1. Ambulatory blood pressure.
2. LV mass: using CMR.
3. GLS: using feature tracking techniques from CMR images.
4. Native T1 time: using MOLLI sequences during CMR imaging.
5. T2 mapping: using three single shot T2 weighted CMR images

Relationships between cardiovascular end points and proteinuria would be examined as continuous variables and results would be corrected for age, body mass index and ambulatory systolic blood pressure.

7.3 Conclusion

The results from the EARNEST and CRIB-DONOR II studies provide cautious reassurance of the longer term consequences of living kidney donation. We have found no evidence of adverse cardiovascular structural or functional consequences of living kidney donation at 5 years after nephrectomy. This is the only study to successfully investigate serial change in cardiovascular structure and function alongside a healthy control group. These encouraging results contribute to ongoing studies of the long term consequences of living kidney donation which ultimately have implications for thousands of donors, potential recipients and the future planning of organ transplantation programs. Furthermore, it provides living kidney donors and clinicians with the knowledge that their health will not inevitably deteriorate as a result of nephrectomy and risk is potentially modifiable. Further research must now focus on the very long term consequences and whether these findings remain applicable to expanded criteria donors, different races and varying age groups. Only then can we maintain a robust and ethical living kidney donation programme for both developed and developing countries in future generations.[480]

CHAPTER 8

APPENDIX

8.1 Published abstracts, presentations and publications directly from this work

8.1.1 Oral communications

- **Price AM,** Moody WE, Stoll VM, Vijapurapu R, Hayer MK, Biasiolli L, Weston CJ, Webster R, Wesolowski R, McGee KC, Kaur A, Edwards NC, Liu B, Baig S, Pickup LC, Radhakrishnan A, Law JP, Steeds RP, Ferro CF, Townend JN Cardiovascular effects of unilateral nephrectomy in living kidney donors at five years. European Renal Association, Milan (Virtual) **June 2020**

- **Price AM,** Greenhall G, Ferro CJ, Wilkinson IB, Tomlinson LA, Townend JN Effect of a reduction in glomerular filtration rate after nephrectomy on arterial stiffness and central haemodynamics: The EARNEST study. British and Irish Hypertension Society, Birmingham **Sept 2019**

- **Price AM**, Law JP, Hayer MK, Pickup LC, Radhakrishnan A, Moody WE, Edwards NC, Ferro CJ, Townend JN Medium term haemodynamic and blood pressure effects of living kidney donation. British and Irish Hypertension Society, Birmingham **Sept 2019**

- **Price AM,** Hayer MK, Fyyaz SA, Moody WE, Ferro CJ, Townend JN, Steeds RP, Edwards NC Patterns of late gadolinium enhancement in chronic kidney disease: A predictor of clinical outcome data? British Cardiovascular Society, Manchester **Jun 2018**

8.1.2 Poster communications

- **Price AM,** Stoll VM, Vijapurapu R, McGee K, Wesolowski R, Hayer MK, Moody WE. Edwards NC, Liu B, Baig S, Kaur A, Pickup LC, Radhakrishnan A, Law JP, Steeds RP, Ferro CJ, Townend JN Myocardial tissue characterisation in living kidney donors 5 years after nephrectomy. European Renal Association, Milan (Virtual) **June 2020**

- **Price AM,** Moody WE, Stoll V, Vijapurapu R, Hayer MK, Biasiolli L, Weston C, Webster R, Wesolowski R, McGee K, Edwards NC, Liu B, Baig S, Pickup LC, Radhakrishnan A, Law JP, Steeds RP, Ferro CJ, Townend JN. Cardiovascular effects of living kidney donation: A five year longitudinal study. British Cardiovascular Society, Manchester (Virtual) **June 2020**

- **Price AM,** Hayer MK, Radhakrishnan A, Pickup LP, Moody WE, Edwards NC, Steeds RP, Ferro CJ, Townend JN. Weight gain and office blood pressure in living kidney donors: A five year follow up study, European Renal Association, Budapest. **June 2019**

- **Price AM,** Hayer MK, Moody W, Law JP, Edwards NC, Steeds RP, Ferro CJ, Cockwell P, Townend JN. Living kidney donation, inflammation and free light chains. European Renal Association, Budapest. **June 2019**

- **Price AM**, Pickup LC, Radhakrishnan A, Law JP, Hayer MK, Moody WE, Edwards NC, Ferro CJ, Townend JN Nocturnal dipping and blood pressure variability in living kidney donors five years after nephrectomy. British and Irish Hypertension Society, Birmingham **Sept 2019**

- **Price AM**, Hopkins L, Wheeldon H, Hayer M, Ferro C, Edwards N, Townend J Relationships between 24 hour pulse wave velocity and blood pressure variability in early stage CKD. British and Irish Hypertension Society Annual Scientific Meeting, Cambridge **Sept 2018**

- **Price AM**, Wheeldon H, Hopkins L, Hayer MK, Edwards NC, Ferro CJ, Townend JN Blood pressure control in non-diabetic patients with early stage CKD. Are we undertreating hypertension? British and Irish Hypertension Society Annual Scientific Meeting, Cambridge **Sept 2018**

8.1.3 Publications

- **Price AM,** Moody WE, Stoll VM, Vijapurapu R, Hayer MK, Biasiolli L, Weston CJ, Webster R, Wesolowski R, McGee KC, Liu B, Baig S, Pickup LC, Radhakrishnan A, Law JP, Edwards NC, Steeds RP, Ferro CJ, Townend JN. Cardiovascular effects of unilateral nephrectomy in living kidney donors at 5 years. *Hypertension.* **In press 2021** doi: 10.1161/hypertensionaha.120.15398

- **Price AM**, Greenhall GHB, Moody WE, Steeds RP, Mark PB, Edwards NC, Hayer MK, Pickup LC, Radhakrishnan A, Law JP, Banerjee D, Campbell T, Tomson CRV, Cockcroft JR, Shrestha B, Wilkinson IB, Tomlinson LA, Ferro CJ, Townend JN. Changes in blood pressure and arterial hemodynamics following living kidney donation. *Clinical Journal of American Society of Nephrology.*15 (9) 1330-1339 **2020** doi.org/10.2215/CJN.15651219

- **Price AM**, Hayer MK, Vijapurapu R, Fyyaz SA, Moody WE, Ferro CJ, Townend JN, Steeds RP, Edwards NC. Myocardial characterization in pre-dialysis chronic kidney disease: A study of prevalence, patterns and outcomes. *BMC Cardiovascular Disorders* 19: 295 **2019**
  doi: 10.1186/s12872-019-1256-3

- **Price AM**, Edwards NC, Hayer MK, Moody WE, Steeds RP, Ferro CJ, Townend JN. Chronic kidney disease as a cardiovascular risk factor: Lessons from kidney donors. *Journal of the American Society of Hypertension.*12: 497-505.e494 **2018**
  doi: 10.1016/j.jash.2018.04.010

- **Price AM,** Ferro CJ, Hayer MK, Steeds RP, Edwards NC, Townend JC. Premature coronary artery disease and early stage chronic kidney disease *QJM* 111(10): 683–686 **2017**
  doi: 10.1093/qjmed/hcx179

8.1.4 Personal awards

- Virtual access grant (top eight abstracts by a young investigator). Oral presentation at European Renal Association **2020.**
- Winner of young investigator research award. Oral presentation at British Irish Hypertension Society **2019.**
- Winner of best abstract for imaging category. Oral presentation at British Cardiac Society **2018.**

- Nominated for Dr Robert Grayson award for research. Poster presentation British Irish Hypertension Society **2018.**
- British Heart Foundation Clinical Training Fellowship **2017-2020.**
- American Society of Nephrology travel bursary **2016.**

8.2 Published abstracts, presentations and publications indirectly from this work

8.2.1 Other abstracts and presentations

- Radhakirsinan A, Pickup L, **Price AM,** Law JP, McGee K, Fabritz L, Senior R, Steeds RP, Ferro CJ, Townend JN Anaemia and coronary microvascular dysfunction in end-stage renal disease. European Association of Cardiovascular Imaging (Virtual) **Dec 2020**

- Radhakirsinan A, Pickup L, **Price AM,** Law JP, Fabritz L, Steeds RP, Ferro CJ, Townend JN Coronary flow reserve is reduced in asymptomatic living kidney donors-results of the Chronic Impairment in Birmingham coronary flow reserve (CRIB-FLOW) study. British Cardiovascular Society, Manchester (Virtual) **Jun 2020**

- Hayer MK, **Price AM,** Liu B, Baig S, Ferro CJ, Townend JN, Steeds RP, Edwards NC Progression of myocardial fibrosis in chronic kidney disease. European Renal Association, Budapest. **Jun 2019**

- Hayer MK, **Price AM,** Liu B, Baig S, Ferro CJ, Townend JN, Steeds RP, Edwards NC. Myocardial ultrastructural changes in progressive CKD: the key intermediaries of 'uraemic' cardiomyopathy? European Society of Cardiology, Munich. **Aug 2018**

- Liu B, Neil AD, Bhabra M, Hayer MK, Baig S, **Price AM,** Edwards NC, Steeds RP. Sex differences in left ventricular remodeling in volume overload due to primary degenerative mitral regurgitation. European Society of Cardiology, Munich. **Aug 2018**

- Hayer MK, **Price AM,** Liu B, Baig S, Ferro CJ, Townend JN, Steeds RP, Edwards NC. Myocardial ultrastructural changes in progressive CKD: the key intermediaries of 'uraemic' cardiomyopathy? British Cardiovascular Imaging Meeting, Edinburgh **May 2018**

- Hayer MK, **Price AM,** Baig S, Liu B, Ferro CJ, Townend JN, Edwards NC, Steeds RP. CPEX testing detects subclinical cardiac limitation to exercise in early stage CKD. British Cardiovascular Society, Manchester **Jun 2017**

- Hayer MK, **Price AM,** Baig S, Liu B, Townend JN, Ferro JN, Steeds RP, Edwards NC. Cardiac alterations after renal transplant; controversies unravelled by cardiac MRI. British Cardiovascular Society, Manchester **Jun 2017**

- Hayer MK, **Price AM,** Baig S, Liu B, Ferro CJ, Edwards NC, Townend JN, Steeds RP. Trends on right ventricular function across stages of CKD in a cohort with

minimal conventional risk factors of cardiovascular disease. European Renal Association, Madrid **May 2017**

- Hayer MK, **Price AM,** Townend JN, Ferro CJ, Steeds RP, Edwards NC. Defining cardiac function early after renal transplantation: challenges unravelled by cardiac magnetic resonance imaging. European Renal Association, Madrid **May 2017**

- Hayer MK, **Price AM,** Baig S, Liu B, Ferro CJ, Edwards NC, Townend JN, Steeds RP. Cardiopulmonary exercise testing detects subclinical cardiac limitation to exercise in early stage CKD. European Renal Association, Madrid **May 2017**

- Hayer MK, Baig S, **Price AM,** Liu B, Ferro CJ, Townend JN, Edwards NC, Steeds RP. Cardiac limitation occurs early in CKD, and cannot be fully explained by ischaemia or reduced LV compliance as measured by diastolic function during exercise. European Renal Association, Madrid **May 2017**

8.2 2 Other publications

- Loutradis C, **Price AM,** Ferro CJ, Sarafidis P Renin-angiotensin system blockade in patients with chronic kidney disease: benefits, problems in everyday clinical use, and open questions for advanced renal dysfunction. *Journal of Human Hypertension.* **In press 2021**
  doi.org/10.1038/s41371-021-00504-9

- Pickup LC, Law JP Radhakrishnan A, **Price AM,** Loutradis C, Smith TO, Edwards **NC,** Steeds RP, Townend JN, Ferro CJ Changes in left ventricular structure and function associated with renal transplantation; a systematic review and meta-analysis. *European Society of Cardiology Heart failure.* **In press 2021**
  doi.org/10.1002/ehf2.13283

- Radhakrishnan A, **Price AM,** Pickup L, Law JP, McGee KC, Fabritz L, Senior R, Steeds RP, Ferro CJ, Townend JN Coronary flow velocity reserve and inflammatory markers in living kidney donors. *International Journal of Cardiology.*320:141-147 **2020**
  doi: 10.1016/j.ijcard.2020.08.013

- Hayer MK, Radhakrishnan A, **Price AM**, Liu B, Baig S, Weston CJ, Biasiolli L, Ferro CJ, Townend JN, Steeds RP, Edwards NC. Defining myocardial abnormalities across the stages of chronic kidney disease. A Cardiac Magnetic Resonance Imaging Study. *JACC: Cardiovascular Imaging*.13(11):2357-2367 **2020**
  doi: 10.1016/j.jcmg.2020.04.021

- Law JP, **Price AM**, Pickup L, Radhakrishnan A, Weston C, Jones AM, McGettrick HM, Chua W, Steeds RP, Fabritz L, Kirchhof P, Pavlovic D, Townend JN, Ferro CJ. Clinical potential of targeting fibroblast growth factor-23 and klotho in the treatment of uremic cardiomyopathy. *Journal of the American Heart Association***.** 9(7):e016041 **2020**
  doi: 10.1161/JAHA.120.016041.

- Vijapurapu R, Baig S, Nordin S, Augusto JB, **Price AM**, Wheeldon N, Lewis N, Kozor R, Kotecha D, Hodson J, Hughes DA, Moon JC, Geberhiwot T, Steeds RP. Longitudinal assessment of cardiac involvement in fabry disease using cardiovascular magnetic resonance imaging. *JACC: Cardiovascular Imaging*.13(8):1850-1852. **2020** doi: 10.1016/j.jcmg.2020.03.004.

- Baig S, Paisey R, Dawson C, Barrett T, Maffei P, Hodson J, Rambhatla SB, Chauhan P, Bolton S, Dassie F, Francomano C, Marshall RP, Belal M, Skordilis K, Hayer M, **Price AM**, Cramb R, Edwards N, Steeds RP, Geberhiwot T. Defining renal phenotype in alström syndrome. *Nephrol Dial Transplant*. 35(6):994-1001 **2020** doi: 10.1093/ndt/gfy293.

- Hayer MK, Radhakrishnan A, **Price AM**, Baig S, Liu B, Ferro CJ, Captur G, Townend JN, Moon JC, Edwards NC, Steeds RP. Early effects of kidney transplantation on the heart - a cardiac magnetic resonance multi-parametric study. *International Journal of Cardiology*.293:272-277 **2019** doi: 10.1016/j.ijcard.2019.06.007.

- **Price AM,** Sarween N, Gupta I, Baharani J. Risk factors and short-term outcomes for methicillin-resistant staphylococcus aureus and methicillin-sensitive staphylococcus aureus colonization among hemodialysis patients. *Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia*.30(6):1351-1363 **2019** doi: 10.4103/1319-2442.275479.

- Radhakrishnan A, Pickup LC, **Price AM**, Law JP, Edwards NC, Steeds RP, Ferro CJ, Townend JN. Coronary microvascular dysfunction: A key step in the development of uraemic cardiomyopathy? *Heart*. 105(17):1302-1309 **2019** doi: 10.1136/heartjnl-2019-315138.

- Liu B, Dardeer AM, Moody WE, Hayer MK, Baig S, **Price AM,** Leyva F, Edwards NC, Steeds RP. Reference ranges for three-dimensional feature tracking cardiac magnetic resonance: Comparison with two-dimensional methodology and relevance of age and gender. *The International Journal of Cardiovascular Imaging*. 34(5):761-775 **2018** doi: 10.1007/s10554-017-1277-x.

- Hayer M, **Price AM**, Liu B, Baig S, Ferro CJ, Townend JN, Steeds RP, Edwards NC. Diffuse myocardial interstitial fibrosis and dysfunction in early chronic kidney disease. *American Journal of Cardiology.* 121(5):656-660 **2017** doi: 10.1016/j.amjcard.2017.11.041.

- Hayer MK, Edwards NC, Slinn G, Moody WE, Steeds RP, Ferro CJ, **Price AM**, Andujar C, Dutton M, Webster R, Webb DJ, Semple S, MacIntyre I, Melville V, Wilkinson IB, Hiemstra TF, Wheeler DC, Herrey A, Grant M, Mehta S, Ives N, Townend JN. A randomized, multicenter, open-label, blinded end point trial comparing the effects of spironolactone to chlorthalidone on left ventricular mass in patients with early-stage chronic kidney disease: Rationale and design of the spiro-ckd trial. *American Heart Journal*. 191:37-46 **2017** doi: 10.1016/j.ahj.2017.05.008.

CHAPTER 9

REFERENCES

1. Price A, Ferro CJ, Hayer MK, Steeds RP, Edwards NC, Townend JN. Premature Coronary Artery Disease and Early Stage Chronic Kidney Disease. *QJM: An International Journal of Medicine*. 2017;111:683-686.
2. Price AM, Edwards NC, Hayer MK, Moody WE, Steeds RP, Ferro CJ and Townend JN. Chronic kidney disease as a cardiovascular risk factor: lessons from kidney donors. *Journal of the American Society of Hypertension*. 2018;12:497-505.e4.
3. Price AM, Hayer MK, Vijapurapu R, Fyyaz SA, Moody WE, Ferro CJ, Townend JN, Steeds RP and Edwards NC. Myocardial characterization in pre-dialysis chronic kidney disease: a study of prevalence, patterns and outcomes. *BMC Cardiovasc Disord*. 2019;19:295.
4. Klatte T, Ficarra V, Gratzke C, Kaouk J, Kutikov A, Macchi V, Mottrie A, Porpiglia F, Porter J, Rogers CG, Russo P, Thompson RH, Uzzo RG, Wood CG and Gill IS. A Literature Review of Renal Surgical Anatomy and Surgical Strategies for Partial Nephrectomy. *European Urology*. 2015;68:980-992.
5. Robson L. The kidney--an organ of critical importance in physiology. *J Physiol*. 2014;592:3953-3954.
6. Levey AS and Coresh J. Chronic kidney disease. *The Lancet*. 2012;379:165-180.
7. Eckardt K-U, Berns JS, Rocco MV and Kasiske BL. Definition and Classification of CKD: The Debate Should Be About Patient Prognosis-A Position Statement From KDOQI and KDIGO. *American Journal of Kidney Diseases*. 2009;53:915-920.
8. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J and Eknoyan G. National Kidney Foundation Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. *Annals of Internal Medicine*. 2003;139:137-147.
9. Levey AS, Eckardt K-U, Tsukamoto Y, Levin A, Coresh J, Rossert J, Zeeuw DDE, Hostetter TH, Lameire N and Eknoyan G. Definition and classification of chronic kidney disease: A position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney International*. 2005;67:2089-2100.
10. Levey AS, de Jong PE, Coresh J, Nahas MEl, Astor BC, Matsushita K, Gansevoort RT, Kasiske BL and Eckardt K-U. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney International*. 2011;80:17-28.
11. Kidney Disease: Improving global outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney International*. 2013;Suppl. 2013:1-150.
12. de Jong PE and Gansevoort RT. Fact or fiction of the epidemic of chronic kidney disease—let us not squabble about estimated GFR only, but also focus on albuminuria. *Nephrology Dialysis Transplantation*. 2008;23:1092-1095.
13. Go AS, Chertow GM, Fan D, McCulloch CE and Hsu C-y. Chronic Kidney Disease and the Risks of Death, Cardiovascular Events, and Hospitalization. *New England Journal of Medicine*. 2004;351:1296-1305.
14. Romagnani P, Remuzzi G, Glassock R, Levin A, Jager KJ, Tonelli M, Massy Z, Wanner C and Anders H-J. Chronic kidney disease. *Nature Reviews Disease Primers*. 2017;3:17088.
15. Matsushita K, van der velde M, Astor BC, Woodward M, Levey AS, de Jong P, Coresh J, Ganesvoort RT. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *The Lancet*.2010; 375:2073-2081.
16. Glassock RJ, Warnock DG and Delanaye P. The global burden of chronic kidney disease: estimates, variability and pitfalls. *Nature Reviews Nephrology*. 2017;13:104-114.

17. Couser WG, Remuzzi G, Mendis S and Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. *Kidney International*. 2011;80:1258-1270.
18. Callaghan CA, Shine B and Lasserson DS. Chronic kidney disease: a large-scale population-based study of the effects of introducing the ckd-epi formula for eGFR reporting. *BMJ Open*. 2011;1:e000308.
19. Eriksson JK, Neovius M, Jacobson SH, Elinder C-G and Hylander B. Healthcare costs in chronic kidney disease and renal replacement therapy: a population-based cohort study in Sweden. *BMJ Open*. 2016;6:e012062.
20. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS and Hobbs FDR. Global Prevalence of Chronic Kidney Disease – A Systematic Review and Meta-Analysis. *PLOS ONE*. 2016;11:e0158765.
21. Aitken GR, Roderick PJ, Fraser S, Mindell JS, O'Donoghue D, Day J and Moon G. Change in prevalence of chronic kidney disease in England over time: comparison of nationally representative cross-sectional surveys from 2003 to 2010. *BMJ Open*. 2014;4:e005480.
22. Hounkpatin HO, Harris S, Fraser SDS, Day J, Mindell JS, Taal MW, O'Donoghue D and Roderick PJ. Prevalence of chronic kidney disease in adults in England: comparison of nationally representative cross-sectional surveys from 2003 to 2016. *BMJ Open*. 2020;10:e038423.
23. Hirst JA, Hill N, O'Callaghan CA, Lasserson D, McManus RJ, Ogburn E, Mena JMO, Shine B, Taylor CJ, Vazquez-Montes MD, Yang Y and Hobbs FR. Prevalence of chronic kidney disease in the community using data from OxRen: a UK population-based cohort study. *British Journal of General Practice*. 2020;70:e285-e293.
24. Rangaswami J, Bhalla V, Blair John EA, Chang Tara I, Costa S, Lentine Krista L, Lerma Edgar V, Mezue K, Molitch M, Mullens W, Ronco C, Tang WHW, McCullough PA. Cardiorenal Syndrome: Classification, Pathophysiology, Diagnosis, and Treatment Strategies: A Scientific Statement From the American Heart Association. *Circulation*. 2019:139:e840-e878.
25. Keith DS, Nichols GA, Gullion CM, Brown J and Smith DH. Longitudinal follow-up and outcomes among a population with chronic kidney disease in a large managed care organization. *Archives of Internal Medicine*. 2004;164:659-663.
26. Cases and Observations Illustrative of Renal Disease, Accompanied with the Secretion of Albuminous Urine. *Med Chir Rev*. 1836;25:23-35.
27. Ronco C, McCullough P, Anker SD, Anand I, Aspromonte N, Bagshaw SM, Bellomo R, Berl T, Bobek I, Cruz DN, Daliento L, Davenport A, Haapio M, Hillege H, House AA, Katz N, Maisel A, Mankad S, Zanco P, Mebazaa A, Palazzuoli A, Ronco F, Shaw A, Sheinfeld G, Soni S, Vescovo G, Zamperetti N, Ponikowski P. Cardio-renal syndromes: report from the consensus conference of the Acute Dialysis Quality Initiative. *European Heart Journal*. 2009;31:703-711.
28. Turin TC, Tonelli M, Manns BJ, Ahmed SB, Ravani P, James M and Hemmelgarn BR. Proteinuria and Life Expectancy. *American Journal of Kidney Diseases*. 2013;61:646-648.
29. Turin TC, Tonelli M, Manns BJ, Ravani P, Ahmed SB and Hemmelgarn BR. Chronic kidney disease and life expectancy. *Nephrology Dialysis Transplantation*. 2012;27:3182-3186.
30. Wen CP, Cheng TYD, Tsai MK, Chang YC, Chan HT, Tsai SP, Chiang PH, Hsu CC, Sung PK, Hsu YH and Wen SF. All-cause mortality attributable to chronic kidney disease: a prospective cohort study based on 462 293 adults in Taiwan. *The Lancet*. 2008;371:2173-2182.

31. Matsushita K, Ballew SH, Astor BC, Jong PE, Gansevoort RT, Hemmelgarn BR, Levey AS, Levin A, Wen CP, Woodward M and Coresh J. Cohort profile: the chronic kidney disease prognosis consortium. *International journal of epidemiology*. 2013;42:1660-8.
32. van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey AS, de Jong PE, Gansevoort RT and the Chronic Kidney Disease Prognosis C. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. *Kidney International*. 2011;79:1341-1352.
33. Thompson S, James M, Wiebe N, Hemmelgarn B, Manns B, Klarenbach S and Tonelli M. Cause of Death in Patients with Reduced Kidney Function. *Journal of the American Society of Nephrology*. 2015;26:2504-2511.
34. Wanner C, Amann K and Shoji T. The heart and vascular system in dialysis. *The Lancet*. 2016;388:276-284.
35. Saran R, Robinson B, Abbott KC, Agodoa LYC, Bragg-Gresham J, Balkrishnan R, Bhave N, Dietrich X, Ding Z, Eggers PW, Gaipov A, Gillen D, Gipson D, Gu H, Guro P, Haggerty D, Han Y, He K, Herman W, Heung M, Hirth RA, Hsiung JT, Hutton D, Inoue A, Jacobsen SJ, Jin Y, Kalantar-Zadeh K, Kapke A, Kleine CE, Kovesdy CP, Krueter W, Kurtz V, Li Y, Liu S, Marroquin MV, McCullough K, Molnar MZ, Modi Z, Montez-Rath M, Moradi H, Morgenstern H, Mukhopadhyay P, Nallamothu B, Nguyen DV, Norris KC, O'Hare AM, Obi Y, Park C, Pearson J, Pisoni R, Potukuchi PK, Repeck K, Rhee CM, Schaubel DE, Schrager J, Selewski DT, Shamraj R, Shaw SF, Shi JM, Shieu M, Sim JJ, Soohoo M, Steffick D, Streja E, Sumida K, Kurella Tamura M, Tilea A, Turf M, Wang D, Weng W, Woodside KJ, Wyncott A, Xiang J, Xin X, Yin M, You AS, Zhang X, Zhou H and Shahinian V. US Renal Data System 2018 Annual Data Report: Epidemiology of Kidney Disease in the United States. *Am J Kidney Dis*. 2019;73:A7-a8.
36. Foley RN, Curtis BM, Randell EW and Parfrey PS. Left Ventricular Hypertrophy in New Hemodialysis Patients without Symptomatic Cardiac Disease. *Clinical Journal of the American Society of Nephrology*. 2010;5:805-813.
37. Coresh J, Wei G, McQuillan G and et al. Prevalence of high blood pressure and elevated serum creatinine level in the united states: Findings from the third national health and nutrition examination survey (1988-1994). *Archives of Internal Medicine*. 2001;161:1207-1216.
38. Alicic RZ, Rooney MT and Tuttle KR. Diabetic Kidney Disease. *Challenges, Progress, and Possibilities*. 2017;12:2032-2045.
39. Collins AJ, Foley RN, Gilbertson DT and Chen S-C. United States Renal Data System public health surveillance of chronic kidney disease and end-stage renal disease. *Kidney International Supplements*. 2015;5:2-7.
40. Weiner DE, Tighiouart H, Elsayed EF, Griffith JL, Salem DN, Levey AS and Sarnak MJ. The Framingham Predictive Instrument in Chronic Kidney Disease. *Journal of the American College of Cardiology*. 2007;50:217-224.
41. Mahmoodi BK, Matsushita K, Woodward M, Blankestijn PJ, Cirillo M, Ohkubo T, Rossing P, Sarnak MJ, Stengel B, Yamagishi K, Yamashita K, Zhang L, Coresh J, de Jong PE and Astor BC. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without hypertension: a meta-analysis. *The Lancet*. 2012;380:1649-1661.
42. Fox CS, Matsushita K, Woodward M, Bilo HJG, Chalmers J, Heerspink HJL, Lee BJ, Perkins RM, Rossing P, Sairenchi T, Tonelli M, Vassalotti JA, Yamagishi K, Coresh J, de Jong PE, Wen C-P and Nelson RG. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. *The Lancet*. 2012;380:1662-1673.

43. Matsushita K, Ballew SH and Coresh J. Cardiovascular risk prediction in people with chronic kidney disease. *Current opinion in nephrology and hypertension*. 2016;25:518-523.
44. Matsushita K, Coresh J, Sang Y, Chalmers J, Fox C, Guallar E, Jafar T, Jassal SK, Landman GWD, Muntner P, Roderick P, Sairenchi T, Schöttker B, Shankar A, Shlipak M, Tonelli M, Townend J, van Zuilen A, Yamagishi K, Yamashita K, Gansevoort R, Sarnak M, Warnock DG, Woodward M and Ärnlöv J. Estimated glomerular filtration rate and albuminuria for prediction of cardiovascular outcomes: a collaborative meta-analysis of individual participant data. *The Lancet Diabetes & Endocrinology*. 2015;3:514-525.
45. Turakhia MP, Blankestijn PJ, Carrero J-J, Clase CM, Deo R, Herzog CA, Kasner SE, Passman RS, Pecoits-Filho R, Reinecke H, Shroff GR, Zareba W, Cheung M, Wheeler DC, Winkelmayer WC, Wanner C and Participants C. Chronic kidney disease and arrhythmias: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *European Heart Journal*. 2018;39:2314-2325.
46. Goldenberg I, Moss AJ, McNitt S, Zareba W, Andrews ML, Hall WJ, Greenberg H and Case RB. Relations among renal function, risk of sudden cardiac death, and benefit of the implanted cardiac defibrillator in patients with ischemic left ventricular dysfunction. *Am J Cardiol*. 2006;98:485-90.
47. Wheeler David C, London Gerard M, Parfrey Patrick S, Block Geoffrey A, Correa-Rotter R, Dehmel B, Drüeke Tilman B, Floege J, Kubo Y, Mahaffey Kenneth W, Goodman William G, Moe Sharon M, Trotman ML, Abdalla S, Chertow Glenn M, Herzog Charles A. Effects of Cinacalcet on Atherosclerotic and Nonatherosclerotic Cardiovascular Events in Patients Receiving Hemodialysis: The EValuation Of Cinacalcet HCl Therapy to Lower CardioVascular Events (EVOLVE) Trial. *Journal of the American Heart Association*. 3:e001363.
48. Sarnak MJ, Amann K, Bangalore S, Cavalcante JL, Charytan DM, Craig JC, Gill JS, Hlatky MA, Jardine AG, Landmesser U, Newby LK, Herzog CA, Cheung M, Wheeler DC, Winkelmayer WC and Marwick TH. Chronic Kidney Disease and Coronary Artery Disease. *Journal of the American College of Cardiology*. 2019;74:1823-1838.
49. Lusis AJ. Atherosclerosis. *Nature*. 2000;407:233-241.
50. London GM, Marchais SJ, Guerin AP, Metivier F and Adda H. Arterial structure and function in end-stage renal disease. *Nephrology Dialysis Transplantation*. 2002;17:1713-1724.
51. Altman R. Risk factors in coronary atherosclerosis athero-inflammation: the meeting point. *Thromb J*. 2003;1:4-4.
52. Tuzcu EM, Kapadia SR, Tutar E, Ziada KM, Hobbs RE, McCarthy PM, Young JB and Nissen SE. High Prevalence of Coronary Atherosclerosis in Asymptomatic Teenagers and Young Adults. *Circulation*. 2001;103:2705-2710.
53. Lindner A, Charra B, Sherrard DJ and Scribner BH. Accelerated Atherosclerosis in Prolonged Maintenance Hemodialysis. *New England Journal of Medicine*. 1974;290:697-701.
54. Manjunath G, Tighiouart H, Ibrahim H, MacLeod B, Salem DN, Griffith JL, Coresh J, Levey AS and Sarnak MJ. Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community. *Journal of the American College of Cardiology*. 2003;41:47-55.
55. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ and Wilson PW. Kidney Disease as a Risk Factor for Development of Cardiovascular Disease. *Hypertension*. 2003;42:1050-1065.
56. Craven TE, Ryu JE, Espeland MA, Kahl FR, McKinney WM, Toole JF, McMahan MR, Thompson CJ, Heiss G and Crouse JR. Evaluation of the associations between carotid

artery atherosclerosis and coronary artery stenosis. A case-control study. *Circulation*. 1990;82:1230-1242.
57. Darabian S, Hormuz M, Latif MA, Pahlevan S and Budoff MJ. The role of carotid intimal thickness testing and risk prediction in the development of coronary atherosclerosis. *Curr Atheroscler Rep*. 2013;15:306-306.
58. Szeto C-C, Chow K-M, Woo K-S, Chook P, Ching-Ha Kwan B, Leung C-B and Kam-Tao Li P. Carotid Intima Media Thickness Predicts Cardiovascular Diseases in Chinese Predialysis Patients with Chronic Kidney Disease. *Journal of the American Society of Nephrology*. 2007;18:1966-1972.
59. Nakano T, Ninomiya T, Sumiyoshi S, Fujii H, Doi Y, Hirakata H, Tsuruya K, Iida M, Kiyohara Y and Sueishi K. Association of Kidney Function With Coronary Atherosclerosis and Calcification in Autopsy Samples From Japanese Elders: The Hisayama Study. *American Journal of Kidney Diseases*.2010; 55:21-30.
60. Sosnov J, Lessard D, Goldberg RJ, Yarzebski J and Gore JM. Differential Symptoms of Acute Myocardial Infarction in Patients With Kidney Disease: A Community-Wide Perspective. *American Journal of Kidney Diseases*. 2006;47:378-384.
61. Ohtake T, Kobayashi S, Moriya H, Negishi K, Okamoto K, Maesato K and Saito S. High Prevalence of Occult Coronary Artery Stenosis in Patients with Chronic Kidney Disease at the Initiation of Renal Replacement Therapy: An Angiographic Examination. *Journal of the American Society of Nephrology*. 2005;16:1141-1148.
62. Chue CD, Townend JN, Steeds RP and Ferro CJ. Arterial stiffness in chronic kidney disease: causes and consequences. *Heart*. 2010;96:817-823.
63. Moody WE, Edwards NC, Chue CD, Ferro CJ and Townend JN. Arterial disease in chronic kidney disease. *Heart*. 2013;99:365-372.
64. Segers P, Rietzschel ER and Chirinos JA. How to Measure Arterial Stiffness in Humans. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2020;40:1034-1043.
65. London GM, Marchais SJ, Safar ME, Genest AF, Guerin AP, Metivier F, Chedid K and London AM. Aortic and large artery compliance in end-stage renal failure. *Kidney International*. 1990;37:137-142.
66. Schwarz U, Buzello M, Ritz E, Stein G, Raabe G, Wiest G, Mall G and Amann K. Morphology of coronary atherosclerotic lesions in patients with end-stage renal failure. *Nephrology Dialysis Transplantation*. 2000;15:218-223.
67. Zanoli L, Lentini P, Briet M, Castellino P, House AA, London GM, Malatino L, McCullough PA, Mikhailidis DP and Boutouyrie P. Arterial Stiffness in the Heart Disease of CKD. *Journal of the American Society of Nephrology*. 2019;30:918-928.
68. Wang J-J, O'Brien AB, Shrive NG, Parker KH and Tyberg JV. Time-domain representation of ventricular-arterial coupling as a windkessel and wave system. *American Journal of Physiology-Heart and Circulatory Physiology*. 2003;284:H1358-H1368.
69. Baksi AJ, Treibel TA, Davies JE, Hadjiloizou N, Foale RA, Parker KH, Francis DP, Mayet J and Hughes AD. A meta-analysis of the mechanism of blood pressure change with aging. *J Am Coll Cardiol*. 2009;54:2087-92.
70. London GM, Guerin AP, Marchais SJ, Pannier B, Safar ME and Day M. Cardiac and arterial interactions in end-stage renal disease. *Kidney Int*. 1996;50.
71. Saeki A, Recchia F and Kass DA. Systolic Flow Augmentation in Hearts Ejecting Into a Model of Stiff Aging Vasculature. *Circulation Research*. 1995;76:132-141.
72. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME and London GM. Impact of Aortic Stiffness on Survival in End-Stage Renal Disease. *Circulation*. 1999;99:2434-2439.
73. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, Clement DL, Coca A, de Simone G, Dominiczak A, Kahan T, Mahfoud F, Redon J, Ruilope L, Zanchetti A, Kerins M, Kjeldsen SE, Kreutz R, Laurent S, Lip GYH, McManus R,

Narkiewicz K, Ruschitzka F, Schmieder RE, Shlyakhto E, Tsioufis C, Aboyans V and Desormais I. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur Heart J*. 2018;39:3021-3104.
74. Guerin Alain P, Blacher J, Pannier B, Marchais Sylvain J, Safar Michel E and London Gérard M. Impact of Aortic Stiffness Attenuation on Survival of Patients in End-Stage Renal Failure. *Circulation*. 2001;103:987-992.
75. Lioufas N, Hawley CM, Cameron JD and Toussaint ND. Chronic Kidney Disease and Pulse Wave Velocity: A Narrative Review. *Int J Hypertens*.2019:9189362-9189362.
76. Vlachopoulos C, Aznaouridis K and Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol*. 2010;55:1318-27.
77. Wang M, Tsai W, Chen J and Huang J. Stepwise increase in arterial stiffness corresponding with the stages of chronic kidney disease. *Am J Kidney Dis*. 2005;45.
78. Edwards NC, Ferro CJ, Townend JN and Steeds RP. Aortic distensibility and arterial-ventricular coupling in early chronic kidney disease: a pattern resembling heart failure with preserved ejection fraction. *Heart*. 2008;94:1038-1043.
79. Baumann M, Wassertheurer S, Suttmann Y, Burkhardt K and Heemann U. Aortic pulse wave velocity predicts mortality in chronic kidney disease stages 2-4. *J Hypertens*. 2014;32.
80. Bailey GL, Hampers CL and Merrill JP. Reversible cardiomyopathy in uremia. *ASAIO Journal*. 1967;13:263-270.
81. Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC and Barre PE. The prognostic importance of left ventricular geometry in uremic cardiomyopathy. *J Am Soc Nephrol*. 1995;5:2024-2031.
82. Nik-Akhtar B, Khonsari H, Hesabi A and Khakpour M. Uremic Cardiomyopathy in Hemodialysis Patients. *Angiology*. 1978;29:758-763.
83. Hung J, Harris PJ, Uren RF, Tiller DJ and Kelly DT. Uremic Cardiomyopathy — Effect of Hemodialysis on Left Ventricular Function in End-Stage Renal Failure. *New England Journal of Medicine*. 1980;302:547-551.
84. de Albuquerque Suassuna PG, Sanders-Pinheiro H and de Paula RB. Uremic Cardiomyopathy: A New Piece in the Chronic Kidney Disease-Mineral and Bone Disorder Puzzle. *Frontiers in Medicine*. 2018;5:206.
85. Drüeke T, Le Pailleur C, Zingraff J and Jungers P. Uremic cardiomyopathy and pericarditis. *Advances in nephrology from the Necker Hospital*. 1980;9:33-70.
86. Ianhez LE, Lowen J and Sabbaga E. Uremic Myocardiopathy. *Nephron*. 1975;15:17-28.
87. Foley RN, Parfrey PS, Harnett JD, Kent GM, Martin CJ and Murray DC. Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. *Kidney Int*. 1995;47:186-192.
88. Stack AG and Saran R. Clinical correlates and mortality impact of left ventricular hypertrophy among new ESRD patients in the United States. *American Journal of Kidney Diseases*. 2002;40:1202-1210.
89. Zoccali C, Benedetto FA, Mallamaci F, Tripepi G, Giacone G, Stancanelli B, Cataliotti A and Malatino LS. Left ventricular mass monitoring in the follow-up of dialysis patients: Prognostic value of left ventricular hypertrophy progression. *Kidney International*. 2004;65:1492-1498.
90. Stewart GA, Foster J, Cowan M, Rooney E, McDonagh T and Dargie HJ. Echocardiography overestimates left ventricular mass in hemodialysis patients relative to magnetic resonance imaging. *Kidney Int*. 1999;56:2248-2253.

91. Mark PB, Johnston N, Groenning BA, Foster JE, Blyth KG, Martin TN, Steedman T, Dargie HJ and Jardine AG. Redefinition of uremic cardiomyopathy by contrast-enhanced cardiac magnetic resonance imaging. *Kidney Int*. 2006;69:1839-1845.
92. Amann K, Rychlik I, Miltenberger-Milteny G and Ritz E. Left ventricular hypertrophy in renal failure. *Kidney Int*. 1998;54:S78-S85.
93. Maron BJ and Pelliccia A. The Heart of Trained Athletes. *Circulation*. 2006;114:1633-1644.
94. Minamino-Muta E, Kato T, Morimoto T, Taniguchi T, Inoko M, Haruna T, Izumi T, Miyamoto S, Nakane E, Sasaki K, Funasako M, Ueyama K, Shirai S, Kitai T, Izumi C, Nagao K, Inada T, Tada E, Komasa A, Ishii K, Saito N, Sakata R, Minatoya K and Kimura T. Impact of the left ventricular mass index on the outcomes of severe aortic stenosis. *Heart*. 2017;103:1992-1999.
95. Mostovaya IM, Bots ML, van den Dorpel MA, Goldschmeding R, den Hoedt CH, Kamp O, Levesque R, Mazairac AHA, Penne EL, Swinkels DW, van der Weerd NC, ter Wee PM, Nubé MJ, Blankestijn PJ and Grooteman MPC. Left Ventricular Mass in Dialysis Patients, Determinants and Relation with Outcome. Results from the COnvective TRansport STudy (CONTRAST). *PLOS ONE*. 2014;9:e84587.
96. Stansfield WE, Ranek M, Pendse A, Schisler JC, Wang S, Pulinilkunnil T and Willis MS. Chapter 4 - The Pathophysiology of Cardiac Hypertrophy and Heart Failure. In: M. S. Willis, J. W. Homeister and J. R. Stone, eds. *Cellular and Molecular Pathobiology of Cardiovascular Disease* San Diego: Academic Press; 2014: 51-78.
97. Bouzas-Mosquera A, Broullón FJ, Álvarez-García N, Peteiro J, Mosquera VX and Castro-Beiras A. Association of left ventricular mass with all-cause mortality, myocardial infarction and stroke. *PloS one*. 2012;7:e45570-e45570.
98. Levy D, Garrison RJ, Savage DD, Kannel WB and Castelli WP. Prognostic Implications of Echocardiographically Determined Left Ventricular Mass in the Framingham Heart Study. *New England Journal of Medicine*. 1990;322:1561-1566.
99. Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Gattobigio R, Zampi I, Reboldi G and Porcellati C. Prognostic Significance of Serial Changes in Left Ventricular Mass in Essential Hypertension. *Circulation*. 1998;97:48-54.
100. Verdecchia P, Angeli F, Borgioni C, Gattobigio R, de Simone G, Devereux RB and Porcellati C. Changes in cardiovascular risk by reduction of left ventricular mass in hypertension: a meta-analysis. *American Journal of Hypertension*. 2003;16:895-899.
101. Paoletti E, Bellino D, Signori A, Pieracci L, Marsano L, Russo R, Massarino F, Ravera M, Fontana I, Carta A, Cassottana P and Garibotto G. Regression of asymptomatic cardiomyopathy and clinical outcome of renal transplant recipients: a long-term prospective cohort study. *Nephrology Dialysis Transplantation*. 2015;31:1168-1174.
102. Odudu A, Eldehni MT, McCann GP and McIntyre CW. Randomized Controlled Trial of Individualized Dialysate Cooling for Cardiac Protection in Hemodialysis Patients. *Clinical Journal of the American Society of Nephrology*. 2015;10:1408-1417.
103. Edwards NC, Steeds RP, Stewart PM, Ferro CJ and Townend JN. Effect of Spironolactone on Left Ventricular Mass and Aortic Stiffness in Early-Stage Chronic Kidney Disease: A Randomized Controlled Trial. *Journal of the American College of Cardiology*. 2009;54:505-512.
104. Aoki J, Ikari Y, Nakajima H, Mori M, Sugimoto T, Hatori M, Tanimoto S, Amiya E and Hara K. Clinical and pathologic characteristics of dilated cardiomyopathy in hemodialysis patients. *Kidney International*. 2005;67:333-340.
105. Mall G, Huther W, Schneider J, Lundin P and Ritz E. Diffuse Intermyocardiocytic Fibrosis in Uraemic Patients. *Nephrology Dialysis Transplantation*. 1990;5:39-44.

106. Hinderer S and Schenke-Layland K. Cardiac fibrosis – A short review of causes and therapeutic strategies. *Advanced Drug Delivery Reviews*. 2019;146:77-82.
107. Frantz C, Stewart KM and Weaver VM. The extracellular matrix at a glance. *Journal of Cell Science*. 2010;123:4195-4200.
108. Kong P, Christia P and Frangogiannis NG. The pathogenesis of cardiac fibrosis. *Cellular and Molecular Life Sciences*. 2014;71:549-574.
109. Murtha LA, Schuliga MJ, Mabotuwana NS, Hardy SA, Waters DW, Burgess JK, Knight DA and Boyle AJ. The Processes and Mechanisms of Cardiac and Pulmonary Fibrosis. *Frontiers in Physiology*. 2017;8:777.
110. Jellis C, Martin J, Narula J and Marwick TH. Assessment of Nonischemic Myocardial Fibrosis. *Journal of the American College of Cardiology.* 2010;56:89-97.
111. Mall G, Rambausek M, Neumeister A, Kollmar S, Vetterlein F and Ritz E. Myocardial interstitial fibrosis in experimental uremia-implications for cardiac compliance. *Kidney International*. 1988;33:804-811.
112. Amann K, Breitbach M, Ritz E and Mall G. Myocyte/capillary mismatch in the heart of uremic patients. *Journal of the American Society of Nephrology*. 1998;9:1018-1022.
113. Shroff GR and Raggi P. Exploring the elusive link between subclinical fibrosis and clinical events in end-stage renal disease: does cardiac magnetic resonance imaging hold the key? *Kidney International*. 2016;90:729-732.
114. Taylor AJ, Salerno M, Dharmakumar R and Jerosch-Herold M. T1 Mapping: Basic Techniques and Clinical Applications. *JACC: Cardiovascular Imaging*. 2016;9:67-81.
115. Haaf P, Garg P, Messroghli DR, Broadbent DA, Greenwood JP and Plein S. Cardiac T1 Mapping and Extracellular Volume (ECV) in clinical practice: a comprehensive review. *Journal of Cardiovascular Magnetic Resonance*. 2016;18:89.
116. Carr DH, Brown J, Bydder GM, Steiner RE, Weinmann HJ, Speck U, Hall AS and Young IR. Gadolinium-DTPA as a contrast agent in MRI: initial clinical experience in 20 patients. *American Journal of Roentgenology*. 1984;143:215-224.
117. Doltra A, Amundsen BH, Gebker R, Fleck E and Kelle S. Emerging concepts for myocardial late gadolinium enhancement MRI. *Current cardiology reviews*. 2013;9:185-190.
118. Matsumoto H, Matsuda T, Miyamoto K, Shimada T, Mikuri M and Hiraoka Y. Peri-Infarct Zone on Early Contrast-Enhanced CMR Imaging in Patients With Acute Myocardial Infarction. *JACC: Cardiovascular Imaging*. 2011;4:610-618.
119. Kuruvilla S, Adenaw N, Katwal AB, Lipinski MJ, Kramer CM and Salerno M. Late Gadolinium Enhancement on CMR Predicts Adverse Cardiovascular Outcomes in Non-ischemic Cardiomyopathy: A Systematic Review and Meta-analysis. *Circulation: Cardiovascular Imaging*. 2014:7:250-258.
120. Satoh H, Sano M, Suwa K, Saitoh T, Nobuhara M, Saotome M, Urushida T, Katoh H and Hayashi H. Distribution of late gadolinium enhancement in various types of cardiomyopathies: Significance in differential diagnosis, clinical features and prognosis. *World journal of cardiology*. 2014;6:585-601.
121. Moon JC, Reed E, Sheppard MN, Elkington AG, Ho SY, Burke M, Petrou M and Pennell DJ. The histologic basis of late gadolinium enhancement cardiovascular magnetic resonance in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;43:2260-4.
122. Grobner T. Gadolinium – a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis? *Nephrology Dialysis Transplantation*. 2006;21:1104-1108.
123. Marckmann P, Skov L, Rossen K, Dupont A, Damholt MB, Heaf JG and Thomsen HS. Nephrogenic Systemic Fibrosis: Suspected Causative Role of Gadodiamide Used for Contrast-Enhanced Magnetic Resonance Imaging. *Journal of the American Society of Nephrology*. 2006;17:2359-2362.

124. Mendoza FA, Artlett CM, Sandorfi N, Latinis K, Piera-Velazquez S and Jimenez SA. Description of 12 Cases of Nephrogenic Fibrosing Dermopathy and Review of the Literature. *Seminars in Arthritis and Rheumatism*. 2006;35:238-249.
125. Mangion K, McDowell K, Mark PB and Rutherford E. Characterizing Cardiac Involvement in Chronic Kidney Disease Using CMR-a Systematic Review. *Current cardiovascular imaging reports*. 2018;11:2-2.
126. Look DC and Locker DR. Time saving in measurement of NMR and EPR relaxation times. *Rev Sci Instrum*. 1970;41:250-251.
127. Messroghli DR, Radjenovic A, Kozerke S, Higgins DM, Sivananthan MU and Ridgway JP. Modified Look-Locker inversion recovery (MOLLI) for high-resolution T1 mapping of the heart. *Magnetic Resonance in Medicine*. 2004;52:141-146.
128. Xue H, Shah S, Greiser A, Guetter C, Littmann A, Jolly M-P, Arai AE, Zuehlsdorff S, Guehring J and Kellman P. Motion correction for myocardial T1 mapping using image registration with synthetic image estimation. *Magnetic resonance in medicine*. 2012;67:1644-1655.
129. Bull S, White S, Piechnik S, Flett A, Ferreira V, Loudon M, Francis J, Karamitsos T, Prendergast B, Robson M, Neubauer S, Moon J and Myerson S. Human non-contrast T1 values and correlation with histology in diffuse fibrosis. *Heart*. 2013;99:932-937.
130. Rutherford E, Talle MA, Mangion K, Bell E, Rauhalammi SM, Roditi G, McComb C, Radjenovic A, Welsh P, Woodward R, Struthers AD, Jardine AG, Patel RK, Berry C and Mark PB. Defining myocardial tissue abnormalities in end-stage renal failure with cardiac magnetic resonance imaging using native T1 mapping. *Kidney International*. 2016;90:845-852.
131. Cui Y, Cao Y, Song J, Dong N, Kong X, Wang J, Yuan Y, Zhu X, Yan X, Greiser A, Shi H and Han P. Association between myocardial extracellular volume and strain analysis through cardiovascular magnetic resonance with histological myocardial fibrosis in patients awaiting heart transplantation. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2018;20:25-25.
132. Treibel TA, López B, González A, Menacho K, Schofield RS, Ravassa S, Fontana M, White SK, DiSalvo C, Roberts N, Ashworth MT, Díez J and Moon JC. Reappraising myocardial fibrosis in severe aortic stenosis: an invasive and non-invasive study in 133 patients. *European Heart Journal*. 2017;39:699-709.
133. Kim PK, Hong YJ, Im DJ, Suh YJ, Park CH, Kim JY, Chang S, Lee H-J, Hur J, Kim YJ and Choi BW. Myocardial T1 and T2 Mapping: Techniques and Clinical Applications. *Korean journal of radiology*. 2017;18:113-131.
134. Arcari L, Hinojar R, Engel J, Freiwald T, Platschek S, Zainal H, Zhou H, Vasquez M, Keller T, Rolf A, Geiger H, Hauser I, Vogl TJ, Zeiher AM, Volpe M, Nagel E and Puntmann VO. Native T1 and T2 provide distinctive signatures in hypertrophic cardiac conditions – Comparison of uremic, hypertensive and hypertrophic cardiomyopathy. *International Journal of Cardiology*. 2020;306:102-108.
135. Graham-Brown MPM, March DS, Churchward DR, Stensel DJ, Singh A, Arnold R, Burton JO and McCann GP. Novel cardiac nuclear magnetic resonance method for noninvasive assessment of myocardial fibrosis in hemodialysis patients. *Kidney International*.2016; 90:835-844.
136. Hayer M, Marie Price A, Liu B, Baig S, Ferro C, Nicholas Townend J, Paul Steeds R and Catherine Edwards N. *Diffuse Myocardial Interstitial Fibrosis and Dysfunction in Early Chronic Kidney Disease*; 2018;121:656-660.
137. Graham-Brown MPM, Rutherford E, Levelt E, March DS, Churchward DR, Stensel DJ, McComb C, Mangion K, Cockburn S, Berry C, Moon JC, Mark PB, Burton JO and

McCann GP. Native T1 mapping: inter-study, inter-observer and inter-center reproducibility in hemodialysis patients. *Journal of Cardiovascular Magnetic Resonance*. 2017;19:21.
138. Graham-Brown M, S. Singh A, S. Gulsin G, Levelt E, A. Arnold J, J. Stensel D, Burton J and P. McCann G. *Defining myocardial fibrosis in haemodialysis patients with non-contrast cardiac magnetic resonance*; 2018;145.
139. Krishnasamy R, Isbel NM, Hawley CM, Pascoe EM, Burrage M, Leano R, Haluska BA, Marwick TH and Stanton T. Left Ventricular Global Longitudinal Strain (GLS) Is a Superior Predictor of All-Cause and Cardiovascular Mortality When Compared to Ejection Fraction in Advanced Chronic Kidney Disease. *PLoS ONE*. 2015;10:e0127044.
140. Bansal N, Keane M, Delafontaine P, Dries D, Foster E, Gadegbeku CA, Go AS, Hamm LL, Kusek JW, Ojo AO, Rahman M, Tao K, Wright JT, Xie D and Hsu, Chi-yuan. A Longitudinal Study of Left Ventricular Function and Structure from CKD to ESRD: The CRIC Study. *Clinical Journal of the American Society of Nephrology*. 2013;8:355-362.
141. Farshid A, Pathak R, Shadbolt B, Arnolda L and Talaulikar G. Diastolic function is a strong predictor of mortality in patients with chronic kidney disease. *BMC Nephrology*. 2013;14:280.
142. Otsuka T, Suzuki M, Yoshikawa H and Sugi K. Left ventricular diastolic dysfunction in the early stage of chronic kidney disease. *Journal of Cardiology*. 2009;54:199-204.
143. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola V-P, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P and Group ESD. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *European Heart Journal*. 2016;37:2129-2200.
144. House AA, Wanner C, Sarnak MJ, Piña IL, McIntyre CW, Komenda P, Kasiske BL, Deswal A, deFilippi CR, Cleland JGF, Anker SD, Herzog CA, Cheung M, Wheeler DC, Winkelmayer WC and McCullough PA. Heart failure in chronic kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int*. 2019;95:1304-1317.
145. Park M, Hsu C-y, Li Y, Mishra RK, Keane M, Rosas SE, Dries D, Xie D, Chen J, He J, Anderson A, Go AS, Shlipak MG and Chronic Renal Insufficiency Cohort Study G. Associations between Kidney Function and Subclinical Cardiac Abnormalities in CKD. *Journal of the American Society of Nephrology : JASN*. 2012;23:1725-1734.
146. Bansal N, Lin F, Vittinghoff E, Peralta C, Lima J, Kramer H, Shlipak M and Bibbins-Domingo K. Estimated GFR and Subsequent Higher Left Ventricular Mass in Young and Middle-Aged Adults With Normal Kidney Function: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *American Journal of Kidney Diseases*. 2016;67:227-234.
147. Dandamudi S SJ, Hodge DO, Glockner JF and Chen HH. The Prognostic Significance of Late-Gadolinium Enhancement Identified by Cardiac MRI in Association with Renal Function. *Journal of Molecular Biology and Molecular Imaging*. 2015;2:1021.

148. Edwards NC, Moody WE, Yuan M, Hayer MK, Ferro CJ, Townend JN and Steeds RP. Diffuse interstitial fibrosis and myocardial dysfunction in early chronic kidney disease. *Am J Cardiol*. 2015;115:1311-1317.
149. Weinreb JC, Rodby RA, Yee J, Wang CL, Fine D, McDonald RJ, Perazella MA, Dillman JR and Davenport MS. Use of Intravenous Gadolinium-based Contrast Media in Patients with Kidney Disease: Consensus Statements from the American College of Radiology and the National Kidney Foundation. *Radiology*. 2021;298:28-35.

150. Panoulas VF, Sulemane S, Konstantinou K, Bratsas A, Elliott SJ and Dawson D. Early detection of subclinical left ventricular myocardial dysfunction in patients with chronic kidney disease. *Eur Heart J Cardiovasc Imaging*. 2015;16:539-548.
151. Edwards NC, Hirth A, Ferro CJ, Townend JN and Steeds RP. Subclinical Abnormalities of Left Ventricular Myocardial Deformation in Early-Stage Chronic Kidney Disease: The Precursor of Uremic Cardiomyopathy? *Journal of the American Society of Echocardiography*. 2008;21:1293-1298.
152. Sulemane S, Panoulas VF, Konstantinou K, Bratsas A, Tam FW, Brown EA and Nihoyannopoulos P. Left ventricular twist mechanics and its relation with aortic stiffness in chronic kidney disease patients without overt cardiovascular disease. *Cardiovascular Ultrasound*. 2016;14:10.
153. Wang X and Shapiro JI. Evolving concepts in the pathogenesis of uraemic cardiomyopathy. *Nature Reviews Nephrology*. 2019;15:159-175.
154. Levin A. Anemia and left ventricular hypertrophy in chronic kidney disease populations: A review of the current state of knowledge. *Kidney International*. 2002;61:S35-S38.
155. Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC and Barre PE. The impact of anemia on cardiomyopathy, morbidity, and and mortality in end-stage renal disease. *Am J Kidney Dis*. 1996;28:53-61.
156. Portolés J, Torralbo A, Martin P, Rodrigo J, Herrero JA and Barrientos A. Cardiovascular effects of recombinant human erythropoietin in predialysis patients. *Am J Kidney Dis*. 1997;29:541-8.
157. Hayashi T, Suzuki A, Shoji T, Togawa M, Okada N, Tsubakihara Y, Imai E and Hori M. Cardiovascular effect of normalizing the hematocrit level during erythropoietin therapy in predialysis patients with chronic renal failure. *Am J Kidney Dis*. 2000;35:250-6.
158. Macdougall IC, White C, Anker SD, Bhandari S, Farrington K, Kalra PA, McMurray JJV, Murray H, Tomson CRV, Wheeler DC, Winearls CG and Ford I. Intravenous Iron in Patients Undergoing Maintenance Hemodialysis. *New England Journal of Medicine*. 2018;380:447-458.
159. Viegas C, Araújo N, Marreiros C and Simes D. The interplay between mineral metabolism, vascular calcification and inflammation in Chronic Kidney Disease (CKD): challenging old concepts with new facts. *Aging (Albany NY)*. 2019;11:4274-4299.
160. Ruospo M, Palmer SC, Natale P, Craig JC, Vecchio M, Elder GJ and Strippoli GF. Phosphate binders for preventing and treating chronic kidney disease-mineral and bone disorder (CKD-MBD). *Cochrane Database Syst Rev*. 2018;8:CD006023-CD006023.
161. Effect of Cinacalcet on Cardiovascular Disease in Patients Undergoing Dialysis. *New England Journal of Medicine*. 2012;367:2482-2494.
162. Lu RJ, Zhu SM, Tang FL, Zhu XS, Fan ZD, Wang GL, Jiang YF and Zhang Y. Effects of vitamin D or its analogues on the mortality of patients with chronic kidney disease: an updated systematic review and meta-analysis. *Eur J Clin Nutr*. 2017;71:683-693.
163. Jean G, Bresson E, Terrat J-C, Vanel T, Hurot J-M, Lorriaux C, Mayor B and Chazot C. Peripheral vascular calcification in long-haemodialysis patients: associated factors and survival consequences. *Nephrology Dialysis Transplantation*. 2008;24:948-955.
164. Seiler S, Rogacev KS, Roth HJ, Shafein P, Emrich I, Neuhaus S, Floege J, Fliser D and Heine GH. Associations of FGF-23 and sKlotho with Cardiovascular Outcomes among Patients with CKD Stages 2–4. *Clinical Journal of the American Society of Nephrology*. 2014;9:1049-1058.
165. Faul C, Amaral AP, Oskouei B, Hu M-C, Sloan A, Isakova T, Gutiérrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, Feldman HI, St. John Sutton M, Ojo

A, Gadegbeku C, Di Marco GS, Reuter S, Kentrup D, Tiemann K, Brand M, Hill JA, Moe OW, Kuro-o M, Kusek JW, Keane MG and Wolf M. FGF23 induces left ventricular hypertrophy. *The Journal of Clinical Investigation*. 2011;121:4393-4408.
166. Hu MC, Shi M, Cho HJ, Adams-Huet B, Paek J, Hill K, Shelton J, Amaral AP, Faul C, Taniguchi M, Wolf M, Brand M, Takahashi M, Kuro-o M, Hill JA and Moe OW. Klotho and Phosphate Are Modulators of Pathologic Uremic Cardiac Remodeling. *Journal of the American Society of Nephrology : JASN*. 2015;26:1290-1302.
167. Xie J, Yoon J, An S-W, Kuro-o M and Huang C-L. Soluble Klotho Protects against Uremic Cardiomyopathy Independently of Fibroblast Growth Factor 23 and Phosphate. *Journal of the American Society of Nephrology*. 2015;26:1150-1160.
168. Johnson RJ, Nakagawa T, Jalal D, Sánchez-Lozada LG, Kang D-H and Ritz E. Uric acid and chronic kidney disease: which is chasing which? *Nephrology Dialysis Transplantation*. 2013;28:2221-2228.
169. Bonino B, Leoncini G, Russo E, Pontremoli R and Viazzi F. Uric acid in CKD: has the jury come to the verdict? *Journal of Nephrology*. 2020;33:715-724.
170. Luo Q, Xia X, Li B, Lin Z, Yu X and Huang F. Serum uric acid and cardiovascular mortality in chronic kidney disease: a meta-analysis. *BMC Nephrology*. 2019;20:18.
171. Kao MP, Ang DS, Gandy SJ, Nadir MA, Houston JG, Lang CC and Struthers AD. Allopurinol Benefits Left Ventricular Mass and Endothelial Dysfunction in Chronic Kidney Disease. *Journal of the American Society of Nephrology*. 2011;22:1382-1389.
172. Struthers AD. Aldosterone: Cardiovascular assault. *American Heart Journal*. 2002;144:S2-S7.
173. Orsborne C, Chaggar PS, Shaw SM and Williams SG. The renin-angiotensin-aldosterone system in heart failure for the non-specialist: the past, the present and the future. *Postgraduate Medical Journal*. 2017;93:29-37.
174. Bomback AS and Klemmer PJ. The incidence and implications of aldosterone breakthrough. *Nature Clinical Practice Nephrology*. 2007;3:486-492.
175. Hostetter TH, Ibrahim HN and Editor F. Aldosterone in Chronic Kidney and Cardiac Disease. *Journal of the American Society of Nephrology*. 2003;14:2395-2401.
176. Jurk D, Wilson C, Passos JF, Oakley F, Correia-Melo C, Greaves L, Saretzki G, Fox C, Lawless C, Anderson R, Hewitt G, Pender SLF, Fullard N, Nelson G, Mann J, van de Sluis B, Mann DA and von Zglinicki T. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. *Nature Communications*. 2014;5:4172.
177. Barnes PJ. Mechanisms of development of multimorbidity in the elderly. *European Respiratory Journal*. 2015;45:790-806.
178. Stenvinkel P and Larsson TE. Chronic Kidney Disease: A Clinical Model of Premature Aging. *American Journal of Kidney Diseases*. 2013;62:339-351.
179. Stenvinkel P, Heimbürger O, Paultre F, Diczfalusy U, Wang T, Berglund L and Jogestrand T. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int*. 1999;55:1899-911.
180. Zimmermann J, Herrlinger S, Pruy A, Metzger T and Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int*. 1999;55:648-58.
181. Carracedo J, Alique M, Vida C, Bodega G, Ceprián N, Morales E, Praga M, de Sequera P and Ramírez R. Mechanisms of Cardiovascular Disorders in Patients With Chronic Kidney Disease: A Process Related to Accelerated Senescence. *Frontiers in Cell and Developmental Biology*. 2020;8:185.
182. Cohen SD, Phillips TM, Khetpal P and Kimmel PL. Cytokine patterns and survival in haemodialysis patients. *Nephrol Dial Transplant*. 2010;25:1239-43.

183. Roger SD, McMahon LP, Clarkson A, Disney A, Harris D, Hawley C, Healy H, Kerr P, Lynn K, Parnham A, Pascoe R, Voss D, Walker R and Levin A. Effects of Early and Late Intervention with Epoetin α on Left Ventricular Mass among Patients with Chronic Kidney Disease (Stage 3 or 4): Results of a Randomized Clinical Trial. *Journal of the American Society of Nephrology*. 2004;15:148-156.
184. Levin A, Djurdjev O, Thompson C, Barrett B, Ethier J, Carlisle E, Barre P, Magner P, Muirhead N, Tobe S, Tam P, Wadgymar JA, Kappel J, Holland D, Pichette V, Shoker A, Soltys G, Verrelli M and Singer J. Canadian Randomized Trial of Hemoglobin Maintenance to Prevent or Delay Left Ventricular Mass Growth in Patients With CKD. *American Journal of Kidney Diseases*. 2005;46:799-811.
185. Parfrey PS, Foley RN, Wittreich BH, Sullivan DJ, Zagari MJ and Frei D. Double-Blind Comparison of Full and Partial Anemia Correction in Incident Hemodialysis Patients without Symptomatic Heart Disease. *Journal of the American Society of Nephrology*. 2005;16:2180-2189.
186. Drüeke TB, Locatelli F, Clyne N, Eckardt K-U, Macdougall IC, Tsakiris D, Burger H-U and Scherhag A. Normalization of Hemoglobin Level in Patients with Chronic Kidney Disease and Anemia. *New England Journal of Medicine*. 2006;355:2071-2084.
187. Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M and Reddan D. Correction of Anemia with Epoetin Alfa in Chronic Kidney Disease. *New England Journal of Medicine*. 2006;355:2085-2098.
188. Pfeffer MA, Burdmann EA, Chen C-Y, Cooper ME, de Zeeuw D, Eckardt K-U, Feyzi JM, Ivanovich P, Kewalramani R, Levey AS, Lewis EF, McGill JB, McMurray JJV, Parfrey P, Parving H-H, Remuzzi G, Singh AK, Solomon SD and Toto R. A Trial of Darbepoetin Alfa in Type 2 Diabetes and Chronic Kidney Disease. *New England Journal of Medicine*. 2009;361:2019-2032.
189. Suki WN, Zabaneh R, Cangiano JL, Reed J, Fischer D, Garrett L, Ling BN, Chasan-Taber S, Dillon MA, Blair AT and Burke SK. Effects of sevelamer and calcium-based phosphate binders on mortality in hemodialysis patients. *Kidney Int*. 2007;72:1130-7.
190. Raggi P, Chertow GM, Torres PU, Csiky B, Naso A, Nossuli K, Moustafa M, Goodman WG, Lopez N, Downey G, Dehmel B and Floege J. The ADVANCE study: a randomized study to evaluate the effects of cinacalcet plus low-dose vitamin D on vascular calcification in patients on hemodialysis. *Nephrol Dial Transplant*. 2011;26:1327-39.
191. Thadhani R, Appelbaum E, Pritchett Y, Chang Y, Wenger J, Tamez H, Bhan I, Agarwal R, Zoccali C, Wanner C, Lloyd-Jones D, Cannata J, Thompson BT, Andress D, Zhang W, Packham D, Singh B, Zehnder D, Shah A, Pachika A, Manning WJ and Solomon SD. Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease: the PRIMO randomized controlled trial. *Jama*. 2012;307:674-84.
192. Chue CD, Townend JN, Moody WE, Zehnder D, Wall NA, Harper L, Edwards NC, Steeds RP and Ferro CJ. Cardiovascular Effects of Sevelamer in Stage 3 CKD. *Journal of the American Society of Nephrology : JASN*. 2013;24:842-852.
193. Wang AY-M, Fang F, Chan J, Wen Y-Y, Qing S, Chan IH-S, Lo G, Lai K-N, Lo W-K, Lam CW-K and Yu C-M. Effect of Paricalcitol on Left Ventricular Mass and Function in CKD—The OPERA Trial. *Journal of the American Society of Nephrology*. 2014;25:175-186.
194. Banerjee D, Chitalia N, Ster IC, Appelbaum E, Thadhani R, Kaski JC and Goldsmith D. Impact of Vitamin D on Cardiac structure and function in CKD patients with hypovitaminosis D, a randomised controlled trial and meta-analysis. *Eur Heart J Cardiovasc Pharmacother*. 2019:pvz080.
195. Vukusich A, Kunstmann S, Varela C, Gainza D, Bravo S, Sepulveda D, Cavada G, Michea L and Marusic ET. A randomized, double-blind, placebo-controlled trial of

spironolactone on carotid intima-media thickness in nondiabetic hemodialysis patients. *Clinical journal of the American Society of Nephrology : CJASN*. 2010;5:1380-1387.
196. Ito Y, Mizuno M, Suzuki Y, Tamai H, Hiramatsu T, Ohashi H, Ito I, Kasuga H, Horie M, Maruyama S, Yuzawa Y, Matsubara T and Matsuo S. Long-Term Effects of Spironolactone in Peritoneal Dialysis Patients. *Journal of the American Society of Nephrology*. 2014;25:1094-1102.
197. Matsumoto Y, Mori Y, Kageyama S, Arihara K, Sugiyama T, Ohmura H, Yakushigawa T, Sugiyama H, Shimada Y, Nojima Y and Shio N. Spironolactone Reduces Cardiovascular and Cerebrovascular Morbidity and Mortality in Hemodialysis Patients. *Journal of the American College of Cardiology*. 2014;63:528-536.
198. Hammer F, Malzahn U, Donhauser J, Betz C, Schneider MP, Grupp C, Pollak N, Störk S, Wanner C and Krane V. A randomized controlled trial of the effect of spironolactone on left ventricular mass in hemodialysis patients. *Kidney Int*. 2019;95:983-991.
199. Charytan DM, Himmelfarb J, Ikizler TA, Raj DS, Hsu JY, Landis JR, Anderson AH, Hung AM, Mehrotra R, Sharma S, Weiner DE, Williams M, DiCarli M, Skali H, Kimmel PL, Kliger AS, Dember LM, Kliger A, Charytan DM, Robinson E, Williams M, Weiner DE, Aurien-Blajeni E, Cinelli MA, Nizam T, Rim S, Seok P, Smith C, Rollins J, Raj D, Regunathan-Shenk R, Sharma S, Ramezani A, Andrews S, Dumadag M, Franco C, Wing M, Himmelfarb J, Mehrotra R, Anderson L, Linke L, Manahan L, Ikizler TA, Hung A, Cavanaugh K, Booker C, Brannon B, Clagett A, Ellis C, Dember L, Landis JR, Anderson A, Hsu J, Cifelli D, Ballard S, Durborow M, Howard T, Kuzla N, Nessel L, Tierney A, Skali H, Solomon S, Rad A, Di Carli M, Gaber M, Foster C, Kimmel P and Kusek J. Safety and cardiovascular efficacy of spironolactone in dialysis-dependent ESRD (SPin-D): a randomized, placebo-controlled, multiple dosage trial. *Kidney International*. 2019;95:973-982.
200. Libório AB, Barros RM, Esmeraldo RM, Oliveira MLMB, Silva GB and Daher EF. Creatinine-Based Equations Predicting Chronic Kidney Disease After Kidney Donation. *Transplantation Proceedings*. 2011;43:2481-2486.
201. Kerr M, Bray B, Medcalf J, O'Donoghue DJ and Matthews B. Estimating the financial cost of chronic kidney disease to the NHS in England. *Nephrology Dialysis Transplantation*. 2012;27:iii73-iii80.
202. Himmelfarb J, Vanholder R, Mehrotra R and Tonelli M. The current and future landscape of dialysis. *Nature Reviews Nephrology*. 2020;16:573-585.
203. Jaar BG, Chang A and Plantinga L. Can We Improve Quality of Life of Patients on Dialysis? *Clinical Journal of the American Society of Nephrology*. 2013;8:1-4.
204. Janki S, Klop KWJ, Kimenai HJAN, van de Wetering J, Weimar W, Massey EK, Dehghan A, Rizopoulos D, Völzke H, Hofman A and Ijzermans JNM. LOng-term follow-up after liVE kidney donation (LOVE) study: a longitudinal comparison study protocol. *BMC Nephrology*. 2016;17:1-5.
205. Cozzi E, Biancone L, López-Fraga M and Nanni-Costa A. Long-term Outcome of Living Kidney Donation: Position Paper of the European Committee on Organ Transplantation, Council of Europe. *Transplantation*. 2016;100:270-271.
206. Randolph Beard T and Osterkamp R. The organ crisis: a disaster of our own making. *The European Journal of Health Economics*. 2014;15:1-5.
207. Cheetham OV, Thomas MJC, Hadfield J, O'Higgins F, Mitchell C and Rooney KD. Rates of organ donation in a UK tertiary cardiac arrest centre following out-of-hospital cardiac arrest. *Resuscitation*. 2016;101:41-43.
208. de Seigneux S, Ponte B, Berchtold L, Hadaya K, Martin P-Y and Pasch A. Living kidney donation does not adversely affect serum calcification propensity and markers of vascular stiffness. *Transplant International*. 2015;28:1074-1080.

209. Muramatsu M, Gonzalez HD, Cacciola R, Aikawa A, Yaqoob MM and Puliatti C. ABO incompatible renal transplants: Good or bad? *World Journal of Transplantation*. 2014;4:18-29.
210. Aubert O, Kamar N, Vernerey D, Viglietti D, Martinez F, Duong-Van-Huyen J-P, Eladari D, Empana J-P, Rabant M, Verine J, Rostaing L, Congy N, Guilbeau-Frugier C, Mourad G, Garrigue V, Morelon E, Giral M, Kessler M, Ladrière M, Delahousse M, Glotz D, Legendre C, Jouven X, Lefaucheur C and Loupy A. Long term outcomes of transplantation using kidneys from expanded criteria donors: prospective, population based cohort study. *BMJ : British Medical Journal*. 2015;351:h3557.
211. Bailey PK, Wong K, Robb M, Burnapp L, Rogers A, Courtney A and Wroe C. Has the UK living kidney donor population changed over time? A cross-sectional descriptive analysis of the UK living donor registry between 2006 and 2017. *BMJ Open*. 2020;10:e033906.
212. NHS Blood and Transplant [internet]. Annual Activity report. Organ Donation and Transplantation Acitvity Report 2019/2020 [cited 2021 Jul]. Available from: www.odt.nhs.uk/statistics-and-reports/annual-activity-report/.
213. Slinin Y, Brasure M, Eidman K, Bydash J, Maripuri S, Carlyle M, Ishani A and Wilt TJ. Long-term Outcomes of Living Kidney Donation. *Transplantation*. 2016;100:1371-1386.
214. Newell KA, Formica RN and Gill JS. Engaging Living Kidney Donors in a New Paradigm of Postdonation Care. *American Journal of Transplantation*. 2016;16:29-32.
215. Ommen ES, Winston JA and Murphy B. Medical Risks in Living Kidney Donors: Absence of Proof Is Not Proof of Absence. *Clinical Journal of the American Society of Nephrology*. 2006;1:885-895.
216. Segev DL, Muzaale AD, Caffo BS and et al. Perioperative mortality and long-term survival following live kidney donation. *JAMA*. 2010;303:959-966.
217. Ibrahim HN, Foley R, Tan L, Rogers T, Bailey RF, Guo H, Gross CR and Matas AJ. Long-Term Consequences of Kidney Donation. *New England Journal of Medicine*. 2009;360:459-469.
218. Garg AX, Prasad GVR, Thiessen-Philbrook HR, Ping L, Melo M, Gibney EM, Knoll G, Karpinski M, Parikh CR, Gill J, Storsley L, Vlasschaert M, Mamdani M and Network ftDNOR. Cardiovascular Disease and Hypertension Risk in Living Kidney Donors: An Analysis of Health Administrative Data in Ontario, Canada. *Transplantation*. 2008;86:399-406.
219. Mjøen G, Reisaeter A, Hallan S, Line P-D, Hartmann A, Midtvedt K, Foss A, Dahle DO and Holdaas H. Overall and cardiovascular mortality in Norwegian kidney donors compared to the background population. *Nephrology Dialysis Transplantation*. 2012;27:443-447.
220. Rizvi SAH, Zafar MN, Jawad F, Aziz T, Hussain Z, Hashmi A, Hussain M, Akhtar F, Ahmed E, Naqvi R and Naqvi SAA. Long-term Safety of Living Kidney Donation in an Emerging Economy. *Transplantation*. 2016;100:1284-1293.
221. Garg AX, Meirambayeva A, Huang A, Kim J, Prasad GVR, Knoll G, Boudville N, Lok C, McFarlane P, Karpinski M, Storsley L, Klarenbach S, Lam N, Thomas SM, Dipchand C, Reese P, Doshi M, Gibney E, Taub K and Young A. Cardiovascular disease in kidney donors: matched cohort study. *BMJ*. 2012;344.
222. Reese PP, Bloom RD, Feldman HI, Rosenbaum P, Wang W, Saynisch P, Tarsi NM, Mukherjee N, Garg AX, Mussell A, Shults J, Even-Shoshan O, Townsend RR and Silber JH. Mortality and Cardiovascular Disease among Older Live Kidney Donors. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2014;14:1853-1861.

223. Mjoen G, Hallan S, Hartmann A, Foss A, Midtvedt K, Oyen O, Reisater A, Pfeffer P, Jenssen T, Leivestad T, Line P-D, Ovrehus M, Dale DO, Pihlstrom H, Holme I, Dekker FW and Holdaas H. Long-term risks for kidney donors. *Kidney Int*. 2014;86:162-167.
224. Kaplan B and Ilahe A. Quantifying Risk of Kidney Donation: The Truth Is Not Out There (Yet). *American Journal of Transplantation*. 2014;14:1715-1716.
225. Thiel GT, Nolte C, Tsinalis D, Steiger J and Bachmann LM. Investigating kidney donation as a risk factor for hypertension and microalbuminuria: findings from the Swiss prospective follow-up of living kidney donors. *BMJ Open*. 2016;6:e010869
226. Maggiore U, Budde K, Heemann U, Hilbrands L, Oberbauer R, Oniscu GC, Pascual J, Schwartz Sorensen S, Viklicky O and Abramowicz D. Long-term risks of kidney living donation: review and position paper by the ERA-EDTA DESCARTES working group. *Nephrology Dialysis Transplantation*. 2017;32:216-223.
227. Williams SL, Oler J and Jorkasky DK. Long-Term Renal Function in Kidney Donors: A Comparison of Donors and Their Siblings. *Annals of Internal Medicine*. 1986;105:1-8.
228. Najarian JS, McHugh LE, Matas AJ and Chavers BM. 20 years or more of follow-up of living kidney donors. *The Lancet*. 1992;340:807-810.
229. Muzaale AD, Massie AB, Wang M and et al. RIsk of end-stage renal disease following live kidney donation. *JAMA*. 2014;311:579-586.
230. Matas AJ, Berglund DM, Vock DM and Ibrahim HN. Causes and timing of end-stage renal disease after living kidney donation. *American Journal of Transplantation*. 2018;18:1140-1150.
231. Boudville N and Garg AX. End-stage renal disease in living kidney donors. *Kidney International*. 2014;86:20-22.
232. Grams ME, Sang Y, Levey AS, Matsushita K, Ballew S, Chang AR, Chow EKH, Kasiske BL, Kovesdy CP, Nadkarni GN, Shalev V, Segev DL, Coresh J, Lentine KL and Garg AX. Kidney-Failure Risk Projection for the Living Kidney-Donor Candidate. *New England Journal of Medicine*. 2016;374:411-421.
233. Boudville N, Ramesh Prasad GV, Knoll G, Muirhead N, Thiessen-Philbrook H, Yang RC, Rosas-Arellano MP, Housawi A and Garg AX. Meta-Analysis: Risk for Hypertension in Living Kidney Donors. *Annals of Internal Medicine*. 2006;145:185-196.
234. Kim SH, Hwang HS, Yoon HE, Kim YK, Choi BS, Moon IS, Kim JC, Hwang TK, Kim YS and Yang CW. Long-Term Risk of Hypertension and Chronic Kidney Disease in Living Kidney Donors. *Transplantation Proceedings*. 2012;44:632-634.
235. Kasiske BL, Anderson-Haag T, Israni AK, Kalil RS, Kimmel PL, Kraus ES, Kumar R, Posselt AA, Pesavento TE, Rabb H, Steffes MW, Snyder JJ and Weir MR. A Prospective Controlled Study of Living Kidney Donors: Three-Year Follow-up. *American Journal of Kidney Diseases*. 2015;66:114-124.
236. Lentine KL, Schnitzler MA, Xiao H, Saab G, Salvalaggio PR, Axelrod D, Davis CL, Abbott KC and Brennan DC. Racial Variation in Medical Outcomes among Living Kidney Donors. *New England Journal of Medicine*. 2010;363:724-732.
237. Chia YC, Lim HM and Ching SM. Long-Term Visit-to-Visit Blood Pressure Variability and Renal Function Decline in Patients With Hypertension Over 15 Years. *Journal of the American Heart Association*. 2016;5:e003825.
238. Mancia G. Short- and Long-Term Blood Pressure Variability. *Present and Future*. 2012;60:512-517.
239. Stevens SL, Wood S, Koshiaris C, Law K, Glasziou P, Stevens RJ and McManus RJ. Blood pressure variability and cardiovascular disease: systematic review and meta-analysis. *BMJ*. 2016;354:i4098.

240. Ternes L A-HT, Weir M, Kasiske B. . Assessment of Visit-to-Visit Blood Pressure Variability in Living Kidney Donors and Healthy Two-Kidney Controls [abstract]. *Am J Transplant*. 2013;13 (suppl 5).
241. Moody WE, Ferro CJ, Edwards NC, Chue CD, Lin ELS, Taylor RJ, Cockwell P, Steeds RP and Townend JN. Cardiovascular Effects of Unilateral Nephrectomy in Living Kidney Donors. *Hypertension*. 2016:67:368-377.
242. Altmann U, Böger CA, Farkas S, Mack M, Luchner A, Hamer OW, Zeman F, Debl K, Fellner C, Jungbauer C, Banas B and Buchner S. Effects of Reduced Kidney Function Because of Living Kidney Donation on Left Ventricular Mass. *Hypertension*. 2017;69:297-303.
243. Maruotti GM, Sarno L, Napolitano R, Mazzarelli LL, Quaglia F, Capone A, Capuano A and Martinelli P. Preeclampsia in women with chronic kidney disease. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2012;25:1367-1369.
244. Ahmed R, Dunford J, Mehran R, Robson S and Kunadian V. Pre-Eclampsia and Future Cardiovascular Risk Among Women: A Review. *Journal of the American College of Cardiology*. 2014;63:1815-1822.
245. Ibrahim HN, Akkina SK, Leister E, Gillingham K, Cordner G, Guo H, Bailey R, Rogers T and Matas AJ. Pregnancy Outcomes after Kidney Donation. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2009;9:825-834.
246. Reisæter AV, Røislien J, Henriksen T, Irgens LM and Hartmann A. Pregnancy and Birth After Kidney Donation: The Norwegian Experience. *American Journal of Transplantation*. 2009;9:820-824.
247. Garg AX, Nevis IF, McArthur E, Sontrop JM, Koval JJ, Lam NN, Hildebrand AM, Reese PP, Storsley L, Gill JS, Segev DL, Habbous S, Bugeja A, Knoll GA, Dipchand C, Monroy-Cuadros M and Lentine KL. Gestational Hypertension and Preeclampsia in Living Kidney Donors. *New England Journal of Medicine*. 2015;372:124-133.
248. Fesler P, Mourad G, du Cailar G, Ribstein J and Mimran A. Arterial stiffness: an independent determinant of adaptive glomerular hyperfiltration after kidney donation. *American Journal of Physiology - Renal Physiology*. 2015;308:F567-F571.
249. Moody WE, Tomlinson LA, Ferro CJ, Steeds RP, Mark PB, Zehnder D, Tomson CR, Cockcroft JR, Wilkinson IB and Townend JN. Effect of A Reduction in glomerular filtration rate after NEphrectomy on arterial STiffness and central hemodynamics: Rationale and design of the EARNEST study. *American Heart Journal*. 2014;167:141-149.e2.
250. Bahous SA, Stephan A, Blacher J and Safar ME. Aortic Stiffness, Living Donors, and Renal Transplantation. *Hypertension*. 2006;47:216-221.
251. Bellavia D, Cataliotti A, Clemenza F, Baravoglia CH, Luca A, Traina M, Gridelli B, Bertani T, Burnett JC and Scardulla C. Long-Term Structural and Functional Myocardial Adaptations in Healthy Living Kidney Donors: A Pilot Study. *PLoS ONE*. 2015;10:e0142103.
252. Kendi Celebi Z, Peker A, Kutlay S, Kocak S, Tuzuner A, Erturk S, Keven K and Sengul S. Effect of unilateral nephrectomy on urinary angiotensinogen levels in living kidney donors: 1 year follow-up study. *Journal of the Renin-Angiotensin-Aldosterone System*. 2017;18:1470320317734082.
253. Kasiske BL, Anderson-Haag T, Ibrahim HN, Pesavento TE, Weir MR, Nogueira JM, Cosio FG, Kraus ES, Rabb HH, Kalil RS, Posselt AA, Kimmel PL and Steffes MW. A Prospective Controlled Study of Kidney Donors: Baseline and 6-Month Follow-up. *American Journal of Kidney Diseases*. 2013;62:577-586.

254. Huan Y, Kapoor S, DeLoach S, Ommen E, Meyers KE and Townsend RR. Changes in Biomarkers Associated With Living Kidney Donation. *American journal of nephrology*. 2013;38:10.1159/000354312.
255. Kasiske BL, Kumar R, Kimmel PL, Pesavento TE, Kalil RS, Kraus ES, Rabb H, Posselt AM, Anderson-Haag TL, Steffes MW, Israni AK, Snyder JJ, Singh RJ and Weir MR. Abnormalities in biomarkers of mineral and bone metabolism in kidney donors. *Kidney International*.2016;90:861-868.
256. Young A, Hodsman AB, Boudville N, Geddes C, Gill J, Goltzman D, Jassal SV, Klarenbach S, Knoll G, Muirhead N, Prasad GVR, Treleaven D and Garg AX. Bone and Mineral Metabolism and Fibroblast Growth Factor 23 Levels After Kidney Donation. *American Journal of Kidney Diseases*.2012; 59:761-769.
257. Thorsen IS, Bleskestad IH, Jonsson G, Skadberg Ø and Gøransson LG. Neutrophil Gelatinase-Associated Lipocalin, Fibroblast Growth Factor 23, and Soluble Klotho in Long-Term Kidney Donors. *Nephron Extra*. 2016;6:31-39.
258. Ponte B, Trombetti A, Hadaya K, Ernandez T, Fumeaux D, Iselin C, Martin PY and de Seigneux S. Acute and long term mineral metabolism adaptation in living kidney donors: A prospective study. *Bone*. 2014;62:36-42.
259. Lam NN, McArthur E, Kim SJ, Prasad GVR, Lentine KL, Reese PP, Kasiske BL, Lok CE, Feldman LS, Garg AX, Arnold J, Boudville N, Bugeja A, Dipchand C, Doshi M, Gill J, Karpinski M, Klarenbach S, Knoll G, Monroy-Cuadros M, Nguan CY, Sontrop J, Storsley L, Treleaven D and Young A. Gout After Living Kidney Donation: A Matched Cohort Study. *American Journal of Kidney Diseases*. 2015;65:925-932.
260. Rossi M, Campbell KL, Johnson DW, Stanton T, Haluska BA, Hawley CM, Dimeski G, McWhinney BC, Ungerer JPJ, Kaisar OM and Isbel NM. Uremic Toxin Development in Living Kidney Donors: A Longitudinal Study. *Transplantation*. 2014;97:548-554.
261. Kielstein JT, Veldink H, Martens-Lobenhoffer J, Haller H, Perthel R, Lovric S, Lichtinghagen R, Kliem V and Bode-Böger SM. Unilateral nephrectomy causes an abrupt increase in inflammatory mediators and a simultaneous decrease in plasma ADMA: a study in living kidney donors. *American Journal of Physiology - Renal Physiology*. 2011;301:F1042-F1046.
262. Bernd Hewing HD, Fabian Knebel, Sebastian Spethmann, Wolfram C. Poller, Anna Maria Dehn, Hans-H. Neumayer, Johannes Waiser, Klemens Budde, Fabian Halleck. Midterm echocardiographic follow-up of cardiac function after living kidney donation. *Clinical Nephrology*. 2015;83:253-261.
263. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJL, Mann JF, Matsushita K and Wen CP. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *The Lancet*.2013;382:339-352.
264. British Transplantation Society/The Renal Association: United Kingdom Guidelines for Living Donor Kidney Transplantation, 3rd ED. British Transplantation Society and The Renal Association. 2011
265. Abramowicz D, Cochat P, Claas FHJ, Heemann U, Pascual J, Dudley C, Harden P, Hourmant M, Maggiore U, Salvadori M, Spasovski G, Squifflet J-P, Steiger J, Torres A, Viklicky O, Zeier M, Vanholder R, Van Biesen W and Nagler E. European Renal Best Practice Guideline on kidney donor and recipient evaluation and perioperative care. *Nephrology Dialysis Transplantation*. 2015;30:1790-1797.
266. Mattu GS, Heran BS and Wright JM. Overall accuracy of the BpTRU™—an automated electronic blood pressure device. *Blood Pressure Monitoring*. 2004;9:47-52.
267. Wright JM, Mattu GS, Perry TLJ, Gelferc ME, Strange KD, Zorn A and Chen Y. Validation of a new algorithm for the BPM-100 electronic oscillometric office blood pressure monitor. *Blood Pressure Monitoring*. 2001;6:161-165.

268. Beckett L and Godwin M. The BpTRU automatic blood pressure monitor compared to 24 hour ambulatory blood pressure monitoring in the assessment of blood pressure in patients with hypertension. *BMC Cardiovasc Disord*. 2005;5:18-18.
269. Parati G, Stergiou G, O'Brien E, Asmar R, Beilin L, Bilo G, Clement D, de la Sierra A, de Leeuw P, Dolan E, Fagard R, Graves J, Head GA, Imai Y, Kario K, Lurbe E, Mallion J-M, Mancia G, Mengden T, Myers M, Ogedegbe G, Ohkubo T, Omboni S, Palatini P, Redon J, Ruilope LM, Shennan A, Staessen JA, vanMontfrans G, Verdecchia P, Waeber B, Wang J, Zanchetti A and Zhang Y. European Society of Hypertension practice guidelines for ambulatory blood pressure monitoring. *Journal of Hypertension*. 2014;32:1359-1366.
270. Levey AS and Inker LA. GFR Evaluation in Living Kidney Donor Candidates. *Journal of the American Society of Nephrology : JASN*. 2017;28:1062-1071.
271. Tent H, Rook M, Stevens LA, van Son WJ, van Pelt LJ, Hofker HS, Ploeg RJ, van der Heide JJH and Navis G. Renal Function Equations before and after Living Kidney Donation: A Within-Individual Comparison of Performance at Different Levels of Renal Function. *Clinical Journal of the American Society of Nephrology : CJASN*. 2010;5:1960-1968.
272. British Transplantation Society/The Renal Association: United Kingdom Guidelines for Living Donor Kidney Transplantation, 4rd ED. British Transplantation Society and The Renal Association. 2018.
273. Rehling M. Measuring glomerular filtration rate from plasma clearance of 51Cr-EDTA: quality assurance. *European Journal of Nuclear Medicine and Molecular Imaging*. 2012;39:713-714.
274. Boele-Schutte E and Gansevoort RT. Measured GFR: not a gold, but a gold-plated standard. *Nephrology Dialysis Transplantation*. 2017;32:ii180-ii184.
275. Oatway WB JA, Holmes S, Watson S, Cabianca T. 2016 Ionising radiation exposure of the UK population: 2010 review. *Public Health England*. 2016.

276. Bird NJ, Peters C, Michell AR and Peters AM. Reproducibilities and responses to food intake of GFR measured with chromium-51-EDTA and iohexol simultaneously and independently in normal subjects. *Nephrology Dialysis Transplantation*. 2008;23:1902-1909.
277. C. Sharp JS, RF Bury. Advice on Exposure to Ionising Radiation during Pregnancy. *Joint guidance from National Radiological Protection Board, College of Radiographers, Royal College of Radiologists.*1998.
278. Murray AW, Barnfield MC, Waller ML, Telford T and Peters AM. Assessment of Glomerular Filtration Rate Measurement with Plasma Sampling: A Technical Review. *Journal of Nuclear Medicine Technology*. 2013;41:67-75.
279. Fleming JS, Zivanovic MA, Blake GM, Burniston M and Cosgriff PS. Guidelines for the measurement of glomerular filtration rate using plasma sampling. *Nuclear Medicine Communications*. 2004;25:759-769.
280. Bröchner-Mortensen J. A Simple Method for the Determination of Glomerular Filtration Rate. *Scandinavian Journal of Clinical and Laboratory Investigation*. 1972;30:271-274.
281. Smith ER, Cai MM, McMahon LP and Holt SG. Biological Variability of Plasma Intact and C-Terminal FGF23 Measurements. *The Journal of Clinical Endocrinology & Metabolism*. 2012;97:3357-3365.
282. Smith ER, Cai MM, McMahon LP and Holt SG. Biological variability of plasma intact and C-terminal FGF23 measurements. *J Clin Endocrinol Metab*. 2012;97:3357-3365
283. Pavik I, Jaeger P, Ebner L, Wagner CA, Petzold K, Spichtig D, Poster D, Wüthrich RP, Russmann S and Serra AL. Secreted Klotho and FGF23 in chronic kidney disease Stage 1 to 5: a sequence suggested from a cross-sectional study. *Nephrology Dialysis Transplantation*. 2013;28:352-359.

284. Yamazaki Y, Imura A, Urakawa I, Shimada T, Murakami J, Aono Y, Hasegawa H, Yamashita T, Nakatani K, Saito Y, Okamoto N, Kurumatani N, Namba N, Kitaoka T, Ozono K, Sakai T, Hataya H, Ichikawa S, Imel EA, Econs MJ and Nabeshima Y-i. Establishment of sandwich ELISA for soluble alpha-Klotho measurement: Age-dependent change of soluble alpha-Klotho levels in healthy subjects. *Biochemical and Biophysical Research Communications*. 2010;398:513-518.
285. Heijboer AC, Blankenstein MA, Hoenderop J, de Borst MH, Vervloet MG and consortium obotN. Laboratory aspects of circulating α-Klotho. *Nephrology Dialysis Transplantation*. 2013;28:2283-2287.
286. Zile MR, Claggett BL, Prescott MF, McMurray JJV, Packer M, Rouleau JL, Swedberg K, Desai AS, Gong J, Shi VC and Solomon SD. Prognostic Implications of Changes in N-Terminal Pro-B-Type Natriuretic Peptide in Patients With Heart Failure. *Journal of the American College of Cardiology*. 2016;68:2425-2436.
287. Bandstein N, Ljung R, Johansson M and Holzmann MJ. Undetectable High-Sensitivity Cardiac Troponin T Level in the Emergency Department and Risk of Myocardial Infarction. *Journal of the American College of Cardiology*. 2014;63:2569-2578.
288. Stoner L, Young JM and Fryer S. Assessments of arterial stiffness and endothelial function using pulse wave analysis. *Int J Vasc Med*. 2012;2012:903107-903107.
289. Savage MT, Ferro CJ, Pinder SJ and Tomson CRV. Reproducibility of derived central arterial waveforms in patients with chronic renal failure. *Clinical Science*. 2002;103:59-65.
290. Agabiti-Rosei E, Mancia G, O'Rourke MF, Roman MJ, Safar ME, Smulyan H, Wang J-G, Wilkinson IB, Williams B and Vlachopoulos C. Central Blood Pressure Measurements and Antihypertensive Therapy. *Hypertension*. 2007;50:154-160.
291. Pauca AL, O'Rourke MF and Kon ND. Prospective Evaluation of a Method for Estimating Ascending Aortic Pressure From the Radial Artery Pressure Waveform. *Hypertension*. 2001;38:932-937.
292. Gallagher D, Adji A and O'Rourke MF. Validation of the transfer function technique for generating central from peripheral upper limb pressure waveform. *American Journal of Hypertension*. 2004;17:1059-1067.
293. Weber T, Ammer M, Gündüz D, Bruckenberger P, Eber B and Wallner M. Association of Increased Arterial Wave Reflections With Decline in Renal Function in Chronic Kidney Disease Stages 3 and 4. *American Journal of Hypertension*. 2011;24:762-769.
294. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K and Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *European Heart Journal*. 2010;31:1865-1871.
295. Frimodt-Moller M, Nielsen AH, Kamper AL and Strandgaard S. Reproducibility of pulse-wave analysis and pulse-wave velocity determination in chronic kidney disease. *Nephrol Dial Transplant*. 2008;23:594-600.
296. Frimodt-Møller M, Kamper A-L, Strandgaard S, Kreiner S and Nielsen AH. Beneficial effects on arterial stiffness and pulse-wave reflection of combined enalapril and candesartan in chronic kidney disease--a randomized trial. *PloS one*. 2012;7:e41757-e41757.
297. Holland DJ, Sacre JW, McFarlane SJ, Coombes JS and Sharman JE. Pulse Wave Analysis Is a Reproducible Technique for Measuring Central Blood Pressure During Hemodynamic Perturbations Induced by Exercise. *American Journal of Hypertension*. 2008;21:1100-1106.
298. Gatzka CD, Cameron JD, Dart AM, Berry KL, Kingwell BA, Dewar EM, Reid CM, Jennings GLR and Investigators ftA. Correction of carotid augmentation index for heart rate in elderly essential hypertensives. *American Journal of Hypertension*. 2001;14:573-577.

299. Townsend Raymond R, Wilkinson Ian B, Schiffrin Ernesto L, Avolio Alberto P, Chirinos Julio A, Cockcroft John R, Heffernan Kevin S, Lakatta Edward G, McEniery Carmel M, Mitchell Gary F, Najjar Samer S, Nichols Wilmer W, Urbina Elaine M and Weber T. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness. *Hypertension*. 2015;66:698-722.
300. Cameron JD, McGrath BP and Dart AM. Use of radial artery applanation tonometry and a generalized transfer function to determine aortic pressure augmentation in subjects with treated hypertension. *Journal of the American College of Cardiology*. 1998;32:1214-1220.
301. Wilkinson IB, Mohammad NH, Tyrrell S, Hall IR, Webb DJ, Paul VE, Levy T and Cockcroft JR. Heart rate dependency of pulse pressure amplification and arterial stiffness. *American Journal of Hypertension*. 2002;15:24-30.
302. Butlin M and Qasem A. Large Artery Stiffness Assessment Using SphygmoCor Technology. *Pulse (Basel)*. 2017;4:180-192.
303. Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR and Webb DJ. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *Journal of Hypertension*. 1998;16:2079-2084.
304. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, Boutouyrie P, Cameron J, Chen C-H, Cruickshank JK, Hwang S-J, Lakatta EG, Laurent S, Maldonado J, Mitchell GF, Najjar SS, Newman AB, Ohishi M, Pannier B, Pereira T, Vasan RS, Shokawa T, Sutton-Tyrell K, Verbeke F, Wang K-L, Webb DJ, Willum Hansen T, Zoungas S, McEniery CM, Cockcroft JR and Wilkinson IB. Aortic Pulse Wave Velocity Improves Cardiovascular Event Prediction: An Individual Participant Meta-Analysis of Prospective Observational Data From 17,635 Subjects. *Journal of the American College of Cardiology*. 2014;63:636-646.
305. Weber T, Ammer M, Rammer M, Adji A, O'Rourke MF, Wassertheurer S, Rosenkranz S and Eber B. Noninvasive determination of carotid–femoral pulse wave velocity depends critically on assessment of travel distance: a comparison with invasive measurement. *Journal of Hypertension*. 2009;27:1624-1630.
306. Townsend RR. Arterial Stiffness: Recommendations and Standardization. *Pulse (Basel)*. 2017;4:3-7.
307. Øygarden H. Carotid Intima-Media Thickness and Prediction of Cardiovascular Disease. *Journal of the American Heart Association*.2017;6:e005313.
308. Pignoli P, Tremoli E, Poli A, Oreste P and Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation*. 1986;74:1399-1406.
309. Baldassarre D, Hamsten A, Veglia F, de Faire U, Humphries SE, Smit AJ, Giral P, Kurl S, Rauramaa R, Mannarino E, Grossi E, Paoletti R and Tremoli E. Measurements of Carotid Intima-Media Thickness and of Interadventitia Common Carotid Diameter Improve Prediction of Cardiovascular Events. *Results of the IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) Study*. 2012;60:1489-1499.
310. Mac Ananey O, Mellotte G and Maher V. Comparison of Semi-Automated and Manual Measurements of Carotid Intima-Media Thickening. *BioMed Research International*. 2014;2014:4.
311. Wikstrand J. Methodological considerations of ultrasound measurement of carotid artery intima–media thickness and lumen diameter. *Clinical Physiology and Functional Imaging*. 2007;27:341-345.
312. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Hernandez RH, Jaff M, Kownator S, Naqvi T, Prati P, Rundek T, Sitzer M, Schminke U, Tardif JC, Taylor A, Vicaut E and Woo KS. Mannheim Carotid

Intima-Media Thickness and Plaque Consensus (2004–2006–2011): An Update on Behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovascular diseases (Basel, Switzerland)*. 2012;34:290-296.
313. Gonzalez J, Wood JC, Dorey FJ, Wren TAL and Gilsanz V. Reproducibility of Carotid Intima-Media Thickness Measurements in Young Adults. *Radiology*. 2008;247:465-471.
314. Luo X, Yang Y, Cao T and Li Z. Differences in left and right carotid intima–media thickness and the associated risk factors. *Clinical Radiology*. 2011;66:393-398.
315. Bussmann S, Luechinger R, Froehlich JM, von Weymarn C, Reischauer C, Koh DM and Gutzeit A. Safety of intrauterine devices in MRI. *PloS one*. 2018;13:e0204220-e0204220.
316. Sammet S. Magnetic resonance safety. *Abdom Radiol (NY)*. 2016;41:444-451.
317. Oshinski JN, Delfino JG, Sharma P, Gharib AM and Pettigrew RI. Cardiovascular magnetic resonance at 3.0T: Current state of the art. *Journal of Cardiovascular Magnetic Resonance*. 2010;12:55.
318. Rajiah P and Bolen MA. Cardiovascular MR Imaging at 3 T: Opportunities, Challenges, and Solutions. *RadioGraphics*. 2014;34:1612-1635.
319. Schär M, Vonken E-J and Stuber M. Simultaneous B(0)- and B(1)+-map acquisition for fast localized shim, frequency, and RF power determination in the heart at 3 T. *Magnetic resonance in medicine*. 2010;63:419-426.
320. Messroghli DR, Moon JC, Ferreira VM, Grosse-Wortmann L, He T, Kellman P, Mascherbauer J, Nezafat R, Salerno M, Schelbert EB, Taylor AJ, Thompson R, Ugander M, van Heeswijk RB and Friedrich MG. Clinical recommendations for cardiovascular magnetic resonance mapping of T1, T2, T2* and extracellular volume: A consensus statement by the Society for Cardiovascular Magnetic Resonance (SCMR) endorsed by the European Association for Cardiovascular Imaging (EACVI). *Journal of Cardiovascular Magnetic Resonance*. 2017;19:75.
321. Moon JC, Messroghli DR, Kellman P, Piechnik SK, Robson MD, Ugander M, Gatehouse PD, Arai AE, Friedrich MG, Neubauer S, Schulz-Menger J and Schelbert EB. Myocardial T1 mapping and extracellular volume quantification: a Society for Cardiovascular Magnetic Resonance (SCMR) and CMR Working Group of the European Society of Cardiology consensus statement. *J Cardiovasc Magn Reson*. 2013;15.
322. Kellman P, Herzka D, Arai A and Hansen M. Influence of off-resonance in myocardial T1-mapping using SSFP based MOLLI method. *J Cardiovasc Magn Reson*. 2013;15:63.
323. Deshpande VS, Shea SM and Li D. Artifact reduction in true-FISP imaging of the coronary arteries by adjusting imaging frequency. *Magnetic Resonance in Medicine*. 2003;49:803-809.
324. Myerson SG, Bellenger NG and Pennell DJ. Assessment of left ventricular mass by cardiovascular magnetic resonance. *Hypertension*. 2002;39:750-755.
325. Maceira AM, Prasad SK, Khan M and Pennell DJ. Normalized Left Ventricular Systolic and Diastolic Function by Steady State Free Precession Cardiovascular Magnetic Resonance. *Journal of Cardiovascular Magnetic Resonance*. 2006;8:417-426.
326. Kramer CM, Barkhausen J, Bucciarelli-Ducci C, Flamm SD, Kim RJ and Nagel E. Standardized cardiovascular magnetic resonance imaging (CMR) protocols: 2020 update. *Journal of Cardiovascular Magnetic Resonance*. 2020;22:17.
327. Miller CA, Naish JH, Bishop P, Coutts G, Clark D, Zhao S, Ray SG, Yonan N, Williams SG, Flett AS, Moon JC, Greiser A, Parker GJM and Schmitt M. Comprehensive

Validation of Cardiovascular Magnetic Resonance Techniques for the Assessment of Myocardial Extracellular Volume. *Circulation: Cardiovascular Imaging*. 2013;6:373-383.
328. Vassiliou VS, Wassilew K, Cameron D, Heng EL, Nyktari E, Asimakopoulos G, de Souza A, Giri S, Pierce I, Jabbour A, Firmin D, Frenneaux M, Gatehouse P, Pennell DJ and Prasad SK. Identification of myocardial diffuse fibrosis by 11 heartbeat MOLLI T1 mapping: averaging to improve precision and correlation with collagen volume fraction. *Magnetic Resonance Materials in Physics, Biology and Medicine*. 2018;31:101-113.
329. Giri S, Chung Y-C, Merchant A, Mihai G, Rajagopalan S, Raman SV and Simonetti OP. T2 quantification for improved detection of myocardial edema. *Journal of Cardiovascular Magnetic Resonance*. 2009;11:56.
330. White S, Sado D, Fontana M, Banypersad S, Maestrini V, Flett A, Piechnik S, Robson M, Hausenloy D, Sheikh A, Hawkins P and Moon J. T1 mapping for myocardial extracellular volume measurement by CMR: bolus only versus primed infusion technique. *JACC Cardiovasc Imaging*. 2013;6:955-962.
331. Chai P and Mohiaddin R. How we perform cardiovascular magnetic resonance flow assessment using phase-contrast velocity mapping. *J Cardiovasc Magn Reson*. 2005;7:705-16.
332. Gupta A, Lee VS, Chung Y-C, Babb JS and Simonetti OP. Myocardial Infarction: Optimization of Inversion Times at Delayed Contrast-enhanced MR Imaging. *Radiology*. 2004;233:921-926.
333. Kellman P, Arai AE, McVeigh ER and Aletras AH. Phase-sensitive inversion recovery for detecting myocardial infarction using gadolinium-delayed hyperenhancement†. *Magnetic Resonance in Medicine*. 2002;47:372-383.
334. Kellman P and Hansen MS. T1-mapping in the heart: accuracy and precision. *J Cardiovasc Magn Reson*. 2014;16.
335. Pennell DJ, Sechtem UP, Higgins CB, Manning WJ, Pohost GM, Rademakers FE, Rossum AC, Shaw LJ and Yucel EK. Clinical indications for cardiovascular magnetic resonance (CMR): Consensus Panel report. *Eur Heart J*. 2004;25:1940-1965.
336. Holtackers RJ, Van De Heyning CM, Nazir MS, Rashid I, Ntalas I, Rahman H, Botnar RM and Chiribiri A. Clinical value of dark-blood late gadolinium enhancement cardiovascular magnetic resonance without additional magnetization preparation. *Journal of Cardiovascular Magnetic Resonance*. 2019;21:44.
337. Schulz-Menger J, Bluemke DA, Bremerich J, Flamm SD, Fogel MA and Friedrich MG. Standardized image interpretation and post processing in cardiovascular magnetic resonance: Society for Cardiovascular Magnetic Resonance (SCMR) board of trustees task force on standardized post processing. *J Cardiovasc Magn Reson*. 2013;15.
338. Katz J, Milliken MC, Stray-Gundersen J, Buja LM, Parkey RW, Mitchell JH and Peshock RM. Estimation of human myocardial mass with MR imaging. *Radiology*. 1988;169:495-498.
339. Bottini PB, Carr AA, Prisant LM, Flickinger FW, Allison JD and Gottdiener JS. Magnetic resonance imaging compared to echocardiography to assess left ventricular mass in the hypertensive patient. *American Journal of Hypertension*. 1995;8:221-228.
340. Grothues F, Smith GC, Moon JC, Bellenger NG, Collins P, Klein HU and Pennell DJ. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *Am J Cardiol*. 2002;90:29-34.
341. Mullally J, Goyal P, Simprini LA, Afroz A, Kochav JD, Codella N, Devereux RB and Weinsaft JW. Marked variability in published CMR criteria for left ventricular basal slice selection - impact of methodological discrepancies on LV mass quantification. *Journal of Cardiovascular Magnetic Resonance*. 2013;15:P101.

342. Simplified Calculation of Body-Surface Area. *New England Journal of Medicine*. 1987;317:1098-1098.
343. Cerqueira Manuel D, Weissman Neil J, Dilsizian V, Jacobs Alice K, Kaul S, Laskey Warren K, Pennell Dudley J, Rumberger John A, Ryan T and Verani Mario S. Standardized Myocardial Segmentation and Nomenclature for Tomographic Imaging of the Heart. *Circulation*. 2002;105:539-542.
344. Bluemke DA, Kronmal RA, Lima JAC, Liu K, Olson J, Burke GL and Folsom AR. The Relationship of Left Ventricular Mass and Geometry to Incident Cardiovascular Events. *Journal of the American College of Cardiology*. 2008;52:2148.
345. Hudsmith LE, Cheng ASH, Tyler DJ, Shirodaria C, Lee J, Petersen SE, Francis JM, Clarke K, Robson MD and Neubauer S. Assessment of Left Atrial Volumes at 1.5 Tesla and 3 Tesla Using FLASH and SSFP Cine Imaging. *Journal of Cardiovascular Magnetic Resonance*. 2007;9:673-679.
346. Khan MA, Yang EY, Zhan Y, Judd RM, Chan W, Nabi F, Heitner JF, Kim RJ, Klem I, Nagueh SF and Shah DJ. Association of left atrial volume index and all-cause mortality in patients referred for routine cardiovascular magnetic resonance: a multicenter study. *Journal of Cardiovascular Magnetic Resonance*. 2019;21:4.
347. Liu B, Dardeer AM, Moody WE, Hayer MK, Baig S, Price AM, Leyva F, Edwards NC and Steeds RP. Reference ranges for three-dimensional feature tracking cardiac magnetic resonance: comparison with two-dimensional methodology and relevance of age and gender. *The International Journal of Cardiovascular Imaging*. 2018;34:761-775.
348. Taylor RJ, Moody WE, Umar F, Edwards NC, Taylor TJ, Stegemann B, Townend JN, Hor KN, Steeds RP, Mazur W and Leyva F. Myocardial strain measurement with feature-tracking cardiovascular magnetic resonance: normal values. *European Heart Journal - Cardiovascular Imaging*. 2015;16:871-881.
349. Bistoquet A, Oshinski J and Skrinjar O. Left Ventricular Deformation Recovery From Cine MRI Using an Incompressible Model. *IEEE Transactions on Medical Imaging*. 2007;26:1136-1153.
350. Wang J, Li W, Sun J, Liu H, Kang Y, Yang D, Yu L, Greiser A, Zhou X, Han Y and Chen Y. Improved segmental myocardial strain reproducibility using deformable registration algorithms compared with feature tracking cardiac MRI and speckle tracking echocardiography. *Journal of Magnetic Resonance Imaging*. 2018;48:404-414.
351. Heidenreich JF, Weng AM, Donhauser J, Greiser A, Chow K, Nordbeck P, Bley TA and Köstler H. T1- and ECV-mapping in clinical routine at 3 T: differences between MOLLI, ShMOLLI and SASHA. *BMC Medical Imaging*. 2019;19:59.
352. Vijapurapu R, Hawkins F, Liu B, Edwards N and Steeds R. 25 A study of the different methodologies used in calculation of extra-cellular volume by CMR imaging. *Heart*. 2018;104:A14-A15.
353. Edwards NC, Moody WE, Yuan M, Hayer MK, Ferro CJ, Townend JN and Steeds RP. Diffuse Interstitial Fibrosis and Myocardial Dysfunction in Early Chronic Kidney Disease. *American Journal of Cardiology*.2015;115:1311-1317.
354. Price AM MW, Stoll VM, Vijapurapu R, Hayer MK, Biasiolli L, Weston CJ, Webster R, Wesolowski R, McGee KC, Liu B, Baig S, Pickup LX, Radhakrishnan A, Law JP, Edwards NC, Steeds RP, Ferro CJ, Townend JN. Cardiovascular Effects of Unilateral Nephrectomy in Living Kidney Donors at 5 Years. *Hypertension*. 2021;77:1273-1284.
355. Flett AS, Hasleton J, Cook C, Hausenloy D, Quarta G, Ariti C, Muthurangu V and Moon JC. Evaluation of Techniques for the Quantification of Myocardial Scar of Differing Etiology Using Cardiac Magnetic Resonance. *JACC: Cardiovascular Imaging*. 2011;4:150-156.

356. Biasiolli L, Hann E, Lukaschuk E, Carapella V, Paiva JM, Aung N, Rayner JJ, Werys K, Fung K, Puchta H, Sanghvi MM, Moon NO, Thomson RJ, Thomas KE, Robson MD, Grau V, Petersen SE, Neubauer S and Piechnik SK. Automated localization and quality control of the aorta in cine CMR can significantly accelerate processing of the UK Biobank population data. *PLOS ONE*. 2019;14:e0212272.
357. Redheuil A, Wu CO, Kachenoura N, Ohyama Y, Yan RT, Bertoni AG, Hundley GW, Duprez DA, Jacobs DR, Daniels LB, Darwin C, Sibley C, Bluemke DA and Lima JAC. Proximal Aortic Distensibility is an Independent Predictor of All-cause Mortality and Incident Cardiovascular Events in the Multi-Ethnic Study of Atherosclerosis. *Journal of the American College of Cardiology*. 2014;64:2619-2629.
358. Jackson CE, Shirodaria CC, Lee JMS, Francis JM, Choudhury RP, Channon KM, Noble JA, Neubauer S and Robson MD. Reproducibility and accuracy of automated measurement for dynamic arterial lumen area by cardiovascular magnetic resonance. *The International Journal of Cardiovascular Imaging*. 2009;25:797.
359. Singh A, Horsfield MA, Bekele S, Greenwood JP, Dawson DK, Berry C, Hogrefe K, Kelly DJ, Houston JG, Guntur Ramkumar P, Uddin A, Suzuki T and McCann GP. Aortic stiffness in aortic stenosis assessed by cardiovascular MRI: a comparison between bicuspid and tricuspid valves. *European Radiology*. 2019;29:2340-2349.
360. Grotenhuis HB, Ottenkamp J, de Bruijn L, Westenberg JJ, Vliegen HW, Kroft LJ and de Roos A. Aortic elasticity and size are associated with aortic regurgitation and left ventricular dysfunction in tetralogy of Fallot after pulmonary valve replacement. *Heart*. 2009;95;1931-1936.
361. Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, Heffernan KS, Lakatta EG, McEniery CM, Mitchell GF, Najjar SS, Nichols WW, Urbina EM and Weber T. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness. *Hypertension*. 2015;66:698-722.
362. Price AM, Greenhall GHB, Moody WE, Steeds RP, Mark PB, Edwards NC, Hayer MK, Pickup LC, Radhakrishnan A, Law JP, Banerjee D, Campbell T, Tomson CRV, Cockcroft JR, Shrestha B, Wilkinson IB, Tomlinson LA, Ferro CJ and Townend JN. Changes in Blood Pressure and Arterial Hemodynamics following Living Kidney Donation. *Clinical Journal of the American Society of Nephrology*. 2020;15:1330-1339.
363. Mitchell GF. Arterial stiffness and hypertension: chicken or egg? *Hypertension (Dallas, Tex : 1979)*. 2014;64:210-214.
364. Kim J, Song TJ, Kim EH, Lee KJ, Lee HS, Nam CM, Song D, Nam HS, Kim YD and Heo JH. Brachial-ankle pulse wave velocity for predicting functional outcome in acute stroke. *Stroke*. 2014;45:2305-10.
365. Krishnasamy R, Tan S-J, Hawley CM, Johnson DW, Stanton T, Lee K, Mudge DW, Campbell S, Elder GJ, Toussaint ND and Isbel NM. Progression of arterial stiffness is associated with changes in bone mineral markers in advanced CKD. *BMC Nephrology*. 2017;18:281.
366. Kim H, Yoo TH, Choi KH, Oh KH, Lee J, Kim SW, Kim TH, Sung S, Han SH and Group K-C. Baseline Cardiovascular Characteristics of Adult Patients with Chronic Kidney Disease from the KoreaN Cohort Study for Outcomes in Patients With Chronic Kidney Disease (KNOW-CKD). *J Korean Med Sci*. 2017;32:231-239.
367. Chen S-C, Chang J-M, Liu W-C, Tsai Y-C, Tsai J-C, Hsu P-C, Lin T-H, Lin M-Y, Su H-M, Hwang S-J and Chen H-C. Brachial-Ankle Pulse Wave Velocity and Rate of Renal Function Decline and Mortality in Chronic Kidney Disease. *Clinical Journal of the American Society of Nephrology*. 2011;6:724-732.
368. Briet M, Bozec E, Laurent S, Fassot C, London G and Jacquot C. Arterial stiffness and enlargement in mild-to-moderate chronic kidney disease. *Kidney Int*. 2006;69:350-357

369. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, Sever PS and Poulter NR. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *The Lancet*. 2010;375:895-905.
370. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, MacLaughlin EJ, Muntner P, Ovbiagele B, Smith SC, Spencer CC, Stafford RS, Taler SJ, Thomas RJ, Williams KA, Williamson JD and Wright JT. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Journal of the American College of Cardiology*. 2018;71:e127-e248.
371. de Luca N, Asmar RG, London GM, O'Rourke MF, Safar ME and investigators obotRP. Selective reduction of cardiac mass and central blood pressure on low-dose combination perindopril/indapamide in hypertensive subjects. *Journal of Hypertension*. 2004;22:1623-1630.
372. Boutouyrie P, Bussy C, Lacolley P, Girerd X, Laloux B and Laurent S. Association Between Local Pulse Pressure, Mean Blood Pressure, and Large-Artery Remodeling. *Circulation*. 1999;100:1387-1393.
373. DeLoach SS, Meyers KEC and Townsend RR. Living Donor Kidney Donation: Another Form of White Coat Effect. *American Journal of Nephrology*. 2012;35:75-79.
374. Izzard AS and Grassi G. Alterations in pulse wave velocity and augmentation index in Australian aboriginals: characteristics and mechanisms. *Journal of Hypertension*. 2007;25:511-512.
375. Fang J and Alderman MH. Serum uric acid and cardiovascular mortality: The nhanes i epidemiologic follow-up study, 1971-1992. *JAMA*. 2000;283:2404-2410.
376. Takashi Y and Fukumoto S. FGF23-Klotho axis in CKD. *Renal Replacement Therapy*. 2016;2:20.
377. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A and Isakova T. FGF23 induces left ventricular hypertrophy. *J Clin Invest*. 2011;121:4393-4408.
378. Issa N, Sánchez OA, Kukla A, Riad SM, Berglund DM, Ibrahim HN and Matas AJ. Weight gain after kidney donation: Association with increased risks of type 2 diabetes and hypertension. *Clinical Transplantation*. 2018;32:e13360.
379. Grupper A, Angel Y, Baruch A, Schwartz IF, Schwartz D, Nakache R, Goykhman Y, Katz P, Nachmany I, Lubezky N, Weinstein T, Shashar M, Ben-Bassat OK, Berliner S, Rogowski O, Zeltser D, Shapira I and Shenhar-Tsarfaty S. Long term metabolic and renal outcomes of kidney donors compared to controls with excellent kidney function. *BMC Nephrology*. 2019;20:30.
380. Rastogi A, Yuan S, Arman F, Simon L, Shaffer K, Kamgar M, Nobakht N, Bromberg JS and Weir MR. Blood Pressure and Living Kidney Donors: A Clinical Perspective. *Transplantation Direct*. 2019;5:e488.
381. Srivastava A, Sinha T, Varma PP, Karan SC, Sandhu AS, Sethi GS, Khanna R, Talwar R and Narang V. Experience with marginal living related kidney donors: Are they becoming routine or are there still any doubts? *Urology*. 2005;66:971-975.
382. Townsend RR, Reese PP and Lim MA. Should living kidney donors with hypertension be considered for organ donation? *Current Opinion in Nephrology and Hypertension*. 2015;24:594-601.
383. Viera AJ, Lingley K and Hinderliter AL. Tolerability of the Oscar 2 ambulatory blood pressure monitor among research participants: a cross-sectional repeated measures study. *BMC Medical Research Methodology*. 2011;11:59.

384. Ernst ME and Bergus GR. Favorable patient acceptance of ambulatory blood pressure monitoring in a primary care setting in the United States: a cross-sectional survey. *BMC Family Practice*. 2003;4:15.
385. Purnell TS, Luo X, Cooper LA, Massie AB, Kucirka LM, Henderson ML, Gordon EJ, Crews DC, Boulware LE and Segev DL. Association of Race and Ethnicity With Live Donor Kidney Transplantation in the United States From 1995 to 2014. Trends in Live Donor Kidney Transplantation Receipt by Race/Ethnicity, 1995-2014. *JAMA*. 2018;319:49-61.
386. Lacolley P, Challande P, Osborne-Pellegrin M and Regnault V. Genetics and pathophysiology of arterial stiffness. *Cardiovascular Research*. 2008;81:637-648.
387. Shlipak MG, Sarnak MJ, Katz R, Fried LF, Seliger SL, Newman AB, Siscovick DS and Stehman-Breen C. Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med*. 2005;352:2049-60.
388. Van Biesen W, De Bacquer D, Verbeke F, Delanghe J, Lameire N and Vanholder R. The glomerular filtration rate in an apparently healthy population and its relation with cardiovascular mortality during 10 years. *Eur Heart J*. 2007;28:478-83.
389. Edwards NC, Moody WE, Chue CD, Ferro CJ, Townend JN and Steeds RP. Defining the natural history of uremic cardiomyopathy in chronic kidney disease: the role of cardiovascular magnetic resonance. *JACC Cardiovasc Imaging*. 2014;7:703-714.
390. Blantz RC and Steiner RW. Benign hyperfiltration after living kidney donation. *The Journal of clinical investigation*. 2015;125:972-974.
391. Lenihan CR, Busque S, Derby G, Blouch K, Myers BD and Tan JC. Longitudinal study of living kidney donor glomerular dynamics after nephrectomy. *The Journal of Clinical Investigation*. 2015;125:1311-1318.
392. Lenth RV. Java Applets for Power and Sample Size [Computer software]. (2006-9).
393. Gidding SS, Liu K, Colangelo LA, Cook NL, Goff DC, Glasser SP, Gardin JM and Lima JA. Longitudinal determinants of left ventricular mass and geometry: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Circ Cardiovasc Imaging*. 2013;6:769-75.
394. London GM, Pannier B, Guerin AP, Blacher J, Marchais SJ and Darne B. Alterations of left ventricular hypertrophy in and survival of patients receiving hemodialysis: follow-up of an interventional study. *J Am Soc Nephrol*. 2001;12:2759-2767.
395. Matas AJ, Vock DM and Ibrahim HN. GFR ≤25 years postdonation in living kidney donors with (vs. without) a first-degree relative with ESRD. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2018;18:625-631.
396. Hampel DJ, Pratschke J, May G, Reinke P and Schindler R. Living Kidney Donation: Anemia and Inflammation in the Early Postoperative Period. *Transplantation Proceedings*. 2006;38:661-663.
397. Shah S, Lankowsky B, Gao T, Zaky Z, Stephany BR and Poggio ED. Postdonation Anemia in Living Kidney Donors. *Transplantation Proceedings*. 2017;49:1733-1738.
398. Ayus JC, Go AS, Valderrabano F, Verde E, de Vinuesa SG, Achinger SG, Lorenzo V, Arieff AI and Luao J. Effects of erythropoietin on left ventricular hypertrophy in adults with severe chronic renal failure and hemoglobin <10 g/dL. *Kidney International*. 2005;68:788-795.
399. Hamunen K, Kontinen V, Hakala E, Talke P, Paloheimo M and Kalso E. Effect of pain on autonomic nervous system indices derived from photoplethysmography in healthy volunteers. *BJA: British Journal of Anaesthesia*. 2012;108:838-844.
400. Alper I and Yüksel E. Comparison of Acute and Chronic Pain after Open Nephrectomy versus Laparoscopic Nephrectomy: A Prospective Clinical Trial. *Medicine*. 2016;95:e3433-e3433.

401. Bachmann A, Wolff T, Giannini O, Dickenman M, Ruszat R, Gürke L, Kaufmann M, Gasser TC, Steiger J, Stief CG and Sulser T. How Painful Is Donor Nephrectomy? Retrospective Analysis of Early Pain and Pain Management in Open versus Laparoscopic versus Retroperitoneoscopic Nephrectomy. *Transplantation*. 2006;81:1735-1738.
402. Lønnebakken MT, Izzo R, Mancusi C, Gerdts E, Losi MA, Canciello G, Giugliano G, De Luca N, Trimarco B and de Simone G. Left Ventricular Hypertrophy Regression During Antihypertensive Treatment in an Outpatient Clinic (the Campania Salute Network). *Journal of the American Heart Association*. 2017;6:e004152.
403. Dyadyk AI, Bagriy AE, Lebed IA, Yarovaya NF, Schukina EV and Taradin GG. ACE inhibitors captopril and enalapril induce regression of left ventricular hypertrophy in hypertensive patients with chronic renal failure. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. 1997;12:945-951.
404. Reichek N, Devereux RB, Rocha RA, Hilkert R, Hall D, Purkayastha D and Pitt B. Magnetic Resonance Imaging Left Ventricular Mass Reduction With Fixed-Dose Angiotensin-Converting Enzyme Inhibitor Vased Regimens in Patients With High-Risk Hypertension. *Hypertension*. 2009;54:731-737.
405. Textor SC, Taler SJ, Driscoll N, Larson TS, Gloor J, Griffin M, Cosio F, Schwab T, Prieto M, Nyberg S, Ishitani M and Stegall M. Blood pressure and renal function after kidney donation from hypertensive living donors. *Transplantation*. 2004;78:276-82.
406. Lentine KL, Kasiske BL, Levey AS, Adams PL, Alberú J, Bakr MA, Gallon L, Garvey CA, Guleria S, Li PK-T, Segev DL, Taler SJ, Tanabe K, Wright L, Zeier MG, Cheung M and Garg AX. KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation*. 2017;101:S7-S105.
407. Hudsmith LE, Petersen SE, Tyler DJ, Francis JM, Cheng ASH, Clarke K, Selvanayagam JB, Robson MD and Neubauer S. Determination of cardiac volumes and mass with FLASH and SSFP cine sequences at 1.5 vs. 3 Tesla: A validation study. *Journal of Magnetic Resonance Imaging*. 2006;24:312-318.
408. Banerjee D. Sudden cardiac death in haemodialysis: clinical epidemiology and mechanisms. *Journal of Electrocardiology*. 2016;49:843-847.
409. Burton JO, Jefferies HJ, Selby NM and McIntyre CW. Hemodialysis-induced repetitive myocardial injury results in global and segmental reduction in systolic cardiac function. *Clin J Am Soc Nephrol*. 2009;4:1925-1931.
410. Diez J, Querejeta R, Lopez B, Gonzalez A, Larman M and Martinez Ubago J. Losartan-dependent regression of myocardial fibrosis is associated with reduction of left ventricular chamber stiffness in hypertensive patients. *Circulation*. 2002;105:2512-2517.
411. Villari B, Campbell SE, Hess OM, Mall G, Vassalli G, Weber KT and Krayenbuehl HP. Influence of collagen network on left ventricular systolic and diastolic function in aortic valve disease. *Journal of the American College of Cardiology*. 1993;22:1477-1484.
412. Assomull RG, Prasad SK, Lyne J, Smith G, Burman ED, Khan M, Sheppard MN, Poole-Wilson PA and Pennell DJ. Cardiovascular magnetic resonance, fibrosis, and prognosis in dilated cardiomyopathy. *J Am Coll Cardiol*. 2006;48:1977-1985.
413. Iles L, Pfluger H, Phrommintikul A, Cherayath J, Aksit P, Gupta S, Kaye D and Taylor A. Evaluation of diffuse myocardial fibrosis in heart failure with cardiac magnetic resonance contrast-enhanced T1 mapping. *J Am Coll Cardiol*. 2008;52:1574-1580.
414. Zhuang B, Sirajuddin A, Wang S, Arai A, Zhao S and Lu M. Prognostic value of T1 mapping and extracellular volume fraction in cardiovascular disease: a systematic review and meta-analysis. *Heart Fail Rev*. 2018;23:723-731.

415. Zhao L, Li S, Ma X, Bai R, Liu N, Li N, Schoenhagen P and Ma C. Prognostic Significance of Left Ventricular Fibrosis Assessed by T1 Mapping in Patients with Atrial Fibrillation and Heart Failure. *Scientific Reports*. 2019;9:13374.
416. Roy C, Slimani A, de Meester C, Amzulescu M, Pasquet A, Vancraeynest D, Vanoverschelde J-L, Pouleur A-C and Gerber BL. Age and sex corrected normal reference values of T1, T2 T2* and ECV in healthy subjects at 3T CMR. *Journal of Cardiovascular Magnetic Resonance*. 2017;19:72.
417. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF and Feldman HI. A new equation to estimate glomerular filtration rate. *Annals of internal medicine*. 2009;150:408
418. Grigoratos C, Pantano A, Meschisi M, Gaeta R, Ait-Ali L, Barison A, Todiere G, Festa P, Sinagra G and Aquaro GD. Clinical importance of late gadolinium enhancement at right ventricular insertion points in otherwise normal hearts. *The International Journal of Cardiovascular Imaging*. 2020;36:913-920.
419. Dabir D, Child N, Kalra A, Rogers T, Gebker R, Jabbour A, Plein S, Yu C-Y, Otton J, Kidambi A, McDiarmid A, Broadbent D, Higgins DM, Schnackenburg B, Foote L, Cummins C, Nagel E and Puntmann VO. Reference values for healthy human myocardium using a T1 mapping methodology: results from the International T1 Multicenter cardiovascular magnetic resonance study. *Journal of Cardiovascular Magnetic Resonance*. 2014;16:69.
420. Kawel N, Nacif M, Zavodni A, Jones J, Liu S and Sibley CT. T1 mapping of the myocardium: intra-individual assessment of post-contrast T1 time evolution and extracellular volume fraction at 3T for Gd-DTPA and Gd-BOPTA. *J Cardiovasc Magn Reson*. 2012;14.
421. Kellman P, Wilson J, Xue H, Ugander M and Arai A. Extracellular volume fraction mapping in the myocardium, Part 1: evaluation of an automated method. *J Cardiovasc Magn Reson*. 2012;14:63.
422. Fernández A and Politei J. Cardiac Manifestation of Fabry Disease: From Hypertrophic Cardiomyopathy to Early Diagnosis and Treatment in Patients Without Left Ventricular Hypertrophy. *Journal of Inborn Errors of Metabolism and Screening*. 2016;4.
423. Sancaktar O, Shahir K, Baruah D, Welsh AC and Rubenstein J. Abnormal myocardial T1 mapping of hypertrophic cardiomyopathy in areas without delayed enhancement, as compared to NICM and controls at both 1.5 and 3T. *Journal of Cardiovascular Magnetic Resonance*. 2016;18:Q45.
424. Diaz T, Pencina MJ, Benjamin EJ, Aragam J, Fuller DL, Pencina KM, Levy D and Vasan RS. Prevalence, clinical correlates, and prognosis of discrete upper septal thickening on echocardiography: the Framingham Heart Study. *Echocardiography*. 2009;26:247-253.
425. Kelshiker MA, Mayet J, Unsworth B and Okonko DO. Basal septal hypertrophy. *Current cardiology reviews*. 2013;9:325-330.
426. von Knobelsdorff-Brenkenhoff F, Prothmann M, Dieringer MA, Wassmuth R, Greiser A, Schwenke C, Niendorf T and Schulz-Menger J. Myocardial T1 and T2 mapping at 3 T: reference values, influencing factors and implications. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2013;15:53-53.
427. Kawel N, Nacif M, Zavodni A, Jones J, Liu S, Sibley CT and Bluemke DA. T1 mapping of the myocardium: intra-individual assessment of the effect of field strength, cardiac cycle and variation by myocardial region. *J Cardiovasc Magn Reson*. 2012;14.
428. Piechnik S, Ferreira V, Lewandowski A, Ntusi N, Banerjee R, Holloway C, Hofman M, Sado D, Maestrini V, White S, Lazdam M, Karamitsos T, Moon J, Neubauer S, Leeson P and Robson M. Normal variation of magnetic resonance T1 relaxation times in the human population at 1.5 T using ShMOLLI. *J Cardiovasc Magn Reson*. 2013;15:13.
429. Chen M, Arcari L, Engel J, Freiwald T, Platschek S, Zhou H, Zainal H, Buettner S, Zeiher AM, Geiger H, Hauser I, Nagel E and Puntmann VO. Aortic stiffness is independently

associated with interstitial myocardial fibrosis by native T1 and accelerated in the presence of chronic kidney disease. *IJC Heart & Vasculature*. 2019;24:100389.
430. Gregg LP, Tio MC, Li X, Adams-Huet B, de Lemos JA and Hedayati SS. Association of Monocyte Chemoattractant Protein-1 with Death and Atherosclerotic Events in Chronic Kidney Disease. *American Journal of Nephrology*. 2018;47:395-405.
431. Hulsmans M, Sager HB, Roh JD, Valero-Muñoz M, Houstis NE, Iwamoto Y, Sun Y, Wilson RM, Wojtkiewicz G, Tricot B, Osborne MT, Hung J, Vinegoni C, Naxerova K, Sosnovik DE, Zile MR, Bradshaw AD, Liao R, Tawakol A, Weissleder R, Rosenzweig A, Swirski FK, Sam F and Nahrendorf M. Cardiac macrophages promote diastolic dysfunction. *Journal of Experimental Medicine*. 2018;215:423-440.
432. Welsh P, Murray HM, Ford I, Trompet S, Craen AJMd, Jukema JW, Stott DJ, McInnes IB, Packard CJ, Westendorp RGJ and Sattar N. Circulating Interleukin-10 and Risk of Cardiovascular Events. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2011;31:2338-2344.
433. Mallio CA, Quattrocchi CC, Rovira À and Parizel PM. Gadolinium Deposition Safety: Seeking the Patient's Perspective. *American Journal of Neuroradiology*. 2020;41:944-946.
434. Kidir V, Aynali A, Altuntas A, Inal S, Aridogan B and Sezer MT. Telomerase activity in patients with stage 2–5D chronic kidney disease. *Nefrología*. 2017;37:592-597.
435. Thang OHD, Serné EH, Grooteman MPC, Smulders YM, ter Wee PM, Tangelder GJ and Nubé MJ. Premature Aging of the Microcirculation in Patients with Advanced Chronic Kidney Disease. *Nephron Extra*. 2012;2:283-292.
436. Hamczyk MR, Nevado RM, Barettino A, Fuster V and Andrés V. Biological Versus Chronological Aging. *Focus seminar: J Am Coll Cardiol*. 2020;75:919-930.
437. Xu C, Wang Z, Su X, Da M, Yang Z, Duan W and Mo X. Association between leucocyte telomere length and cardiovascular disease in a large general population in the United States. *Scientific Reports*. 2020;10:80.
438. Ramírez R, Carracedo J, Soriano S, Jiménez R, Martín-Malo A, Rodríguez M, Blasco M and Aljama P. Stress-induced premature senescence in mononuclear cells from patients on long-term hemodialysis. *American Journal of Kidney Diseases*. 2005;45:353-359.
439. Tsirpanlis G, Chatzipanagiotou S, Boufidou F, Kordinas V, Alevyzaki F, Zoga M, Kyritsis I, Stamatelou K, Triantafyllis G and Nicolaou C. Telomerase Activity Is Decreased in Peripheral Blood Mononuclear Cells of Hemodialysis Patients. *American Journal of Nephrology*. 2006;26:91-96.
440. Kłoda K, Domański L and Mierzecki A. Telomere Length Assessment for Prediction of Organ Transplantation Outcome. Future or Failure: A Review of the Literature. *Medical science monitor : international medical journal of experimental and clinical research*. 2017;23:158-162.
441. Yildirim M, Karahan M, Kucuk HF, Demir T, Demir H, Turan H, Yavuz A and Ari E. Increased Oxidative Stress in Living Kidney Donors: Correlation of Renal Functions With Antioxidant Capacity. *Transplant Proc*. 2017;49:407-410.
442. Kasiske BL, Anderson-Haag TL, Duprez DA, Kalil RS, Kimmel PL, Pesavento TE, Snyder JJ and Weir MR. A prospective controlled study of metabolic and physiologic effects of kidney donation suggests that donors retain stable kidney function over the first nine years. *Kidney International*. 2020;98:168-175.
443. Astor BC, Matsushita K, Gansevoort RT, van der Velde M, Woodward M, Levey AS, Jong PEd, Coresh J, Chronic Kidney Disease Prognosis C, Astor BC, Matsushita K, Gansevoort RT, van der Velde M, Woodward M, Levey AS, de Jong PE, Coresh J, El-Nahas M, Eckardt K-U, Kasiske BL, Wright J, Appel L, Greene T, Levin A, Djurdjev O, Wheeler DC, Landray MJ, Townend JN, Emberson J, Clark LE, Macleod A, Marks A, Ali T, Fluck N,

Prescott G, Smith DH, Weinstein JR, Johnson ES, Thorp ML, Wetzels JF, Blankestijn PJ, van Zuilen AD, Menon V, Sarnak M, Beck G, Kronenberg F, Kollerits B, Froissart M, Stengel B, Metzger M, Remuzzi G, Ruggenenti P, Perna A, Heerspink HJL, Brenner B, de Zeeuw D, Rossing P, Parving H-H, Auguste P, Veldhuis K, Wang Y, Camarata L, Thomas B and Manley T. Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. *Kidney international*. 2011;79:1331-1340.
444. Nitsch D, Grams M, Sang Y, Black C, Cirillo M, Djurdjev O, Iseki K, Jassal SK, Kimm H, Kronenberg F, Oien CM, Levey AS, Levin A, Woodward M, Hemmelgarn BR and Chronic Kidney Disease Prognosis C. Associations of estimated glomerular filtration rate and albuminuria with mortality and renal failure by sex: a meta-analysis. *BMJ (Clinical research ed)*. 2013;346:f324-f324.
445. Stevens LA, Coresh J, Feldman HI, Greene T, Lash JP, Nelson RG, Rahman M, Deysher AE, Zhang Y, Schmid CH and Levey AS. Evaluation of the Modification of Diet in Renal Disease Study Equation in a Large Diverse Population. *Journal of the American Society of Nephrology*. 2007;18:2749-2757.
446. Levey AS, Coresh J, Greene T and et al. USing standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Annals of Internal Medicine*. 2006;145:247-254.
447. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T and Coresh J. A New Equation to Estimate Glomerular Filtration Rate. *Annals of internal medicine*. 2009;150:604-612.
448. Matsushita K, Mahmoodi BK, Woodward M, Emberson JR, Jafar TH, Jee SH, Polkinghorne KR, Shankar A, Smith DH, Tonelli M, Warnock DG, Wen C-P, Coresh J, Gansevoort RT, Hemmelgarn BR, Levey AS and Chronic Kidney Disease Prognosis C. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. *JAMA*. 2012;307:1941-1951.
449. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J and Levey AS. Estimating Glomerular Filtration Rate from Serum Creatinine and Cystatin C. *New England Journal of Medicine*. 2012;367:20-29.
450. Lees JS, Welsh CE, Celis-Morales CA, Mackay D, Lewsey J, Gray SR, Lyall DM, Cleland JG, Gill JMR, Jhund PS, Pell J, Sattar N, Welsh P and Mark PB. Glomerular filtration rate by differing measures, albuminuria and prediction of cardiovascular disease, mortality and end-stage kidney disease. *Nature Medicine*. 2019;25:1753-1760.
451. Shlipak MG, Matsushita K, Ärnlöv J, Inker LA, Katz R, Polkinghorne KR, Rothenbacher D, Sarnak MJ, Astor BC, Coresh J, Levey AS and Gansevoort RT. Cystatin C versus Creatinine in Determining Risk Based on Kidney Function. *New England Journal of Medicine*. 2013;369:932-943.
452. Matsushita K, Kwak L, Sang Y, Ballew Shoshana H, Skali H, Shah Amil M, Coresh J and Solomon S. Kidney Disease Measures and Left Ventricular Structure and Function: The Atherosclerosis Risk in Communities Study. *Journal of the American Heart Association*. 6:e006259.
453. Henry RM, Kamp O, Kostense PJ, Spijkerman AM, Dekker JM and Nijpels G. Mild renal insufficiency is associated with increased left ventricular mass in men, but not in women: an arterial stiffness-related phenomenon--the Hoorn Study. *Kidney Int*. 2005;68.
454. Coresh J. CKD Prognosis: Beyond the Traditional Outcomes. *American Journal of Kidney Diseases*. 2009;54:1-3.

455. Guo Y, Cui L, Ye P, Li J, Wu S and Luo Y. Change of Kidney Function Is Associated With All-Cause Mortality and Cardiovascular Diseases: Results From the Kailuan Study. *Journal of the American Heart Association*. 2018;7:e010596.
456. Zoccali C and Mallamaci F. The overdriven glomerulus as a cardiovascular risk factor. *Kidney International*. 2018;93:13-15.
457. Kiberd BA and Tennankore KK. Lifetime risks of kidney donation: a medical decision analysis. *BMJ Open*. 2017;7:e016490.
458. Yamada Y, Ikenoue T, Saito Y and Fukuma S. Undiagnosed and untreated chronic kidney disease and its impact on renal outcomes in the Japanese middle-aged general population. *Journal of Epidemiology and Community Health*. 2019;73:1122-1127.
459. Asp AM, Wallquist C, Rickenlund A, Hylander B, Jacobson SH, Caidahl K and Eriksson MJ. Cardiovascular changes in patients with mild-to-moderate chronic kidney disease compared with healthy subjects: a 5-year follow-up study. *Clinical Physiology and Functional Imaging*. 2020;40:91-98.
460. Cai Q-Z, Lu X-Z, Lu Y and Wang AY-M. Longitudinal Changes of Cardiac Structure and Function in CKD (CASCADE Study). *Journal of the American Society of Nephrology*. 2014;25:1599-1608.
461. Wassertheurer S, Kropf J, Weber T, van der Giet M, Baulmann J, Ammer M, Hametner B, Mayer CC, Eber B and Magometschnigg D. A new oscillometric method for pulse wave analysis: comparison with a common tonometric method. *Journal Of Human Hypertension*. 2010;24:498.
462. Alivon M, Vo-Duc Phuong T, Vignon V, Bozec E, Khettab H, Hanon O, Briet M, Halimi J-M, Hallab M, Plichart M, Mohammedi K, Marre M, Boutouyrie P and Laurent S. A novel device for measuring arterial stiffness using finger-toe pulse wave velocity: Validation study of the pOpmètre®. *Archives of Cardiovascular Diseases*. 2015;108:227-234.
463. Rodríguez-Gómez I, Wangensteen R, Pérez-Abud R, Quesada A, Moral RGd, Osuna A, O'Valle F, Luna JdD and Vargas F. Long-Term Consequences of Uninephrectomy in Male and Female Rats. *Hypertension*. 2012;60:1458-1463.
464. Bae EH, Kim IJ, Park JW, Ma SK, Lee JU and Kim SW. Renoprotective effect of rosuvastatin in DOCA–salt hypertensive rats. *Nephrology Dialysis Transplantation*. 2009;25:1051-1059.
465. Iyer A, Chan V and Brown L. The DOCA-Salt Hypertensive Rat as a Model of Cardiovascular Oxidative and Inflammatory Stress. *Current cardiology reviews*. 2010;6:291-297.
466. Wang X, Johnson AC, Sasser JM, Williams JM, Woods LCS and Garrett MR. Spontaneous one-kidney rats are more susceptible to develop hypertension by DOCA-NaCl and subsequent kidney injury compared with uninephrectomized rats. *American Journal of Physiology-Renal Physiology*. 2016;310:F1054-F1064.
467. Ahmadi AR, Lafranca JA, Claessens LA, Imamdi RMS, Ijzermans JNM, Betjes MGH and Dor FJMF. Shifting paradigms in eligibility criteria for live kidney donation: a systematic review. *Kidney International*. 2015;87:31-45.
468. Cantarelli C and Cravedi P. Criteria for Living Donation from Marginal Donors: One, No One, and One Hundred Thousand. *Nephron*. 2019;142:227-232.
469. Lafranca JA, Spoon EQW, van de Wetering J, JNM IJ and Dor F. Attitudes among transplant professionals regarding shifting paradigms in eligibility criteria for live kidney donation. *PLoS One*. 2017;12:e0181846.
470. Carbone A DAA. Cardiac dysfunction and athlete's heart: new insights into pathophysiology and treatment. *e-journal of Cardiology Practice*. 2017;14.
471. McDiarmid AK, Swoboda PP, Erhayiem B, Lancaster RE, Lyall GK, Broadbent DA, Dobson LE, Musa TA, Ripley DP, Garg P, Greenwood JP, Ferguson C and Plein S. Athletic

Cardiac Adaptation in Males Is a Consequence of Elevated Myocyte Mass. *Circulation: Cardiovascular Imaging*. 2016;9:e003579.
472. Mihai S, Codrici E, Popescu ID, Enciu A-M, Albulescu L, Necula LG, Mambet C, Anton G and Tanase C. Inflammation-Related Mechanisms in Chronic Kidney Disease Prediction, Progression, and Outcome. *Journal of Immunology Research*. 2018;2018:2180373.
473. Haase VH. Inflammation and hypoxia in the kidney: friends or foes? *Kidney international*. 2015;88:213-215.
474. Roubicek T, Bartlova M, Krajickova J, Haluzikova D, Mraz M, Lacinova Z, Kudla M, Teplan V and Haluzik M. Increased production of proinflammatory cytokines in adipose tissue of patients with end-stage renal disease. *Nutrition (Burbank, Los Angeles County, Calif)*. 2009;25:762-8.
475. O'Shaughnessy MM, Liu S, Montez-Rath ME, Lafayette RA and Winkelmayer WC. Cause of kidney disease and cardiovascular events in a national cohort of US patients with end-stage renal disease on dialysis: a retrospective analysis. *European Heart Journal*. 2018;40:887-898.
476. Hayer MK, Radhakrishnan A, Price AM, Liu B, Baig S, Weston CJ, Biasiolli L, Ferro CJ, Townend JN, Steeds RP and Edwards NC. Defining Myocardial Abnormalities Across the Stages of Chronic Kidney Disease. *A Cardiac Magnetic Resonance Imaging Study*. 2020:3435.
477. Agrawal V, Marinescu V, Agarwal M and McCullough PA. Cardiovascular implications of proteinuria: an indicator of chronic kidney disease. *Nature Reviews Cardiology*. 2009;6:301-311.
478. Levey AS, Gansevoort RT, Coresh J, Inker LA, Heerspink HL, Grams ME, Greene T, Tighiouart H, Matsushita K, Ballew SH, Sang Y, Vonesh E, Ying J, Manley T, de Zeeuw D, Eckardt K-U, Levin A, Perkovic V, Zhang L and Willis K. Change in Albuminuria and GFR as End Points for Clinical Trials in Early Stages of CKD: A Scientific Workshop Sponsored by the National Kidney Foundation in Collaboration With the US Food and Drug Administration and European Medicines Agency. *American Journal of Kidney Diseases*. 2020;75:84-104.
479. Stehouwer CDA and Smulders YM. Microalbuminuria and Risk for Cardiovascular Disease: Analysis of Potential Mechanisms. *Journal of the American Society of Nephrology*. 2006;17:2106-2111.
480. Reese PP, Boudville N and Garg AX. Living kidney donation: outcomes, ethics, and uncertainty. *The Lancet*. 385:2003-2013.