

**THE EFFECT OF SPIRONOLACTONE ON CARDIOVASCULAR  
DISEASE IN EARLY CHRONIC KIDNEY DISEASE**



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## **ABSTRACT**

This thesis examined whether patients with early stage chronic kidney disease (CKD) have abnormalities of arterial stiffness and cardiac function. Aldosterone is known to promote inflammation and fibrosis in the arteries, heart and kidney. Treatment with spironolactone, which blocks aldosterone at the mineralocorticoid receptor might be effective at reducing such injury. To address these hypotheses, I performed an observational study in patients with early stage CKD without known cardiovascular disease and healthy volunteers. Aortic stiffness and myocardial structure and function were assessed using cardiac MRI and echocardiography. I demonstrated reductions in aortic distensibility, increased ventricular and arterial stiffness and impairment of markers of left ventricular systolic and diastolic function in CKD. I complemented this work by performing a randomised double blind controlled trial examining the effect of spironolactone on intermediate prognostic markers of cardiovascular function. Spironolactone reduced arterial stiffness and left ventricular mass and improved markers of systolic and diastolic function independent of changes in blood pressure. Spironolactone was well tolerated with low rates of hyperkalaemia and renal dysfunction. These findings support a role for spironolactone in reducing aldosterone mediated cardiovascular injury in early stage CKD. A clinical outcome study evaluating spironolactone in CKD is now required.

*To Matthew, Olivia & Alexander*

*My precious family who make everyday an adventure*

## **ACKNOWLEDGEMENTS**

This research was conducted under the direct supervision of Dr Jonathan Townend (Consultant Cardiologist and Reader), Dr Charles Ferro (Consultant Nephrologists and Honorary Senior Lecturer), Dr Richard Steeds (Consultant Cardiologist and Honorary Senior Lecturer) and Professor Paul Stewart (Professor of Medicine) at the University of Birmingham and University Hospital Birmingham.

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Thanks also to my co-supervisor Dr Charlie Ferro who with John Townend devised this project. Your support and advice have been unlimited throughout this research period. Charlie bravely persuaded his nephrology colleagues and ethics committee to allow us to use spironolactone in renal patients for the first time. Without his planning and attention to detail this project might still be an idea on paper. Finally to the third musketeer Dr Rick Steeds, you have been at the imaging coal face with me throughout this project, teaching me the beauty of cardiac MRI and the complexities of echo. You have always remained in good humour and have inspired my career path into academic and clinical non-invasive cardiology.

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## **EXTENT OF PERSONAL CONTRIBUTION**

Assisted by a research nurse, I screened all nephrology out-patient clinics for potential subjects at University Hospital Birmingham between March 2005 and February 2007. I was responsible for recruiting and consenting all patients, for arranging and performing all study visits, reporting any serious adverse events and for the clinical care of patients during their participation in the studies. I also maintained all the regulatory documents and was responsible for ethics committee amendments. I performed all applanation tonometry measurements and echocardiography examinations. Cardiac MRI studies were performed by me and Dr Richard Steeds. I analysed all data prior to un-blinding of the study and performed statistical analyses with assistance from the statistician Dr Peter Nightingale. Cardiac MRI data was double analysed with Dr Steeds and echo data with Dr Asle Hirth. Both physicians were blinded to my results and all clinical data in the studies. Tagging analysis from cardiac MRI was analysed at the Centre for Advanced MRI, University of Auckland, New Zealand.

Blood tests, ECGs and patient biochemical monitoring throughout the study were performed by research nurses from the Wellcome Clinical Research Facility. All results were discussed with me. Routine blood and urine samples and markers of collagen turnover were analysed at University Hospital Birmingham. Specialist blood samples were analysed at the following institutions; renin, angiotensin II and aldosterone - MRC Unit in Glasgow; NT-proBNP - University of Leicester, hsCRP – Binding Site, University of Birmingham.

The original research hypothesis, grant funding and ethics approval were obtained by Dr Jonathan Townend, Dr Charles Ferro, Dr Richard Steeds and Professor Paul Stewart. All provided assistance throughout the study with general advice, guidance and writing of manuscripts.



## ABBREVIATIONS

|             |                                                                                                  |
|-------------|--------------------------------------------------------------------------------------------------|
| ABPM        | twenty four hour ambulatory blood pressure monitoring                                            |
| ACR         | albumin-creatinine ratio                                                                         |
| ARB         | angiotensin receptor blocker                                                                     |
| Aug         | aortic augmentation pressure                                                                     |
| AIx         | augmentation index                                                                               |
| AIx 75      | augmentation index corrected for a heart rate of 75bpm                                           |
| BP          | blood pressure                                                                                   |
| BSA         | body surface area (Mosteller method)                                                             |
| Cardiac MRI | cardiac magnetic resonance imaging                                                               |
| CKD         | chronic kidney disease                                                                           |
| EDV         | end-diastolic volume                                                                             |
| EF          | ejection fraction                                                                                |
| ESKD        | end-stage kidney disease (requiring renal replacement therapies ie. dialysis or transplantation) |
| eGFR        | estimated glomerular filtration rate                                                             |
| GR          | glucocorticoid receptor                                                                          |
| hsCRP       | high sensitivity C-reactive protein                                                              |
| LV          | left ventricle                                                                                   |
| MR          | mineralocorticoid receptor                                                                       |
| MRB         | mineralocorticoid receptor blocker                                                               |
| PAC         | plasma aldosterone concentration                                                                 |
| PWA         | pulse wave analysis                                                                              |
| PWV         | pulse wave velocity                                                                              |
| TDI         | tissue Doppler Imaging                                                                           |
| SV          | stroke volume                                                                                    |

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**Chapter 3    Aortic distensibility and arterial-ventricular coupling in early chronic kidney disease**

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**Chapter 7 The safety and tolerability of spironolactone in patients with mild-moderate chronic kidney disease**

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**Chapter 8 Assessment of reproducibility**

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## **CHAPTER 1**

### **INTRODUCTION – CARDIOVASCULAR DISEASE IN EARLY STAGE CHRONIC KIDNEY DISEASE- THE ROLE OF ALDOSTERONE**

Contributory publication; NC Edwards, RP Steeds, CJ Ferro, JN Townend. The Treatment of Coronary Artery Disease in patients with Chronic Kidney Disease. *Quarterly Journal of Medicine*. 2006 Nov;99(11):723-36.

## 1.1 OVERVIEW

Chronic kidney disease (CKD) is a global public health problem affecting over 1 in 10 adults in developed countries. The early stages of CKD are the most prevalent and continue to increase exponentially, exceeding rates of end-stage kidney disease (ESKD) by 100-200 times. Cardiovascular disease is a major cause of mortality and morbidity in these patients and poses a far greater risk than progression to ESKD.

The nature of cardiovascular disease in CKD is characterised by unique differences from the general population with a preponderance of non-atheromatous pathogenic processes such as arterial stiffening and abnormalities of cardiac structure such as left ventricular hypertrophy and fibrosis. These observations might explain why traditional cardiovascular risk factors which account for the “atheroma burden” fail to account for the high rates of cardiovascular mortality in CKD.

The mechanisms promoting adverse changes within the cardiovascular system appear complex and are incompletely understood. Experimental data have implicated activation of the renin-angiotensin-aldosterone system (RAAS) and specifically aldosterone as one potential cause. Despite routine use of ACE inhibitors and angiotensin receptor blockers, aldosterone levels frequently rise in CKD, a phenomenon known as “aldosterone breakthrough”. Aldosterone is a powerful mediator of vascular inflammation and subsequently promotes the development of fibrosis in the arteries, heart and kidney. Treatment strategies which block the action of aldosterone are effective at preventing these adverse effects.

Research is clearly warranted to provide insight into the natural history of cardiovascular disease in early stage CKD and the mechanisms which might be responsible

for these pathological changes. Such knowledge might allow the opportunity to develop preventative treatment strategies and eventually reduce the high clinical and economic cost of cardiovascular disease in CKD.

## **1.2 THE KIDNEYS**

### **1.2.1 Anatomy**

The kidneys are a pair of organs located in a retroperitoneal position on the posterior abdominal wall around the 12<sup>th</sup> thoracic vertebra (Snell, 1992).

### **1.2.2 Structure and function**

The kidneys are surrounded by a fibrous capsule which is closely applied to the outer cortex. The inner medulla is composed of approximately 12 renal pyramids, the apices of which project medially as the renal papillae. These are indented by 2-3 minor calyces which combine together as the major calyces and subsequently form the funnel shaped renal pelvis which serves as the upper end of the ureter (Snell, 1992).

Each kidney is composed of approximately 1 million functional units known as nephrons. All nephrons originate in the cortex and consist of vascular and tubular components which are related structurally and functionally and are pivotal in the regulation of fluid and electrolyte homeostasis. The glomerulus is a highly specialised capillary system situated within Bowman's capsule. The glomerular membrane serves as a fine molecular sieve made up of three layers; a single layer of flattened capillary endothelial cells perforated by fenestrae, an acellular gelatinous layer of basement membrane and an inner epithelial layer of the Bowman's capsule consisting of podocytes which interdigitate to form slits along the capillary wall. This arrangement permits passive filtration of water and solute up to 4 nm in diameter but excludes those with diameter greater than 8 nm such as plasma proteins and blood cells.

Filtered fluid passes into the renal tubule which is divided into several parts i) the proximal tubule within the cortex ii) the U-shaped loop of Henle extending into the medulla and traversing back into the cortex to form iii) the distal tubule. The latter coalesce to form the collecting ducts which pass through the cortex and medulla and empty into the renal pelvis (Ganong WF, 2005).

In health, the kidneys control sodium and volume homeostasis primarily through alterations in the activity of the renin-angiotensin-aldosterone system (RAAS). Other important functions include; i) biochemical homeostasis; selective resorption of glucose and amino acids in the glomerular filtrate, selective resorption of water under control of anti-diuretic hormone (ADH), selective resorption or secretion of sodium, potassium, calcium, phosphate and hydrogen ions, ii) secretion of erythropoietin, iii) conversion of vitamin D into its active form 1,25-dihydroxycholecalciferol (Ganong WF, 2005).

### **1.3 DEFINITION AND MEASUREMENT OF CHRONIC KIDNEY DISEASE**

#### **1.3.1 Definition of chronic kidney disease**

Chronic kidney disease (CKD) describes abnormal kidney function or structure. In 2002, the National Kidney Foundation (NKF) published the Kidney Disease Outcome Quality Initiative (K/DOQI™) providing guidelines for the evaluation, classification and stratification of CKD (National Kidney Foundation, 2002). This has led to a standardised classification of CKD into 5 stages based upon i) evidence of kidney damage for  $\geq 3$  months; defined by structural or functional abnormalities of the kidney with or without decrease in glomerular filtration rate (GFR) or ii) a reduction in GFR below  $60 \text{ ml/min/1.73m}^2$  for  $\geq 3$  months with or without kidney damage (Table 1.1).



**Table 1.1** Stages of chronic kidney disease (Adapted from National Kidney Foundation K/DOQI clinical practice guidelines for chronic kidney disease)  
(National Kidney Foundation, 2002)

| <b>Stage</b> | <b>Description</b>                        | <b>GFR ml/min/1.73m<sup>2</sup></b> |
|--------------|-------------------------------------------|-------------------------------------|
| 1            | Kidney damage with normal / increased GFR | Normal / increased GFR $\geq$ 90    |
| 2            | Kidney damage with mildly decreased GFR   | 60 to 89                            |
| 3            | Moderately decreased GFR                  | 30 to 59                            |
| 4            | Severely decreased GFR                    | 15 to 29                            |
| 5            | Kidney failure                            | $\geq$ 15                           |

Kidney damage include; abnormal renal biopsy, markers of renal damage (persistent proteinuria, albuminuria, haematuria) or structural renal abnormality on imaging studies.

Kidney failure: requirement for renal replacement therapy.

### 1.3.2 Measurement of glomerular filtration rate

The level of glomerular filtration rate (GFR) is widely accepted as the best overall index of renal function. However, an appreciation that the normal level of GFR will vary according to age, gender, body size and haemodynamic factors is important in its interpretation. Among healthy adults the inter-individual coefficient of variation is approximately 15-20%. It is conventional therefore to adjust GFR where possible to account for such variation: body size is adjusted to “standard body size” ( $1.73\text{m}^2$ ), normal values in women are assumed to be 8% lower than in males and appreciation of an age-related mean decrease  $1\text{ ml/min}/1.73\text{m}^2$  reduction per year in GFR after the age of 20-30 years. Not all individuals with a “decreased” GFR between  $60\text{-}89\text{ ml/min}/1.73\text{m}^2$  will have evidence of kidney damage. Causes include; age, vegetarian diets, unilateral nephrectomy, extracellular fluid volume depletion, and systemic illnesses associated with reduced kidney perfusion (National Kidney Foundation, 2002).

Glomerular filtration can be measured directly by radioisotope filtration markers such as  $^{125}\text{I}$ othalamate,  $^{99\text{m}}\text{Tc}$ -diethylenetriamine-pentaacetic acid (DTPA), and  $^{51}\text{Cr}$ -EDTA (iGFR) or estimated (eGFR) using prediction equations such as Modification of Diet in Renal Disease (MDRD) equation which rely on serum creatinine, demographic and clinical variables. The Cockcroft-Gault (CG) equation was the most widely used equation following its introduction in 1976. It provided an estimate of urinary creatinine clearance and was subsequently re-expressed to estimate GFR with acceptable performance (Cockcroft and Gault, 1976). Its use has been largely superseded after the NKF K/DOQI guidelines advocated the use of the Modification of Diet in Renal Disease (MDRD) equation which has been shown

to be more accurate than CG when compared to iGFR in patients with diabetic and non-diabetic moderate to advanced CKD (GFRs  $\leq 60$  ml/min/1.73m<sup>2</sup>) (Poggio et al., 2005).

The original 6-variable MDRD equation was derived in a study of 1,628 CKD patients using multiple regression analysis to determine a set of variables that estimated GFR (Levey et al., 1999). The level of measured GFR was related to the serum concentration of the endogenous filtration marker creatinine and to observed clinical and demographic variables (age, sex, ethnicity, urea and albumin), that serve as surrogates for creatinine generation and which affect serum creatinine concentration independent of GFR. This equation was later simplified to the 4-variable formula (4-variable MDRD) with the exclusion of albumin and urea and re-expressed for a standardised serum creatinine assay to improve and normalise serum creatinine results used in the equation (Levey et al., 2006). It has since been validated in multiple populations with and without CKD (Lewis et al., 2001;Stevens et al., 2007a).

The CG and MDRD equations offer a rapid method for assessing renal function but are limited by a lack of validation in the full range of GFRs to which they are frequently applied. This is most evident in healthy individuals where both equations calculate lower eGFRs than their iGFR value. The underestimation of eGFR can increase the number of healthy individuals labelled with CKD (false positive) and might reflect higher levels of creatinine generation, as well as the fact that both equations were derived in populations which did not include people without CKD (Poggio et al., 2005).

In serum creatinine based equations small numerical differences between laboratories have a significant effect on the calculation of eGFR. These inaccuracies have been improved by implementation of standardised calibration of serum creatinine assays to an isotope dilution mass spectroscopy (IDMS) method which on average leads to lower values for serum creatinine and hence higher values for eGFR (Levey et al., 2006).The precision of eGFR is

likely to be further improved by the introduction of new estimation equations such as Chronic Kidney Disease Epidemiology (CKD-EPI) equation (Levey and Stevens, 2010) or by the use of novel filtration markers such as cystatin C (Shlipak et al., 2005) and biomarkers of renal tubular damage such as kidney injury molecule-1 and clusterin (Vaidya et al., 2010).

## **1.4 EPIDEMIOLOGY AND HEALTH BURDEN OF CHRONIC KIDNEY DISEASE**

### **1.4.1 Prevalence**

Chronic kidney disease is a common worldwide condition with increasing epidemiological importance. It affects 10-16% of the adult population in Europe, Australia, Asia and the USA (Chadban et al., 2003; National Collaborating Centre for Chronic Conditions, 2008; Wen et al., 2008). Over the past two decades data from the National Health and Nutrition Examination Surveys (NHANES) in the USA have demonstrated that the prevalence of CKD is high and increasing. These data have provided the bench-mark for the subsequent development of clinical practice guidelines and health care planning.

The NHANES studies are a large nationally representative programme of surveys which have provided extensive data on kidney disease in the USA. In early surveys the prevalence of CKD was determined from data collected on albuminuria and serum creatinine. Subsequent re-calibration of serum creatinine to standardised creatinine measurements has enabled estimation of eGFR by the MDRD formula and hence greater precision in prevalence estimates. In NHANES 1988-1994, the prevalence for stages 1-4 was 10.0%. This increased to 13.1% in NHANES 1999-2004, with the greatest increases observed in stage 2 (2.2% to

3.2%) and 3 (5.4% to 7.7%) and female preponderance in both studies (Coresh et al., 2007).

Within age categories, individuals aged  $\geq 70$  years had the highest prevalence of CKD at every stage of disease and nearly two-thirds had evidence of stage 3 or 4 disease in NHANES 1999-2004.

Prevalence data from the UK is less extensive but a retrospective analysis involving over 160,000 patients in primary care between 1990 and 2003, reported an overall prevalence of CKD stage 3-5 of 8.5% with preponderance in females as observed in NHANES (Stevens et al., 2007b).

#### **1.4.2 Health care and economic burden**

Over the past three decades, improvements in detection of CKD and advances in therapies to reduce or prevent complications have improved renal outcomes. These include pharmacological treatment of cardiovascular risk factors, proteinuria, bone metabolism and anaemia as well as the wider availability of renal replacement therapies (RRT) with dialysis and transplantation (USRDS). A paradox of this success has been the increased prevalence of CKD and particularly the early stages 2 and 3, which exceed rates of end-stage kidney disease (ESKD) by 100-200 times (Sarnak et al., 2003). This is in part explained and in part biased by improved awareness and detection following publication of national clinical guidelines (National Collaborating Centre for Chronic Conditions, 2008; National Institute for Health and Clinical Excellence, 2008; National Kidney Foundation, 2002). The increasing incidence of CKD risk factors specifically an ageing population, obesity, diabetes and hypertension are also likely to be important factors in the exponential rise in observed prevalence. Hence, these patients represent the “true economic health burden” of CKD which is set to increase exponentially in the future.

## 1.5 THE NATURAL HISTORY OF CHRONIC KIDNEY DISEASE

Chronic kidney disease is a progressive condition usually characterised by an asymptomatic period in the earliest stages of disease. Renal function may deteriorate over months to years depending on the aetiology although the mechanisms which determine the rate of progression of CKD remain incompletely understood. The NHANES surveys have demonstrated increased rates of CKD risk factors including hypertension, diabetes mellitus and cardiovascular disease as well as age and obesity with the progression of CKD (United States Renal Data System, 2010). This has led to recent UK clinical guidelines focussing on identification and aggressive treatment of such risk factors as well as avoiding nephrotoxic agents such as NSAIDs and treatment of obvious precipitants such as reflux or bladder outflow obstruction (National Collaborating Centre for Chronic Conditions, 2008).

Data on the association of proteinuria and progression of CKD are more robust. Over the past decade several clinical studies have shown proteinuria to be both a marker and a mechanism for CKD progression (Matsushita et al., 2010). In most proteinuric kidney diseases (exceptions being glomerulopathies with highly selective protein loss without evidence of kidney damage), GFR loss occurs at between 4 to 10 ml/min per year (Peterson et al., 1995). Treatments which reduce proteinuria will slow GFR decline by about 1-2 ml/min/year and thus delay progression to ESKD (Matsushita et al., 2010). Ultimately only a very small proportion of patients progress to CKD stage 5 with approximately 50% of these requiring treatment with RRT, estimated at 0.05% of the UK population. These treatments represent a huge cost in terms of finance and healthcare resources estimated at between 4-6% of the total NHS budget. In 2005 approximately 694 adult patients per million population in

the UK were treated with RRT and it is anticipated that this will increase by a further 5% per annum (National Institute for Health and Clinical Excellence, 2008).

## **1.6     CARDIOVASCULAR RISK AND CHRONIC KIDNEY DISEASE**

Over the past 20 years several observational studies have increased awareness that CKD might confer an elevated risk of cardiovascular disease (Culleton et al., 1999). These data led to the publication of a report in 1988 by the National Kidney Foundation (NKF) Task Force on Cardiovascular Disease in Chronic Renal Disease emphasising the high prevalence of cardiovascular disease in CKD and the extreme mortality associated with ESKD. The Task Force recommended that patients with CKD be considered in the “highest risk group” for cardiovascular events and be treated accordingly for their high risk status (Levey et al., 1998).

Table 1.2 provides some published estimates of the increased risk of cardiovascular disease at different stages of CKD. These rates are approximates and must be interpreted in the context of the patient’s age and presence of risk factors (particularly proteinuria) which significantly influence the relative cardiovascular risk.

**Table 1.2** Cardiovascular risk according to stage of chronic kidney disease compared to normal renal function (Schiffrin et al., 2007)

| Stage of CKD | Odds ratio                           |
|--------------|--------------------------------------|
| 1            | Dependent upon degree of proteinuria |
| 2            | 1.5                                  |
| 3            | 2-4                                  |
| 4            | 4-10                                 |
| 5            | 10-1000                              |

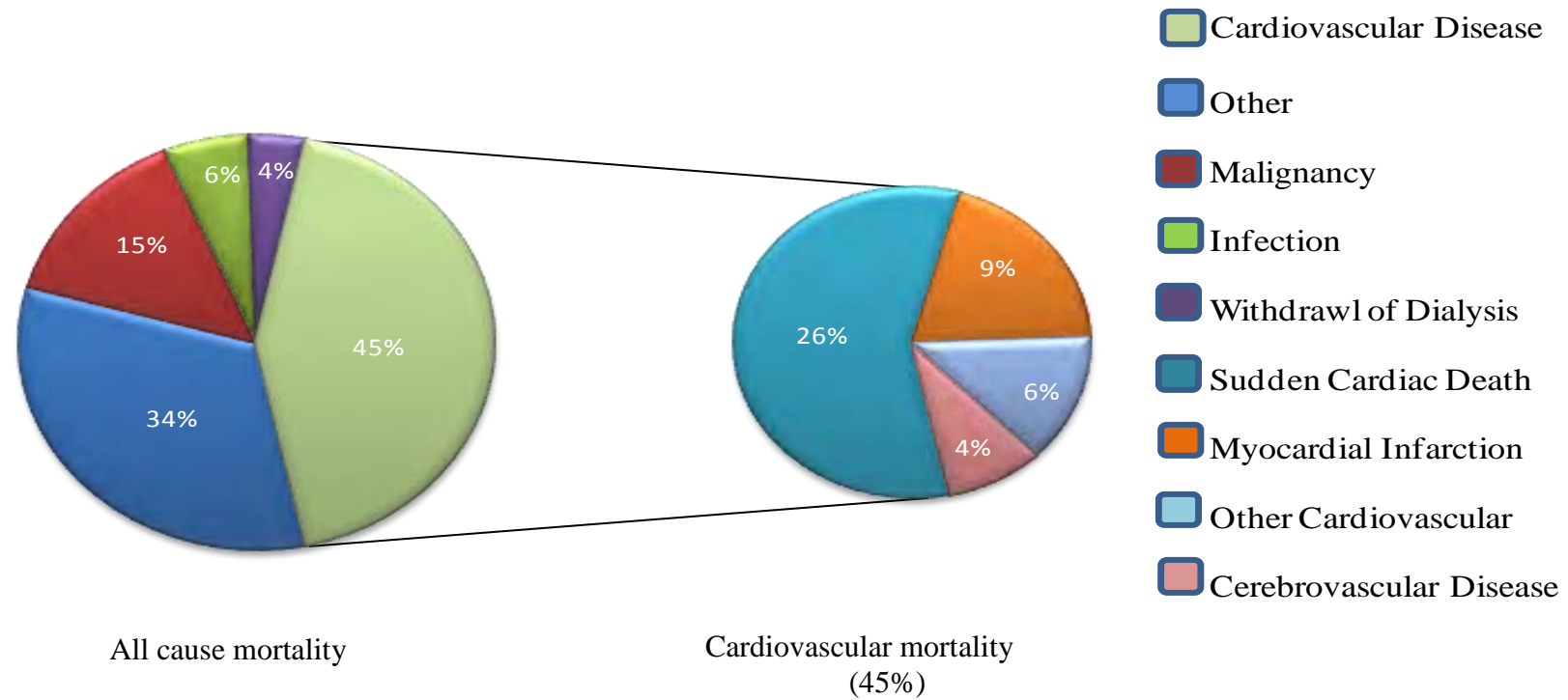
The younger the age group the greater the disparity



### **1.6.1 Cardiovascular risk in end-stage kidney disease**

In the 1990's, seminal epidemiological studies firmly established the extreme mortality rates associated with ESKD (Foley and Parfrey, 1998). The annual risk of mortality for patients on dialysis is approximately 20%; about half of these deaths due to cardiovascular disease (Figure 1.1). Age adjusted mortality is 10-100 times higher than in the general population. This risk is present throughout all age ranges but is most marked in the younger dialysis patients where cardiovascular mortality is up to 500 times that observed in age-matched normal population (Figure 1.2) (Levey et al., 1998). Symptomatic cardiovascular disease and structural cardiac abnormalities associated with an adverse prognosis, principally left ventricular hypertrophy (LVH), are prevalent in over two thirds of patients before the onset of RRT (and are discussed in more detail in section 1.7), and indicate that the mechanisms promoting accelerated cardiovascular disease are operating at much earlier stages of CKD (Foley et al., 1995a).

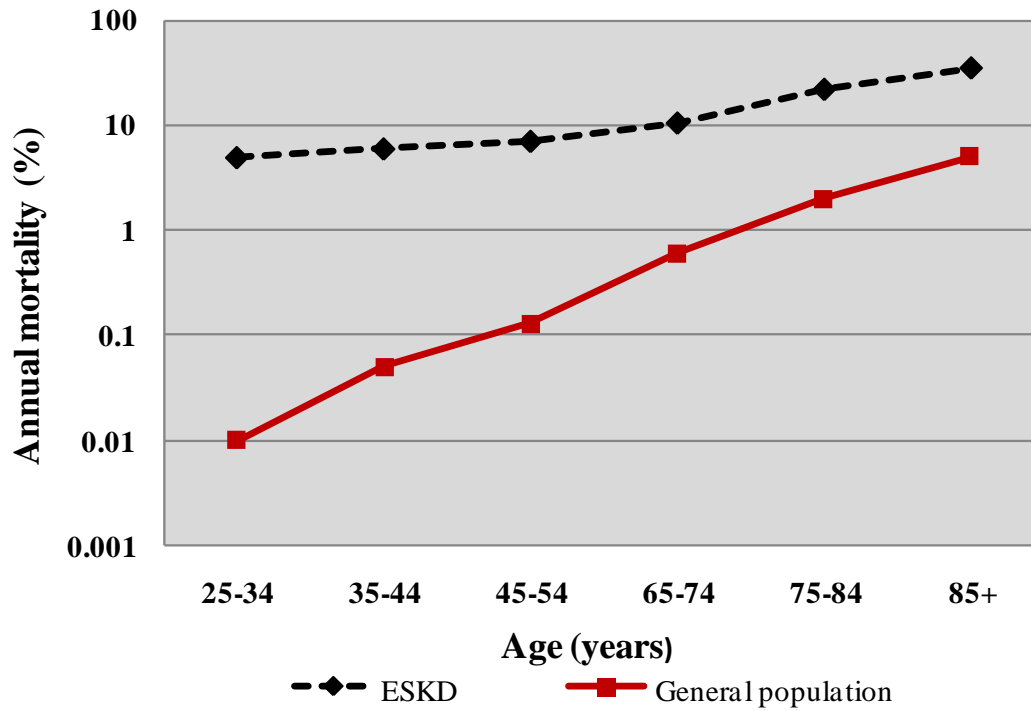
**Figure 1.1** Causes of death in end-stage kidney disease in USA between 2003-2006.



Data Source: USRDS. Adapted from Hage et al., 2009

Data are absolute percentages (%)

**Figure 1.2** Cardiovascular mortality in end-stage kidney disease on dialysis compared with the general population in the United States



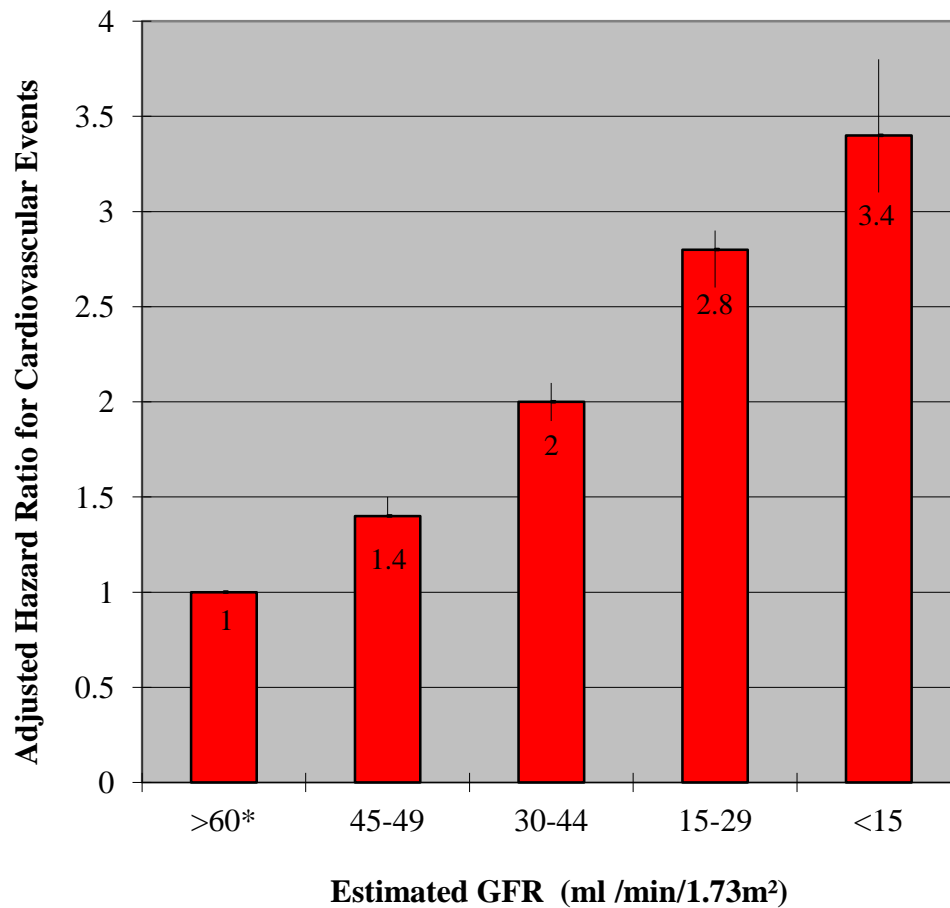
Adapted using data from Foley et al. (1998) (Foley et al., 1998)

CVD mortality defined by death due to arrhythmias, cardiomyopathy, cardiac arrest, myocardial infarction, atherosclerotic heart disease, and pulmonary oedema in the general population

### **1.6.2 Cardiovascular risk in early stage chronic kidney disease**

Recent observational data have confirmed that CKD confers an elevated risk of cardiovascular disease. A major study to demonstrate CKD (based on staging as defined by eGFR) as an independent predictor of cardiovascular mortality was a population based cohort study from the USA involving over 1 million low-risk adults (not treated by dialysis or transplantation) (Go et al., 2004). Go et al. estimated longitudinal GFR using the MDRD equation in patients who had serum creatinine measured between 1996 and 2000. Over a median 3 year follow-up, increased rates of death from any cause, cardiovascular events, and adverse hospitalisation outcomes exhibited an inverse graded relationship with eGFR below 60 ml/min/1.73m<sup>2</sup> (Figure 1.3) independent of other risk factors. This data is in keeping with the findings from the NHANES study demonstrating that patients with early stage CKD are at far higher risk of dying from cardiovascular disease than of ever progressing to ESKD (Coresh et al., 2007). Five year cardiovascular mortality rates for CKD stages 2, 3 and 4 were 19.4%, 24.5% and 19.9% respectively compared to the rate of progression to ESKD which only increased at more advanced stages of CKD; 1.1%, 1.3% and 45.7% respectively (Coresh et al., 2007).

**Figure 1.3** Adjusted hazard ratio for cardiovascular events among 1,120 295 adults according to estimated glomerular filtration rate (eGFR)



(Adapted from Go et al. 2004)

Data adjusted for demographics and co-morbidities. eGFR estimated from serum creatinine by abbreviated Modification of Diet in Renal Disease equation (MDRD).

\*This group serves as a reference group.

### **1.6.3 Glomerular filtration rates and cardiovascular risk in early stage chronic kidney disease**

At present, consensus from international organisations (NKF K/DOQI and Kidney Disease: Improving Global Outcomes) state that individuals with a GFR of  $\leq 60$  ml/min/1.73m<sup>2</sup> are at increased risk of cardiovascular morbidity and mortality, and that the risk increases with decreasing GFR below this level. The use of this “threshold value” is controversial as it may simply reflect the consistent use of a cut-off value  $\geq 60$  ml/min/1.73m<sup>2</sup> as the reference population in studies considering cardiovascular risk. Indeed two recent studies with long-term follow-up (median follow-up 7.9 and 10 years respectively) have suggested that cardiovascular mortality might be increased at even higher levels of eGFR (Matsushita et al., 2010; Van et al., 2007).

A collaborative meta-analysis derived from 21 multi-national studies and involving 1,234,182 participants from the general population assessed the independent relationship between eGFR, proteinuria and mortality (Matsushita et al., 2010). Using splines of eGFR; at 45 ml/min/1.73 m<sup>2</sup>, 60 ml/min/1.73 m<sup>2</sup>, 75 ml/min/1.73 m<sup>2</sup>, 90 ml/min/1.73 m<sup>2</sup>, and 105 ml/min/1.73 m<sup>2</sup>, rates of cardiovascular mortality increased progressively at levels of eGFR below 75 ml/min/1.73 m<sup>2</sup> independent of traditional cardiovascular risk factors and proteinuria. Mortality rates were disproportionately higher in participants with associated proteinuria and in younger participants ( $\leq 65$  years) at low eGFR. This study emphasises the additional important prognostic information associated proteinuria. An ACR higher than 3.4 mg/mmol was associated with a two-fold increase in mortality in all categories of eGFR except  $\geq 105$  mL/min/1.73 m<sup>2</sup> and an ACR  $\geq 1.1$  mg/mmol was a predictor of mortality independent of eGFR and risk factors (Matsushita et al., 2010).

The Belgian Inter-University Research on Nutrition and Health Population Study followed 8,913 apparently healthy participants for a total of 10 years (Van et al., 2007). Interestingly, the results suggested excess cardiovascular mortality began even earlier at an eGFR  $\leq 90$  ml/min/1.73m<sup>2</sup>, a level commonly regarded as indicating near normal renal function. The cardiovascular mortality risk increased linearly by 8% for every 10 mL/min/1.73 m<sup>2</sup> reduction in eGFR. This is a potentially very important finding given the high prevalence of eGFR values in this range, particularly in the elderly in whom the loss of renal function has hitherto been regarded as benign (Van et al., 2007).

Critics of the threshold value  $\leq 60$  ml/min/1.73m<sup>2</sup> emphasise the impact of the following observations:

i) Age- GFR is known to decline with age at an average rate of 1 ml/min/year between 40-80 years of age. As age and serum creatinine are the principle factors in determining eGFR calculated by the MDRD formula, it is possible to underestimate true renal function. Thus in studies with older cohorts such as that of Van Biesen et al. the association of cardiovascular mortality may be related to age rather than true GFR itself.

ii) The imprecision and under-estimation of eGFR by MDRD equation at levels  $\geq 60$  ml/min/1.73m<sup>2</sup> and over-estimation of eGFR  $\leq 30$  ml/min/1.73m<sup>2</sup> is well established (Poggio et al., 2005). These limitations of eGFR calculated by the MDRD equation may explain some of the heterogeneity between GFR and cardiovascular mortality at extremes of eGFR in the cohort studied by Matsushita et al.

iii) The rate of change in eGFR may be more important than a single threshold value in predicting cardiovascular mortality. In a prospective population based study involving over 13,000 participants without a history of heart disease, participants in the quartile with the

steepest annual decline in eGFR (average reduction > 5% / yr) had a higher risk for all-cause mortality. This relationship was present in patients with mild-moderate CKD as defined by eGFR 30-89 ml/min/1.73m<sup>2</sup> and was independent of eGFR and other known risk factors at baseline or follow-up (Matsushita et al., 2009).

These criticisms and publication of recent data have prompted proposals for lower eGFR thresholds or age / gender-specific thresholds values, and use of biomarkers such as cystatin C which might provide better estimates for clinical risk at eGFR  $\geq$  60 ml/min/1.73 m<sup>2</sup> (Shlipak et al., 2005).

## **1.7 THE CAUSES OF CARDIOVASCULAR DEATH IN CHRONIC KIDNEY DISEASE**

Cardiovascular disease is the commonest cause of death in the general population and in ESKD. In the UK, coronary heart disease is the most prevalent form of cardiovascular disease in the general population. It accounts for 46% of all cardiovascular deaths, equating to over 94,000 deaths per annum; 1 in 5 deaths in men and 1 in 7 deaths in women (British Heart Foundation, 2011). In contrast, the most prevalent cause of cardiovascular death in ESKD is sudden arrhythmic cardiac death (Figure 1.1). This is defined as “unexpected natural death from a cardiac cause within a short time period, generally less than an hour from the onset of symptoms”. In ESKD this accounts for approximately 60% of cardiovascular deaths (and 26% of total mortality) (Hage et al., 2009).

These observations suggest mechanistic differences in the nature of cardiovascular death in CKD. In the general population atheromatous coronary heart disease is the driving



pathology and standard cardiovascular risk factor assessments such as the Framingham risk score have proved accurate in predicting cardiovascular mortality rates. Furthermore, pharmacological treatments such as statins (which reduce total cholesterol and low-density lipoprotein levels) and anti-hypertensives agents, both of which reduce the “atheroma burden” have proved highly efficacious in reducing cardiovascular mortality.

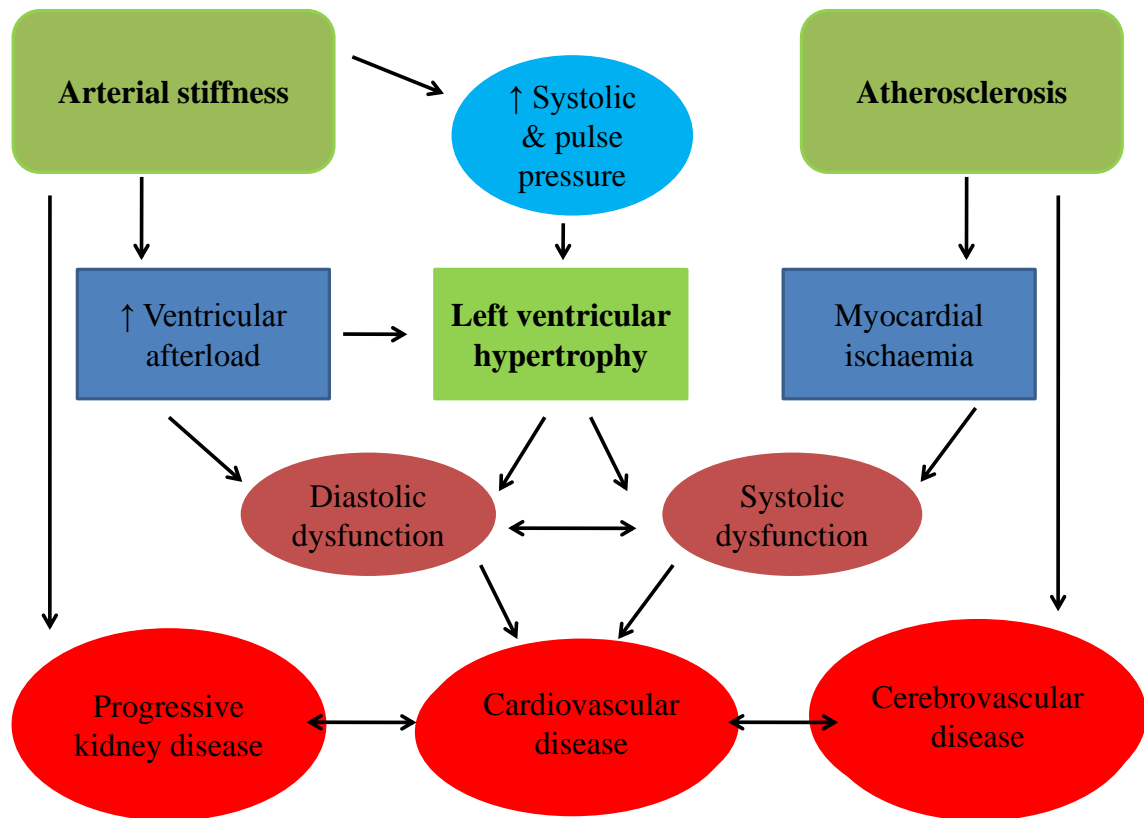
In CKD, non-atheromatous pathogenic processes such as arterial stiffening and abnormalities of cardiac structure appear to predominate and might explain why standard cardiovascular risk scores which assess “atheroma risk” have been shown to be inaccurate. Indeed, risk factors such as blood pressure, obesity and cholesterol have a negative association with mortality in ESKD (Baigent et al., 2000). This is thought to reflect the phenomenon of reverse causality whereby patients with the worst outcomes have chronic disease states characterised by low blood pressure and cholesterol. More recently, prospective cardiovascular screening of ESKD patients as part of a renal transplant assessment have provided further insight into the high prevalence and deleterious effects of non-atheromatous disease such as LVH and fibrosis (Mark et al., 2006).

Despite these observations and recognition from the NKF Task Force that patients with CKD are the “highest risk group for subsequent development of cardiovascular disease” (Levey et al., 1998), data regarding the pathogenesis of cardiovascular mortality in early stage CKD remain sparse.

## **1.8 THE NATURE OF CARDIOVASCULAR DISEASE IN CHRONIC KIDNEY DISEASE**

The nature of cardiovascular disease in CKD is characterised by pathological and clinical differences from that observed in the general population (Figure 1.4). These characteristic differences and their impact on cardiac mortality are outlined in the following three sections.

**Figure 1.4** Structural and functional changes within the cardiovascular system associated with chronic kidney disease.



### **1.8.1 Changes in cardiac structure and function - ureamic cardiomyopathy**

Abnormalities of cardiac structure – left ventricular hypertrophy (LVH), left ventricular (LV) dilatation and changes in cardiac function – left ventricular systolic dysfunction are common in ESKD. These changes were originally described in echocardiographic studies on patients starting dialysis. Foley et al. reported a prevalence of 74% for LVH, 32% for LV dilatation and 15% for LV systolic dysfunction and collectively termed these characteristic changes “ureamic cardiomyopathy” (UC). Only 16% of patients had normal echocardiograms (Foley et al., 1995a).

### **1.8.2 Left ventricular hypertrophy**

Left ventricular hypertrophy is the most prevalent sub-type of UC in ESKD (Foley et al., 1995a). It is present in all age groups including children treated with dialysis and is an independent predictor of cardiac death (Foley et al., 1995b; Mitsnefes et al., 2000).

The pathogenesis in ESKD is postulated to reflect an adaptive response that follows an increase in cardiac work from chronic pressure (hypertension, arterial stiffness) and volume overload (fluid status). The original descriptions of “early phase” LVH in response to increased systolic blood pressure, viewed the increase in ventricular wall thickening as a beneficial compensatory response to maintain wall tension. According to the Law of Laplace, wall tension is directly proportional to wall radius and inversely wall thickness; thus distributing tension amongst more sarcomeres, reduces the individual fibre load and maintaining wall stress and oxygen consumption (Sasayama et al., 1976). However, these “early beneficial changes” have now been challenged. Epidemiological studies have consistently demonstrated a continuous relationship between increased LV mass and adverse cardiovascular outcomes (Schillaci et al., 2000). Furthermore therapeutic strategies designed

to inhibit the development of LVH in response to pressure overload in animal models, prevented subsequent maladaptive changes such as LV dilatation and systolic dysfunction (Drazner, 2011; Hill et al., 2000). Thus, it is now evident that LVH at any stage is a highly adverse structural change and that arbitrary thresholds used to define the presence of hypertrophy based on levels of LV mass are an inappropriate method to define cardiac risk (Drazner, 2011).

Pathological changes of LVH include; i) an increase in the size of cardiomyocytes, ii) alterations in the extracellular matrix and subsequent development of fibrosis, iii) reductions in the intramyocardial microvasculature and myocardial capillary density leading to cardiomyocyte/capillary mismatch and development of chronic ischaemia (Amann et al., 1998). These changes promote a state of chronic small vessel myocardial ischaemia, myocyte death, progressive myocardial fibrosis, ventricular remodelling and systolic dysfunction, all of which act as substrates for ventricular dysfunction and increased risk of arrhythmias (London, 2003).

Histological abnormalities on myocardial biopsies from patients with ESKD but without coronary artery disease have provided further support for these pathophysiological mechanisms. These demonstrated severe myocyte hypertrophy, myocyte disarray and extensive interstitial fibrosis which were strong predictors of cardiac death (Aoki et al., 2005). In a further study of post-mortem myocardial biopsies from patients with ESKD, renal transplantation and CKD not requiring dialysis, diffuse non-coronary intermyocardiocytic fibrosis was demonstrated in over 90% of patients (Mall et al., 1990).

### **1.8.3 Re-defining ureamic cardiomyopathy**

Over the past decade, the use of cardiac MRI in ESKD has brought about an improved understanding of UC. Early studies used echo to calculate LV mass. However, this method relies on mathematical formulae incorporating LV internal cavity dimensions (such as end-diastolic diameter) and assumes a normal prolate ellipsoid shape of the LV. Both can be highly variable depending on the fluid status of the patient. Indeed, LV mass calculated before dialysis can lead to an over-estimation of LV mass by as much as 10g due to volume dependent errors (Hunold et al., 2003). Cardiac MRI allows direct measurement of LV mass and LV volumes and therefore avoids such geometric assumptions and mathematical errors. Furthermore, when combined with an intravenous gadolinium contrast agents, it is possible to detect areas of myocardial tissue damage such as infarction and fibrosis within the myocardium.

In a pivotal paper, Mark et al. used cardiac MRI and intravenous gadolinium contrast in 134 patients with ESKD undergoing renal transplant assessment (Mark et al., 2006). In keeping with previous echo studies, the investigators confirmed a high prevalence of UC with LVH as the predominant subtype; 72% of patients had LVH, 11% LV dilatation and 8% LV dysfunction. In addition, nearly one third of patients had evidence of myocardial scarring detected by late gadolinium enhancement. Two patterns were described; discrete subendocardial enhancement indicative of myocardial infarction was observed in 14.2% of patients. This appearance was associated with a history of ischaemic heart disease and conventional cardiovascular risk factors including cholesterol and diabetes but not blood pressure. Furthermore, these patients had greater LV mass, LV dilatation, LV systolic dysfunction and coronary artery disease on angiography than patients without this pattern of scarring. The second pattern of gadolinium enhancement was within the mid wall of the

myocardium. It was more diffuse in appearance and postulated to reflect areas of regional diffuse fibrosis. This pattern was associated with an increased LV mass but not with risk factors of atheromatous coronary artery disease, LV systolic dysfunction or angiographic coronary artery disease.

This study helped better define UC in ESKD. Firstly, abnormalities of cardiac structure and function are present in nearly 90% of patients with ESKD. The prevalence remained unchanged from the early studies in the 1990s using echo, despite modern pharmacological therapies for heart disease and anaemia. Secondly, LVH is the predominate sub-type of UC and is associated with the development of myocardial fibrosis but does not appear to be “driven” by atheromatous cardiovascular risk factors or coronary artery disease. Finally, LV dilatation and systolic dysfunction occur more often in patients with (often silent) myocardial infarction and ischaemia heart disease.

#### **1.8.4 The significance of increased left ventricular mass and prognosis**

The importance of defining the presence and degree of LVH has been known for over 30 years (Ghali et al., 1992;Levy et al., 1990). The Framingham Heart Study established increased LV mass as a predictor of cardiovascular morbidity and mortality independent of age, hypertension and other cardiovascular risk factors in the general population (Levy et al., 1990). Subsequent studies have confirmed increased LVH as a predictor of mortality in other disease states including myocardial infarction, congestive cardiac failure, stroke and hypertension (Ghali et al., 1992;Koren et al., 1991). Furthermore, pharmacological treatments which cause regression of LVH such as ACE inhibitors (Dahlof et al., 1992) and ARBs (Devereux et al., 2004), have been shown to improve cardiovascular outcomes independent of blood pressure lowering effects.

The classic paradigm of LVH is as an intermediate phenotype in the progression of heart disease. The subsequent cardiac structural and functional changes however, show considerable inter-individual variation. Pressure overload (hypertension) has been widely viewed as the major stimulus for the development of LVH but recent data have challenged this view showing that blood pressure only contributes about 25% to the variability in LV mass observed in the population (Fraser, 2003). Furthermore, LVH associated with a normal blood pressure confers the same cardiovascular risk as that with hypertension (Brown et al., 2000). These data support the view that LVH from any cause is a highly adverse structural change and might develop in response to mechanisms other than blood pressure.

Left ventricular hypertrophy in ESKD is also strongly linked to poor cardiovascular prognosis and is thought to reflect pathological changes within the myocardium, specifically the development of fibrosis (Aoki et al., 2005;Foley et al., 1995b). In an observational study of 432 patients commencing dialysis, median survival with LVH was 45 months compared with  $\geq 66$  months in patients with a normal echocardiogram (Parfrey et al., 1996). On dialysis LVH progresses over time and is associated with increased cardiac mortality independent of baseline LV mass and cardiac risk factors (Zoccali et al., 2004a). The degree of LVH is more strongly associated with hypertension and age and contrasts with the degree of LV dilatation and systolic dysfunction in which co-existing ischaemic heart disease is the primary predictor (Parfrey et al., 1996). Intensive treatment of risk factors known to promote LVH such as hypertension, have been shown to significantly reduce LV mass and cardiovascular mortality (London et al., 2001). To date, there is no comparable prognostic data for LVH in early stage CKD.



### **1.8.5 Detection of early changes in cardiac structure and function in end-stage kidney disease**

The echocardiographic and MRI studies described above have demonstrated that advanced changes in cardiac structure and function in ESKD are present in nearly 90% of patients and predict worse cardiac prognosis. In a recent 5 year prospective analysis of patients with ESKD, LV systolic dysfunction was the most powerful predictor of SCD with an ejection fraction  $\leq 48\%$  being the best cut-off threshold for predicting increased risk (Wang et al., 2010). These observations have prompted an increasing focus on identifying early structural and functional changes in the myocardium which might precede more advanced changes in ventricular function detectable using conventional measures such as ejection fraction.

The ventricular myocardium has a complex anatomical arrangement of myocardial fibres with longitudinal but slightly oblique orientated fibres in the subendocardium, circumferentially orientated in the mid-wall and an oblique orientation in the subepicardium. This arrangement is responsible for the twisting rotational motion of the ventricle in systole and untwisting in diastole (described in detail in chapter 2) (Sengupta et al., 2008).

The subendocardial longitudinal fibres which move the base of the heart toward a relatively fixed apex in systole (long-axis function), contribute approximately 60 % of left ventricular systolic ejection and the speed of relaxation is important for diastolic filling. However, the subendocardial longitudinal fibres are particularly vulnerable to changes in perfusion from coronary ischaemia, hypertrophy and fibrosis. Initially, mid-wall and epicardial function are often preserved or even increased to compensate for the reduction in longitudinal contraction and less sensitive “conventional” markers of systolic function such as ejection fraction are maintained. However, systolic and diastolic function are already

abnormal with compromised longitudinal contraction and relaxation of the LV and increased LV filling pressures (Mor-Avi et al., 2011).

These early abnormalities are detectable using newer echo techniques such as tissue Doppler imaging (TDI) and myocardial deformation imaging (analysis of ventricular mechanics or shape during the cardiac cycle using strain and strain rate) as well as MRI myocardial tagging sequences. Both imaging modalities provide a more comprehensive assessment of ventricular systolic and diastolic function and have been used to detect reductions in LV systolic function before the onset of symptoms in a number of disease states including systolic heart failure, heart failure with preserved ejection fraction, hypertension, hypertrophic cardiomyopathy and diabetes (Yu et al., 2007).

In CKD, studies using such techniques are limited. Rahkit et al. studied 129 patients with stage 4 and 5 CKD (62% on dialysis) with a normal LV mass and without evidence of myocardial ischaemia (on dobutamine stress echo) or LV dysfunction (mean ejection fractions 59%) (Rahkit et al., 2007). Using advanced echocardiographic techniques they demonstrated the presence of at least one “sub-clinical” abnormal change in left ventricular systolic function (strain and strain rate) or myocardial relaxation (tissue Doppler) in every patient. These changes independently predicted an increase in all-cause mortality and cardiac mortality over 2.4 years follow-up. Furthermore, patients who remained on dialysis over follow-up had further deterioration of these early changes before the onset of more characteristic features of UC. In contrast, 45 patients who underwent renal transplantation had a reduction in ventricular wall thickness, ventricular volume and an improvement in systolic and diastolic function suggesting that transplantation might reverse early features of UC associated with advanced CKD.

This study has further improved our understanding of the natural history of UC by identifying prognostically significant markers of reduced LV function before the onset of overt changes characteristic of UC. A strikingly similar pattern of “subclinical” changes have been demonstrated in other population groups including; the elderly, hypertension and heart failure with a normal ejection fraction suggesting that there might be a common aetiology. A characteristic feature in all these conditions is increased large artery stiffness (Blacher et al., 1999a; Lam et al., 2007; Mitchell et al., 2004). This has focused attention on the interaction between the LV and arterial system, termed arterial-ventricular interaction as a possible cause of cardiac dysfunction (Kass, 2002). Arterial-ventricular interaction is a key determinant of cardiovascular performance. In health, the properties of both systems are matched or coupled (as a ratio) to achieve optimal cardiac metabolic efficiency; an optimal transfer of blood from the left ventricle to the periphery without excessive changes in pressure and optimal stroke work. As the vascular tree becomes stiffer, the load on the heart increases such that the left ventricle must generate greater systolic pressures to maintain the coupling ratio. These compensatory adaptations initially maintain cardiac performance (and ejection fraction) through enhanced contractility at rest. However with chronic increases in ventricular stress (either with or without chamber hypertrophy), both systolic and diastolic ventricular stiffness increase and have detrimental effects on myocardial oxygen consumption, haemodynamic stability and cardiac reserve (Kass, 2002). To date, arterial-ventricular interaction has not been assessed in ESKD or earlier stages of CKD.

## **1.9 Coronary heart disease**

Coronary heart disease (CHD) is the second most common cause of cardiovascular death in ESKD after sudden cardiac death (Figure 1.1) (United States Renal Data System, 2010).

Acute myocardial infarction (AMI) is the primary mode of CHD death in ESKD. It accounts for approximately 9% of CHD deaths, less than in the general population where the rate is estimated at approximately 15-20% (British Heart Foundation, 2011). However, mortality rates after index AMI in ESKD are approximately double those in the general population over the subsequent two years (Herzog et al., 1998). Furthermore, revascularisation strategies such as percutaneous intervention and coronary artery bypass surgery do not significantly improve cardiac mortality rates in ESKD (Herzog et al., 2008). This data implies that while coronary artery disease is a significant burden in ESKD it is not the primary cause of cardiac death.

There are distinct morphological differences in the pathology of atherosclerotic coronary artery disease in ESKD compared to the typical fibroatheromatous plaques seen in the general population. Post-mortem quantitative and histopathological analysis of coronary arteries from patients with ESKD have demonstrated significantly increased intimal hyperplasia and increased numbers of densely calcified plaques (Schwarz et al., 2000). The plaques are seen to extend circumferentially as arcs as well extending into the medial arterial layer (often referred to by the unifying term Monckeberg's sclerosis). As a result the overall luminal area is significantly reduced promoting a chronic reduction in blood flow and hence ischaemia (McCullough et al., 2008). Histological evaluation of plaque composition has also shown lower levels of lipid and inflammation in contrast to fibroatheromatous plaques seen in the general population, postulated to account for lower rates of plaque rupture (Schwarz et al., 2000).

High levels of low density lipoprotein cholesterol (LDL-C) are a major cause of atherosclerosis in the general population but ESKD is characterised by very different lipid profiles with frequently low or normal total cholesterol and LDL levels. This in part may be explained by a “reverse causality” whereby the high burden of co-existing chronic illness including chronic inflammation and malnutrition can cause artefactual negative associations between standard risk factors and mortality. By correcting for non-specific markers of chronic ill-health such as albumin, a direct relationship with outcome may be re-established. In addition, ESKD is characterised by low levels of high density lipoprotein cholesterol (HDL-C) and increased triglycerides rich low density lipoprotein cholesterol which reflect preceding alterations in the apolipoproteins (Apo) metabolism and composition (Quaschnig et al., 2001).

These difference might explain why mortality reductions observed using LDL-C lowering statin therapy in the general population in patients with or at risk of cardiovascular disease (MRC/BHF, 2002) have not been replicated in ESKD (Tonelli et al., 2004). The AURORA (Fellstrom et al., 2009) and 4D (Wanner et al., 2005) studies have prospectively randomised over 4,000 patients receiving dialysis (a third with diabetic ESKD) to receive rosuvastatin or atorvastatin respectively or placebo. Both studies demonstrated significant and sustained reductions in LDL-C without reductions in cardiovascular mortality. These data have highlighted two important points. Firstly, the pattern of pathogenic mechanisms driving cardiovascular death in ESKD is different from the general population. Secondly, these findings in ESKD contrast to data from retrospective sub-group analyses of trials using statins in patients with earlier stages of CKD where the cardiovascular benefits of statins are comparable to those observed in the general population (Tonelli et al., 2004). This raises the question of whether statin therapy become less effective with advancing kidney disease.

Recent data from the Study of Heart and Renal Protection (SHARP) study has helped address this latter point by prospectively recruiting over 9,000 patients over a spectrum of renal dysfunction; dialysis-dependent ESKD and with moderate-severe CKD defined as a serum creatinine  $\geq 150$   $\mu\text{mol}$  in men and  $\geq 130$   $\mu\text{mol}$  in women (Baigent C, 2010). The combination of simvastatin and ezetimibe significantly reduced the primary outcome measure of major atherosclerotic events (a composite of coronary death, myocardial infarction, stroke and revascularisation) in all patients although the greatest reduction was observed in the non-dialysis patients over 5 years of follow-up compared to placebo. Thus the different pathogenic mechanisms which predominate at earlier stages of CKD may alter with advancing disease such that atheromatous disease makes a lesser contribution. Secondly, statins may improve outcomes by effects on non-atheromatous pathogenic mechanisms such as endothelial function and arterial stiffness (Baigent C, 2010).

### **1.10 Arterial stiffness and arteriosclerosis**

Increased arterial stiffness is the third distinct feature of cardiovascular disease in CKD. In ESKD, arterial stiffness and specifically aortic stiffness is significantly increased compared to healthy controls matched for age and blood pressure (Ibels et al., 1979) and is a powerful independent prognostic marker of all-cause and cardiovascular mortality (Blacher et al., 1999b).

### **1.10.1 Arterial functions**

The major functions of the aorta and large arteries are:

- i) Conduit function – to deliver an adequate supply of blood to peripheral organs and tissues to meet metabolic demands. In health, changes in the width of the arterial lumen and a low peripheral arterial resistance allow rapid changes in arterial flow to ensure supply meets metabolic demand (McEniery et al., 2007).
- ii) “Buffer” or dampen oscillatory changes in blood pressure that result from intermittent ventricular ejection (known as the Windkessel effect). In health, the highly distensible arterial system ensures that most peripheral tissues receive near steady flow with no exposure to peak systolic pressures and almost no reduction in diastolic pressure from ascending aorta to peripheral arteries. This is achieved by approximately 50% of stroke volume reaching the peripheral tissues in systole while the remaining stroke volume distends the large capacitance arteries such as the aorta. In diastole, energy transferred to the arterial walls makes the aorta recoil and “squeezes” blood into the peripheral tissue ensuring continuous perfusion. Changes in the visco-elastic properties of the arterial wall which occur with age and disease states make the artery stiffer and alter the dampening function (McEniery et al., 2007). The pathology of these changes and patho-physiological consequences are discussed in sections 1.7.3.2 – 3.

Normal arterial function is dependent on the ability of the arteries and more specifically the visco-elastic properties of the wall, to accommodate ventricular ejection. These properties can be assessed using different indices including: i) compliance; a measure of the local vessel capacity to respond to changes in blood volume, ii) distensibility; a measure of the elastic properties of an artery and iii) Young’s elastic incremental modulus ( $E_{inc}$ ); direct information

on the intrinsic elastic properties of the materials that make up the arterial wall independently of vessel geometry. These are differentiated in Table 1.3.



**Table 1.3** Indices of arterial stiffness calculated from geometric measurements of large arteries

| <b>Term</b>                               | <b>Definition</b>                                                                                                                                            | <b>Formula</b>                                        |
|-------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| <b>Distensibility</b>                     | A measure of the elastic properties of an artery: relative change in diameter (or area) for a given pressure change                                          | $\Delta A/A \times \Delta P$                          |
| <b>Compliance</b>                         | A measure of the local vessel capacity to respond to changes in blood volume: Absolute diameter (or area) change for a given pressure step                   | $\Delta A / \Delta P$                                 |
| <b>Pulse wave velocity</b>                | A measure of the speed of a forward pressure wave propagated along the arterial tree determined by the geometric and elastic properties of the arterial wall | $D / \Delta t$                                        |
| <b>Elastic modulus (elasticity)</b>       | The pressure change driving an increase in relative lumen diameter (or area)                                                                                 | $A \times \Delta P / \Delta A$                        |
| <b>Young's incremental modulus (Einc)</b> | Evaluates the elastic properties of the material of the arterial wall. Elastic modulus per unit area                                                         | $(\Delta P \times A) / \Delta A \times h$             |
| <b>Moens-Korteweg equation</b>            | Intrinsic elastic properties of arterial wall material (stiffness / thickness)                                                                               | $\sqrt{(V \times \Delta dP / \rho \times \Delta dV)}$ |

Adapted from Laurent S et al. 2006

$\Delta P$  = pulse pressure,  $\Delta A$  = change in area,  $\Delta t$  = time,  $D$  = distance,  $v$ ; volume,  $\Delta dP$ ; change in pressure driving the change in volume,  $\Delta dV$ ; change in arterial volume,  $\rho$  density of fluid,  $h$ ; wall thickness

### **1.10.2 Pathology of arterial stiffness in chronic kidney disease**

The elastic properties of the arterial system are heterogeneous with more elastic central arteries and stiffer peripheral arteries reflecting differences in cellular and histological structure of arterial wall at different levels. In health, the central arteries such as the aorta have a high elastin-collagen ratio and a decreased influence of smooth muscle tone compared to the peripheral arteries. However, with ageing (particularly after the 5<sup>th</sup> decade) stiffness of the central arteries increases reflecting characteristic changes of; progressive physiological arterial dilatation (arterial remodelling), diffuse hypertrophy and degeneration of the elastic properties of the medial arterial layer (Avolio et al., 1983). These changes are commonly termed arteriosclerosis and result in a two-fold increase in stiffness between the ages of 20 and 80 years when assessed using pulse wave velocity (PWV). Furthermore, increased stiffening reduces the dampening function and results in various adverse haemodynamic consequences including; increased pulse pressure and systolic hypertension. In contrast, the peripheral arteries change little with ageing, which reflects the lower elastin to smooth muscle ratio and lower pulsatile pressure stress.

In ESKD, the physiological processes seen with ageing are accelerated compared to healthy age and blood pressure matched controls. Remodelling in the capacitance arteries is characterised by a predominance of arterial dilatation (secondary to the inability of elastic fibres to sustain pulsatile stress) and to a lesser extent by intimal-medial hypertrophy (Ibels et al., 1979). Furthermore, quantitative and qualitative alterations in the intrinsic properties of arterial wall materials have been observed in CKD. These changes include; diffuse fibro-elastic intimal thickening, hyperplasia and hypertrophy of vascular smooth muscle cells (VSMC), increased medial collagen content, fragmentation of elastic lamellae secondary to mechanical failure and fatigue, fibrosis and calcification of the internal elastic lamellae and

non-enzymatic cross-links between microfibrils (London, 2000;Schwarz et al., 2000). These changes increase arterial stiffness and contribute to creating and amplifying a pressure load. They contrast with increased stiffness in essential hypertension which is driven by the distending pressure rather than changes in wall composition and architecture (Laurent et al., 1994).

According to Laplace's Law, the arterial wall and lumen ratio should increase in proportion to an increase in blood pressure in order to maintain wall stress. However, in ESKD the dilatation predominates and circumferential wall stress remains chronically elevated reflecting abnormalities of smooth muscle cell growth and migration, and production of extracellular matrix in response to diameter enlargement (Blacher et al., 1998). It is important to emphasise that these changes are apparent in the early stages of CKD (Briet et al., 2006) and in muscular brachial and radial arteries which are not typically affected by atherosclerosis or ageing processes. Furthermore no consistent associations have been established between arterial stiffness and common atherogenic risk factors including lipids suggesting that arterial wall properties in CKD are affected by other factors related to uraemia. These will be discussed in more detail in section 1.8.

### **1.10.3 The pathophysiological consequences of arterial stiffness**

Arterial dampening function is impaired with increasing aortic / arterial stiffness such that the more rigid aorta that is less able to accommodate the volume of blood ejected by the left ventricle. The pathophysiological consequences include: (London et al., 2000;O'Rourke and Safar, 2005):

- i) An increase in left ventricular afterload which promotes the development of LVH and increased myocardial oxygen demand.

- ii) Increased systolic pressure and pulse pressure, both powerful predictors of cardiovascular mortality. A wide pulse pressure is transmitted to more peripheral arteries, reducing shear stress and promoting remodelling with an increase in intimal-medial thickness.
- iii) Reduced coronary perfusion secondary to lower diastolic pressure. Reduced sub-endocardial perfusion promotes chronic ischaemia and fibrosis.
- iv) Microvascular damage to smooth muscle and endothelial cells in the renal and cerebral arteries. This reflects continuous perfusion throughout systole and diastole and low peripheral resistance which exposes these arterial beds to increased pulsatile pressures.
- v) Increased arterial stiffening augments cyclical stresses within the wall, accelerating elastic fibre fatigue–fracture and further stiffening the vessel.

An improved understanding of the pathophysiology of large artery stiffness and its risk factors may help in the prevention and progression. The increasing use of different indices of arterial stiffness such as central pressure, aortic augmentation index and aortic pulse wave velocity have identified differences in function and therapeutic responses between arterial beds which are not evident using brachial cuff sphygmomanometry. In the Conduit Artery Function Evaluation (CAFE) study of patients with hypertension, treatment with amlodipine and perindopril significantly reduced central aortic pressure compared to atenolol and thiazide-based therapy despite similar differences in brachial blood pressure (Williams et al., 2006). In ESKD, use of ACE inhibitors have been shown to attenuate aortic stiffness and improve cardiovascular outcomes (Guerin et al., 2001) while peripheral conduit stiffness has no prognostic value (Pannier et al., 2005).

#### **1.10.4 Explanatory models of the physiological mechanisms underlying arterial stiffness**

Two main models / theories exist to explain the physiological mechanisms underlying arterial stiffness and the accompanying increase in systolic pressure (Laurent et al., 2006). The European Network for Non-invasive Investigation of Large Arteries consensus document published in 2006 supported the use of the wave propagation model. This provides an assessment of regional arterial stiffness at various sites along the arterial tree. It is based on the concept of a simple distensible tube which terminates at a point where peripheral resistance exceeds forward pressure wave. The elastic properties of the tube permit the generation of a pressure wave along the tube which is inversely related to the distensibility of the tube.

At a physiological level, a forward wave of ejected blood from the left ventricle is propagated through the arterial tree. A reflected wave is generated in peripheral muscular arteries or sites of arterial bifurcation where pressure exceeds that of the forward wave. In healthy elastic arteries, the reflected wave returns in the aortic root in diastole thereby increasing central diastolic pressure and augmenting coronary artery perfusion. In stiff arteries, the reflected wave returns earlier in systole with more overlap between forward and reflected waves thereby augmenting systolic pressure, pulse pressure and ventricular afterload.

An alternative model of the arterial tree is the Windkessel model which provides a global assessment of systemic arterial stiffness. The model combines two functional properties of the arterial tree - a reservoir function (aorta) which cushions the pressure fluctuations of ventricular ejection and a wide bore pipe acting as a conduit which tapers to the smaller muscular peripheral arterioles which act as resistance vessels to ensure constant

peripheral flow with little reduction in diastolic pressure throughout the arterial tree. When conduit resistance increases, both systolic and diastolic pressures increase but with an additional reduction in compliance of the reservoir, there is a disproportionate increase in systolic pressure.

Both models have theoretical limitations which continue to be debated in the literature(O'Rourke et al., 2010). A recent meta-analysis examining the change in blood pressure with ageing has contentiously challenged the mechanistic importance of the reflected wave theory. The authors evaluated 64 studies involving over 13,000 patients with various diseases and showed that the reflected waves always returned in systole regardless of age and could not solely explain the augmentation of systolic pressure which occurs with ageing and disease (Baksi et al., 2009). The authors proposed a more direct mechanism (based on the Windkessel model) relating to the reduction in aortic compliance with age and hence the reduced buffering capacity to absorb pulsatile LV ejection. However, supporters of the wave propagation model have questioned these data, specifically the interpretation of timing of the returning wave and the confounding nature of different diseases states included in the meta-analysis (O'Rourke et al., 2010).

#### **1.10.5 Assessment of arterial stiffness**

Indices of arterial stiffening can be assessed by a variety of invasive and non-invasive techniques. Cardiac MRI and ultrasound derived distensibility and compliance are used to assess discrete arterial locations at a point in time. Pulse wave velocity techniques which follow the propagative model provide an assessment of regional function. The modified Windkessel model and wave reflection techniques allow a systemic compliance to be assessed.

Recommendations from the European Network for Non-invasive Investigation of Large Arteries Consensus document in 2006 suggest combined measurement of aortic PWV (a direct measure) coupled with central pressures and arterial wave reflection indices as the most comprehensive approach to arterial function. These data are complementary; a low PWV from an elastic artery generally results in the later arrival of the reflected wave in the aorta while a stiff artery increases the transmission velocity of both forward (PWV) and reflected waves which return earlier in systole and augment aortic pressure in systole. Both techniques are independent predictors of cardiovascular mortality in longitudinal studies of patients with ESKD (Blacher et al., 1999b;Safar et al., 2002b)

#### **1.10.5.1 Pulse wave velocity**

The measurement of PWV is generally accepted as the “gold standard” measurement of arterial stiffness being simple, non-invasive, robust and reproducible. It is based on the principle that the forward pressure wave generated by ventricular ejection is propagated along the arterial tree at a speed determined by the geometric (diameter) and elastic properties (elastic modulus and/or wall thickness) of the arterial wall. In simple terms, the stiffer the vessel the faster the wave travels and the higher PWV. This can be calculated by the Moens-Korteweg equation or by the simpler Bramwell-Hill equation in which PWV is inversely related to the distensibility of the arterial tube, expressed as:

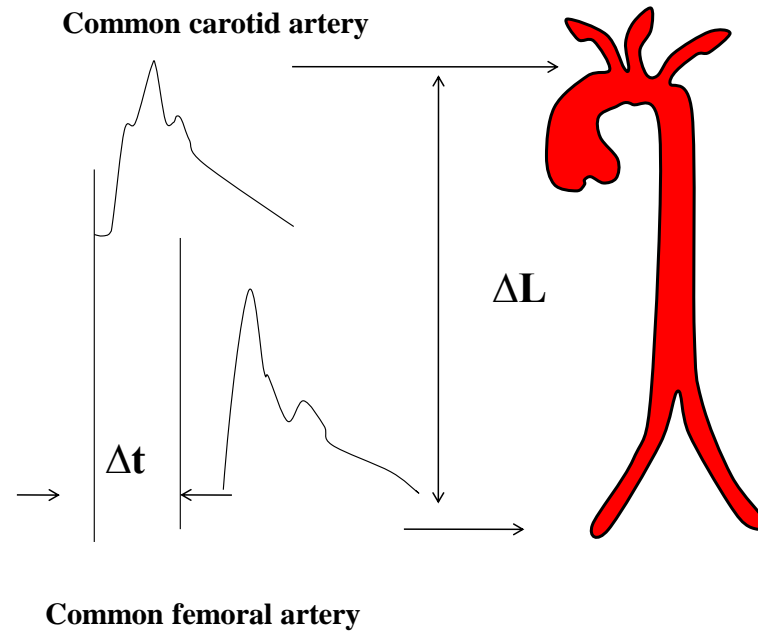
$$PWV = \sqrt{V \cdot dP / \rho \cdot dV}$$

where dV is the change in arterial volume, dP is the change in arterial pressure and  $\rho$  density of fluid.

The technique relies on the detection of two pressure waveforms measured at a known distance apart, with PWV calculated as distance (metres) / time (seconds) and expressed as m/s (Figure 1.5). Several devices, techniques and locations have been described including the use of pressure transducers, Doppler ultrasound, applanation tonometry, magnetic resonance imaging and oscillometric pressure cuffs. However, measurement between the carotid and femoral pulses (Ca-Fem PWV) is recognised as the most reliable and reproducible measure of regional arterial stiffness and is highly predictive of cardiac mortality (Laurent et al., 2006).



**Figure 1.5** Schematic of diagram of the measurement of carotid-femoral pulse wave velocity using the foot-foot method



Adapted from Laurent et al. 2006

$PWV \text{ (m/s)} = \Delta L / \Delta t$  where;  $\Delta L$  = distance between the carotid artery site and femoral artery site, minus the distance between the sternal notch and carotid artery site.  $\Delta t$  = time between the foot of the carotid arterial up-stroke and foot of the femoral artery up-stroke

### **1.10.5.2 Pulse wave analysis**

Measurement of central aortic pressures by pulse wave analysis (PWA) provides an indirect surrogate measure of aortic stiffness and represents the true load “seen” by the ventricle and proximal aorta. The central arterial pressure waveform is a composite of the forward pressure wave derived from ventricular ejection and a reflected wave generated from the periphery either at a branch point or at a site where impedance exceeds forward pressure (Figure 2.3). In addition to derived central aortic pressures, the generated central waveform provides information on; the amplification or augmentation of central systolic pressure (augmentation pressure) and the augmentation index which is the ratio of augmented pressure to pulse pressure (Agabiti-Rosei et al., 2007).

Several non-invasive techniques have been developed which derive central pressures and arterial waveforms from analysis of applanated peripheral arteries (usually radial or carotid artery) using pencil-like micromanometers which incorporate a strain gauge transducer. Each device applies a “transfer function” which describes the mathematical model used to non-invasively derive a central aortic pressure wave form from peripheral tonometry pressure data. This defines the relationship between the peripheral and central pressures using the properties of the connecting arterial system (Chen et al., 1997). The characteristics of the transfer function are determined by the physical properties of the connecting arterial system including diameter, elasticity, wall thickness and branching pattern. Despite individual differences in such properties, studies validating the transfer function found that the main components did not change markedly in adults allowing a generalised transfer function to be applied rather than a requirement of an individual transfer function (individualised to a particular subset of patient characteristics).

There are limitations to the technique; radial tonometry is most accurate when calibrated against intra-arterial pressure. Practically however, it is necessary to use cuff-derived brachial artery pressures as surrogates of radial artery pressures which might introduce some error for calibration of central pressures. This is particularly true in young subjects in whom the amplitude of the pressure wave is higher in the peripheral arteries than the central arteries - the so-called amplification phenomenon. Differences in heart rate must be corrected. An inverse linear relationship exists between heart rate and augmentation index with an approximate 4% reduction for each 10 beat increase. This reflects a reduction in the duration of systole such that the reflected wave is effectively shifted into diastole and reduces the augmentation index. Other sources of variability include; interpretation of the first systolic shoulder (dependent upon the amplitude of the forward wave), gender and height differences (females are generally shorter and have earlier reflection bifurcation points).

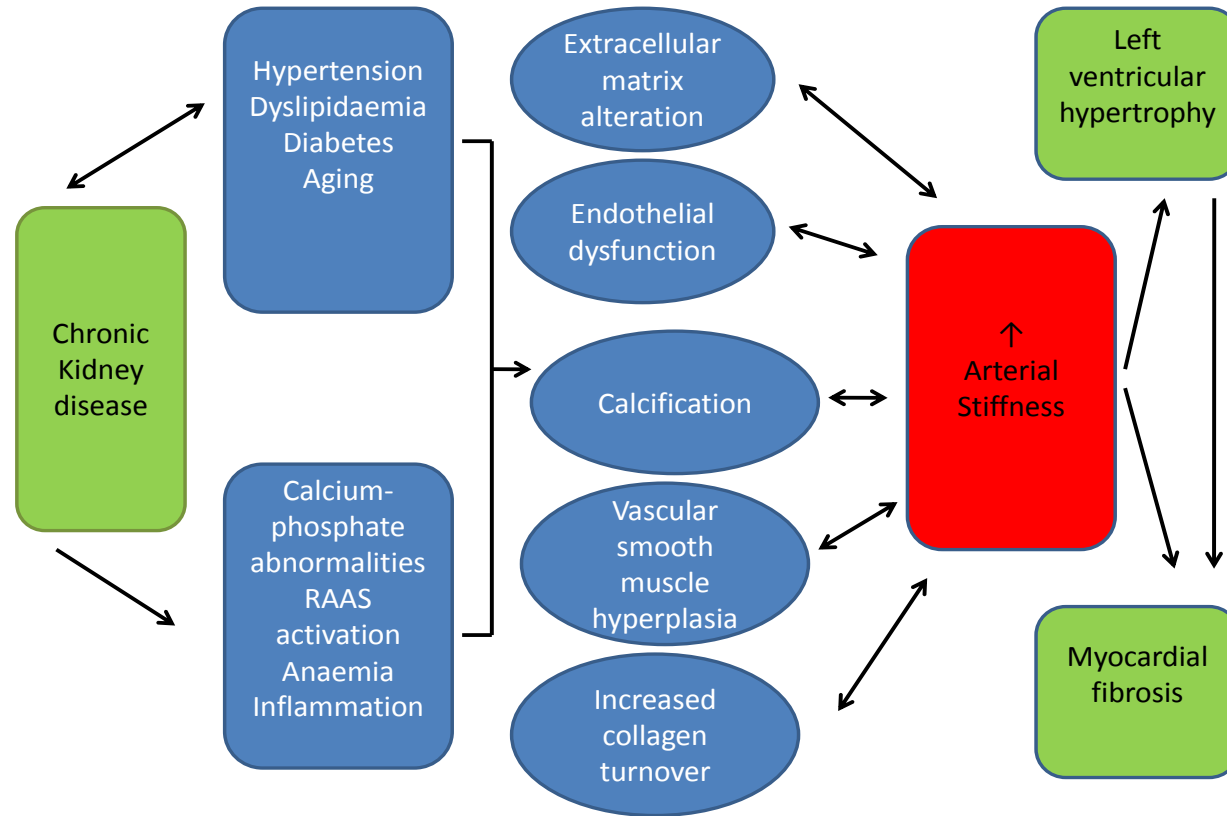
## **1.11 POTENTIAL MECHANISMS PROMOTING ARTERIAL STIFFNESS IN CHRONIC KIDNEY DISEASE**

There are many potential inter-related mechanisms proposed to explain the relationship between increased arterial stiffness and CKD. A summary of the mechanisms known to affect arterial functional and structural / mechanical properties based on the existing evidence base is presented in the following section (Figure 1.6). The role of the renin-angiotensin-aldosterone system will be discussed in more detail in section 1.9.

### **1.11.1 Hypertension**

Hypertension is one of the major risk factors for the development and progression of arterial stiffness. It is both a cause of arterial stiffness and a consequence of its development. Identifying the primary stimuli is challenging with many contributory factors including aberrant activation of the renin-angiotensin system promoting sodium retention and plasma volume expansion and over-activity sympathetic nervous causing vasoconstriction. These haemodynamic alterations provide the signals (cyclic tensile stress and shear stress) for arterial remodelling (London et al., 2004). According to Laplace's Law ( $\sigma = Pr/h$ ) tensile stress ( $\sigma$ ) is directly proportional to arterial transmural pressure (P) and radius (r) of the vessel and inversely proportional to arterial wall thickness (h). Thus to maintain stable tensile stress following an increase in systolic blood and arterial dilatation, the arterial wall must hypertrophy and shear stress which is directly proportional to blood flow and blood viscosity must also increase. As a result, characteristic changes in the intrinsic properties of the arterial wall and geometry of the vessel are observed (as described in section 1.7) which increase arterial stiffness.

**Figure 1.6** Potential mechanisms linking chronic kidney disease and the development of arterial stiffness



There are distinct differences between arterial stiffness in essential hypertension and arterial stiffness in CKD: i) Aortic stiffness in CKD is significantly higher than in hypertensive patients matched for age and blood pressure ii) CKD is characterised by a predominant increase in arterial dilatation and to a lesser degree intimal-medial hypertrophy, whereas in hypertension arterial wall thickening predominates. This reflects intrinsic changes in the elastic properties of the wall in CKD including calcification of elastin fibres which results in an inability of elastic fibres to sustain physiological pulsatile stress. These data suggest that mechanisms other than the haemodynamic stimulus of increased systolic pressure are involved in promoting stiffness in CKD (Briet et al., 2006).

### **1.11.2 Diabetes mellitus**

Diabetes is the leading cause of CKD worldwide and accounts for 40% of patients requiring dialysis (United States Renal Data System, 2010). It is well established that diabetic patients have increased arterial stiffness (Aoun et al., 2001) which increases with advancing stages of CKD (Kimoto et al., 2006) and predicts cardiac mortality (Shoji et al., 2001). Diabetes promotes accelerated arterial stiffness by several mechanisms including: i) hyperglycemia-induced endothelial dysfunction: results from decreased production of nitric oxide (NO), inactivation of NO by oxygen-derived free radicals, and/or increased production of endothelium-derived contracting factors, which oppose the protective activity of NO (Cosentino and Luscher, 1998), ii) inflammation (discussed in section 1.8.4), iii) accumulation of advanced glycation end products (AGE). In brief, AGE are a heterogeneous group of compounds, formed by non-enzymatic oxidative and non-oxidative reactions between proteins and sugar residues (known as the Maillard reaction). The final step is catalysed by oxidative stress which cause increases in AGE. Accumulation of AGEs causes

excessive irreversible covalent cross-linking in connective tissue, extracellular matrix and between collagen and elastin which alter the mechanical properties of the vasculature (Airaksinen et al., 1993). Furthermore, AGE activate the AGE receptors which promote increased fibrosis via the up-regulation of transforming growth factor- $\beta$  (TGF- $\beta$ ). AGE-accumulation in CKD is postulated to occur by two different pathways; decreased renal excretion of AGE degradation products and increased oxidative stress. In turn, an increase in AGEs causes acceleration of oxidation and further increasing AGEs. Accumulation of circulating AGE in ESKD has been independently associated with increased aortic PWV and mortality (Ueno et al., 2008).

### **1.11.3 Endothelial dysfunction**

The endothelium is an important regulator of arterial stiffness (Wilkinson et al., 2004) and arterial stiffening affects endothelial mechano-signalling and cytoprotection to oxidant stress through reduced expression of nitric oxide synthase (Endemann and Schiffrin, 2004). These effects create a self-perpetuating vicious circle of structural and functional vascular injury.

The endothelium releases a number of mediators including NO and endothelin-1 (ET-1) which influence the smooth muscle cells and thereby affect the elastic and collagenous fibres of the arterial wall. An extensive evidence base have confirmed endothelium-derived NO to have a pivotal effect in controlling resting tone in resistance vessels and regulating large artery stiffness (Wilkinson et al., 2002b). In animal models in which the NO synthase (NOS) gene was “knocked out” pulse pressure was seen to be disproportionately increased compared to 24 hour blood pressure, suggesting a greater effect on large arterial stiffness than peripheral resistance (Van Vliet et al., 2003). In vivo studies using NO donors such as glyceryl trinitrate have been shown to reduce augmentation index in healthy volunteers and

disease states such as hypertension (Wilkinson et al., 2002a), while exogenous inhibitors of endothelial NOS (eNOS) such as L-NG-monomethyl-L-arginine (L-NMMA) increased aortic and systemic arterial stiffness to a similar extent as has been reported with noradrenaline and angiotensin II (Wilkinson et al., 2002b).

Early stage CKD is characterised by impaired endothelium dependent vasodilator activity and enhanced endothelium dependent vasoconstriction (Endemann & Schiffrin, 2004). Although endothelial dysfunction might reflect relatively high levels of oxidative stress from conventional cardiovascular risk factors such as hypertension, newer risk factors such as asymmetrical dimethylarginine (ADMA), ET-1 and isoprostanes have been shown to independently predict arterial stiffness and endothelial dysfunction respectively in early CKD (Lilitkarntakul et al., 2011).

Reductions in the clearance of endogenous inhibitors of nitric oxide synthase such as asymmetrical dimethylarginine (ADMA) have received considerable attention in CKD. It is synthesised in the heart, endothelium and smooth muscle cells and levels are inversely related to GFR reaching 7.5 times above normal limits in ESKD (Vallance et al., 1992). Furthermore, ADMA has been shown to independently predict total mortality, arterial stiffness cardiovascular complications and progression of renal disease (Zoccali et al., 2002).

Endothelin peptides such as ET-1 which act on VSMC to cause vasoconstriction have also been implicated in the progression of CKD. In a recent study of patients with early stage CKD and minimal co-morbidity, ET-1 was inversely associated with creatinine clearance and independently predicted endothelial dysfunction assessed by brachial artery flow-mediated dilatation (Lilitkarntakul et al., 2011). Furthermore, short-term endothelin-A receptor antagonism in non-diabetic CKD reduced proteinuria and arterial stiffness independently of a change in blood pressure (Dhaun et al., 2009).



Inflammation also impairs endothelial function although the exact mechanisms remain uncertain. In vitro studies have postulated a role for inducible nitric oxide synthase which might promote endothelial dysfunction by depleting the bioavailability of eNOS (Gunnnett et al., 2005). C-reactive protein (CRP) has also been shown to independently predict endothelial function in inflammatory conditions such as rheumatoid arthritis and might reflect a direct effect on NO bioavailability possibly by decreasing eNOS expression (Venugopal et al., 2002).

#### **1.11.4 Inflammation**

Chronic kidney disease is associated with a chronic state of low-grade inflammation (Stenvinkel et al., 2005). Serological levels of circulating inflammatory biomarkers such acute phase reactants including CRP and other pro-inflammatory biomarkers such as cytokines (interleukins, tumour necrosis factor- $\alpha$ ) are increased in up to 50% of patients with ESKD (Jofre et al., 2006). C-reactive protein provides an overall measure of systemic inflammatory activity with levels reported as high as 10-times above the general population. It has been shown to independently predict an increase in cardiovascular mortality in both ESKD (Wanner and Metzger, 2002) and at earlier stages of CKD (Menon et al., 2005), although a direct cause-and-effect relationship for such biomarkers is yet to be established.

Inflammation is a recognised risk factor for arterial stiffness in CKD (Cachafeiro et al., 2008) but identification of specific mechanisms of injury in renal disease are complicated by the presence of co-existing diseases such as diabetes and hypertension which are also associated with low-grade chronic inflammation. Postulated inflammatory mechanisms which might increase arterial stiffness in CKD include; reduced circulating levels of potent inhibitors of vascular calcification, such as fetuin-A, matrix Gla protein (MGP) and

osteoprotegrin, (Cachofeiro et al., 2008) accumulation of AGE which are normally renally excreted (discussed in section 1.8.2) and increased oxidative stress leading to increase production of reactive oxygen species and endothelial dysfunction (described in section 1.8.3).

#### **1.11.5 Alterations in the extracellular matrix**

As discussed in section 1.7.3.2, arteriosclerosis is prominent in CKD and has an important role in the pathogenesis of arterial stiffness. It is characterised by increases in the extracellular matrix volume, vascular smooth muscle and collagen in the medial arterial layer. The mechanisms responsible are yet to be fully elucidated but appear to be independent of increases in blood pressure (Amann et al., 1995). Matrix metalloproteinases (MMPs) are up-regulated in CKD and have been implicated as a potential mechanism of this adverse vascular remodelling (Chung et al., 2009). Matrix metalloproteinases are endopeptidase enzymes which are abundantly expressed in the adventitia and media of arterial wall following oxidative stress, reduced NO bioavailability and secretion from inflammatory and smooth muscle cells. They adversely affect vascular remodelling by degradation of elastic laminae (essential for arterial distensibility) which promotes reduced elasticity, structural weakening and induction of calcium deposition (Chung et al., 2009). In-vitro work is supported by clinical studies demonstrating that increased levels of MMP-9 and MMP-2 independently predict aortic stiffness in normotensive and hypersensitive individuals (Yasmin et al., 2005).

#### **1.11.6 Vascular calcification and bone-mineral disorder**

Vascular calcification refers to deposition of calcium phosphate mineral (hydroxyapatite) in cardiovascular tissues. Medial calcification is prominent in CKD and has an important role in

the pathogenesis of arterial stiffness. The extent of arterial calcification correlates with severity of arterial stiffness independently of age and blood pressure in ESKD and CKD. Furthermore it is a strong predictor of all cause and cardiovascular mortality in ESKD (Toussaint et al., 2008).

Traditionally, vascular calcification was thought to reflect passive precipitation of calcium and phosphate in the presence of high extracellular concentrations secondary to hyperparathyroidism of renal disease. More recent in-vitro data, have suggested that vascular calcification is an actively regulated process (Ketteler and Giachelli, 2006). Up-regulation of transcription factors secondary to hyperphosphataemia and oxidative stress in CKD, induce osteogenic gene activation in vascular smooth muscle cells, hydroxyapatite formation and suppression of calcification inhibitors which promote matrix mineralisation and calcium deposition. Furthermore, low levels of inhibitors of mineralisation such as fetuin A, osteoprotegerin and matrix Gla protein in CKD are also associated with progressive arterial and ectopic soft tissue calcification with phosphate levels within the normal reference range (Adeney et al., 2009) and an increased cardiovascular mortality (Ketteler et al., 2003).

## **1.12 THE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN PROMOTING CARDIOVASCULAR DISEASE IN CHRONIC KIDNEY DISEASE**

Activation of the renin-angiotensin-aldosterone system is a feature of several disease states including heart failure (Struthers, 2004) and CKD (Bomback and Klemmer, 2007; Hene et al., 1982). Historically, angiotensin II has been viewed as a primary factor causing tissue damage, and ACE inhibitors and ARBs have been used to protect against its detrimental effects on target organs, including the cardiovascular system. There is now substantial evidence to show that aldosterone plays an independent and perhaps equally significant role in the development of cardiovascular tissue damage (Rocha et al., 2002a).

The following sections summarise the current literature regarding the physiology of aldosterone and angiotensin II, proposed mechanisms of end-organ damage and the implications for treatment specifically in CKD.

### **1.12.1 Angiotensin II**

Angiotensin II (Ang II) is an important mediator of injury in many conditions including heart failure, hypertension and CKD. It exerts deleterious effects via angiotensin II type 1 (AT-1) receptors found extensively in vascular smooth muscle cells of the cardiac, vascular and renal tissues but also via complex signalling cascades and cross-talk with aldosterone (Lemarie et al., 2008). It is a powerful vasoconstrictor but also promotes end-organ fibrosis through cellular proliferation, activation of inflammatory cytokines and vascular remodelling, vascular smooth muscle hypertrophy and proliferation, increased production of MMPs and collagen synthesis (Touyz and Schiffrin, 2000).

The efficacy of ACE inhibitors and ARBs in landmark randomised clinical trials provides strong support for the deleterious pathophysiological consequences of angiotensin II in the cardiovascular system. In high-risk patients with heart failure and with heart failure complicating myocardial infarction, use of ACE inhibitors and ARBs reduced mortality and rates of subsequent cardiovascular events (Pfeffer et al., 1992;Pfeffer et al., 2003;The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators, 1993;The SOLVD Investigators, 1991). Improved mortality was postulated to reflect reductions in neurohumoral activation which promote direct (myocardial fibrosis) and indirect effects (catecholamine activation) on adverse LV remodelling. In high-risk patients with heart failure, ACE inhibitors and ARBs exert beneficial effects via reductions in angiotensin II and / or blockade of the AT-1 receptor which also inhibits the action of angiotensin II generated by non-ACE pathways (this is discussed further in section 1.9.3.1). These effects are mediated independent of a reduction in blood pressure. In all but the SOLVD study, blood pressure was seen to increase with treatment indicative of improved cardiac function. Indeed, a reduction in blood pressure using combination treatment with an ACE inhibitor and ARB occurred in the VALLIANT study of patients with heart failure after MI. This treatment had no effect on mortality but increased rates of intolerance (Pfeffer et al., 2003).

In studies of stable patients with high-risk vascular disease (Fox, 2003;Yusuf et al., 2000) but without evidence of heart failure, the contribution of the small blood pressure reduction (-3mmHg systolic in HOPE study and -5 mmHg systolic in EUROPA study) remains much debated. Detailed post-hoc analyses have suggested that the observed benefits of treatment with an ACE inhibitor were about three times greater than expected from blood-pressure-related risk calculations generated from blood-pressure reduction in earlier studies or

from the placebo-group patients. Furthermore, similar benefits were also observed in normotensive patients (Sleight et al., 2001).

Data advocating for the use of ACE inhibitor and ARBs are limited to retrospective sub-group analyses of patients with mild and moderate CKD due to the systematic exclusion of patients with severe renal dysfunction from landmark studies. However, these data confirm cardiovascular efficacy and safety with ACE inhibitors and ARBs in CKD which appears comparable to patients with normal renal function (Mann et al., 2001). The renoprotective effects of ACE inhibitor and ARBs were postulated from experimental studies showing reductions in intra-glomerular capillary and systemic hypertension as well as prevention of direct proliferative effects on mesangial and tubular cells which might lead to matrix production and tubulointerstitial fibrosis (Del et al., 2007). Data from clinical studies reporting the superiority of ACE inhibitors and ARBs at slowing progression of renal disease is more contentious. A number of placebo controlled trials have demonstrated significant reductions in proteinuria in diabetic CKD with these agents (Brenner et al., 2001; REIN Study, 1997). However, a large meta-analysis which assessed the renoprotective effects of ACE inhibitors or ARBs, reported that the observed reductions in proteinuria in such studies were in the range expected by their blood pressure lowering effect. Furthermore, when compared with other antihypertensives, there was no additional benefit using ACE inhibitors or ARBs to suggest a class specific effect (Casas et al., 2005).

### **1.12.2. Aldosterone**

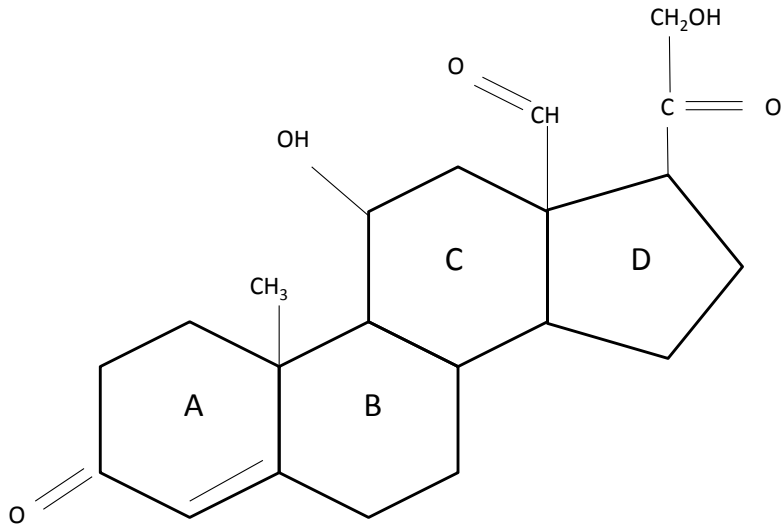
Aldosterone was originally isolated in 1953 from the adrenal glands of cows. It was initially termed electrocortin reflecting its effects on trans-epithelial transport of urinary electrolytes

(Simpson SA, 1953) but was renamed aldosterone after its molecular structure was established (Figure 1.7). It is a mineralocorticoid class of steroid, derived from cholesterol and synthesised in the glomerulosa layer from corticosterone (with 18-hydroxycorticosterone as an intermediate). Its molecular structure consists of a cyclopentanoperhydrophenanthrene nucleus (ABCD rings) with an aldehyde (CHO) group rather than a methyl (CH<sub>3</sub>) group on carbon 18 of the steroid skeleton.

#### **1.12.2.1 Epithelial actions of aldosterone**

The “classical” description of aldosterone action involves unidirectional electrolyte transport via mineralocorticoid receptors (MR) located in the cytosol epithelial cells of the collecting duct in the kidney in response to stimuli from angiotensin II, adrenocorticotrophic hormone (ACTH) and reduced sodium and increased potassium plasma concentrations. Binding to the MR induces conformational activation of the receptor and translocation into the nucleus. The increase in the transcription of MR-responsive genes, results in an increased expression and activity of the basolateral sodium / potassium-ATPase which drives entry of sodium (and water) and excretion of potassium from the cell to the lumen via the potassium channel. The net effect is increased sodium and water reabsorption, potassium excretion and an increase in blood pressure by expanding the extracellular volume (Rogerson and Fuller, 2000). Other mediators of aldosterone action in epithelial cells include the parotid gland, apical membrane (sodium / hydrogen exchanger) in the colon and luminal sodium / chloride co-transporter in the distal renal tubule which both mediate sodium reabsorption in response to volume depletion (Connell and Davies, 2005).

**Figure 1.7** Biochemical structure of aldosterone



Adapted from (Ganong WF, 2005)



### **1.12.2.2 Non-epithelial actions of aldosterone**

In recent years synthesis of aldosterone and expression of MRs have been localised in non-epithelial tissues. These include the heart (cardiomyocytes, macrophages, endothelial cells, fibroblasts), brain (hippocampus and hypothalamus) adipocytes, kidney (pre-glomerular vasculature, mesangial cells and fibroblasts) and systemic vasculature (endothelium, smooth muscle cells of the media and adventitia). The properties of MRs and the actions mediated by aldosterone in these tissues continue to be debated (see sections 1.9.2.4 and 1.9.2.5). Data from experimental studies have suggested that aldosterone synthesis at these extra-renal sites is not sufficient for systemic needs but that local paracrine activity might have functional and pathological consequences (Lemarie et al., 2008). In contrast to the effects on fluid and electrolyte balance in epithelial tissue, aldosterone appears to exert adverse effects on the cardiovascular system in non-epithelial tissue particularly in the presence of high salt. These include:

1. Vasculature – abnormal endothelial function, perivascular and interstitial inflammation, increased collagen deposition, increased vasomotor tone (Brown, 2008; Rocha and Stier, Jr., 2001; Schiffrin et al., 1985)
2. Heart – ventricular hypertrophy and fibrosis (Brilla and Weber, 1992)
3. Central nervous system – increased sympathetic tone, hypertension (Schiffrin et al., 2007)
4. Kidney – intra-glomerular capillary hypertension, proteinuria and tubulointerstitial fibrosis (Greene et al., 1996)

Salt status appears to be of particular importance for non-epithelial MR-mediated effects of aldosterone. Seminal work by Brilla et al. (1992) in which rats received chronic infusion of

exogenous aldosterone in combination with a high salt diet induced interstitial / perivascular fibrosis, ventricular hypertrophy and myocardial scarring. However, exogenous aldosterone combined with a low-salt diet induced neither blood pressure elevation, ventricular hypertrophy or cardiac fibrosis (Brilla & Weber, 1992). This early work has been extended to studies of the vascular injury where inflammatory cells and genes of pro-inflammatory molecules cannot be activated / expressed without concomitant infusion of salt (Rocha et al., 2002b). Thus the presence of high salt, is postulated to sensitise the cardiovascular system to the deleterious inflammatory effects of aldosterone.

Clinical studies examining the role of salt in aldosterone mediated cardiovascular injury are limited. Schlaich et al. (2000) examined plasma levels and urinary excretion of aldosterone after progressive salt loading in young normotensive controls and patients with mild hypertension (mean 24 hour blood pressure 132 / 78 mmHg). Suppression of serum aldosterone was less in hypertension than in controls. Furthermore, patients with least suppression of aldosterone had increased LV mass and impaired markers of LV systolic function (compared to baseline) independent of baseline 24 hour blood pressure (Schlaich et al., 2000). A recent small study of patients with ESKD examined changes in plasma aldosterone levels in response to changes in extracellular fluid status at various stages of dialysis. Aldosterone levels remained inappropriately elevated for the degree of extracellular volume expansion leading the authors to postulate that the hyperaldosteronaemic hypervolumaemic state in ESKD might result from loss of the negative feedback mechanism and thus could continue to promote the adverse non-epithelial effects of aldosterone. A role for dietary sodium advice and pharmacological treatment with aldosterone blockade was suggested (Bomback et al., 2009a).

### 1.12.2.3 Specificity of the mineralocorticoid receptor

Until 1988, the specificity of aldosterone action was believed to be conferred by the presence of high-affinity type 1 MRs which are present in the renal cytosol, colon and salivary glands and are distinguishable from the type 2 glucocorticoid receptors (GR) (Arriza et al., 1987). However, further studies of the MR in cultured pituitary cells demonstrated that it had an equally high affinity for glucocorticoids. The lack of specificity of the MR and the high physiological levels of circulating glucocorticoids which are 1000 times greater than aldosterone would suggest that MRs in epithelial tissue should be occupied by glucocorticoids. This has not shown to be the case however (Connell & Davies, 2005).

Mechanisms proposed to explain how aldosterone maintains its specificity for the MR in epithelial tissue include:

- i) Co-expression of the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ -HSD2) at the MR in target epithelial tissues. This enzyme catalyses the conversion of active glucocorticoids (cortisol in humans, corticosterone in rats) capable of binding with high affinity to MRs, into inactive metabolites (cortisone and 11-dehydrocorticosterone) which have negligible affinity for the MR. Thus, 11 $\beta$ -HSD2 effectively protects the MR from illicit occupation by glucocorticoids (Edwards et al., 1988).
- ii) High local concentrations of NADH are produced by 11 $\beta$ -HSD2 during the conversion of cortisol to cortisone might inactivate the MR-glucocorticoid complexes (Funder, 2004).
- iii) Molecular studies of the cloned MR showed that despite mineralocorticoids and glucocorticoids binding the MR with equivalent affinity, aldosterone achieves

greater transactivation suggesting that ligands can have differential effects on gene transcription at the same receptor (Connell & Davies, 2005).

- iv) The majority of glucocorticoids are bound to proteins in plasma (Connell & Davies, 2005).

The mechanism of MR specificity for aldosterone in non-epithelial tissue is less clear. These tissues appear to express little or no 11 $\beta$ -HSD2 enzyme despite often abundant MRs and under normal conditions total plasma glucocorticoid levels are  $\geq 1000$ -fold higher than aldosterone and plasma free levels are  $\geq 100$ -fold higher. Given these high circulating concentrations of glucocorticoids and “unprotected” nature of MRs, over 90% are occupied by glucocorticoids (cortisol / corticosterone) in-vivo. The effect of glucocorticoid-MR complexes remains controversial. Some experimental studies have reported that glucocorticoid occupation of the MR in non-epithelial tissue is potentially protective as they antagonise the adverse MR effects mediated by mineralocorticoids. Chronic exogenous infusion of low-dose aldosterone in rats elevated blood pressure over days / weeks but infusion of corticosterone had no effect. When co-administered with aldosterone, corticosterone blocked the hypertensive and fibrotic response seen with aldosterone alone, suggesting that it might act as an MR antagonist (Young and Funder, 2000). Furthermore, transgenic mice over-expressing 11 $\beta$ -HSD2 (preventing glucocorticoid effects) develop cardiac hypertrophy, fibrosis and heart failure on a normal salt diet (Qin et al., 2003).

Subsequent studies have failed to replicate such findings and have instead hypothesised that the glucocorticoid–MR complex is activated, allowing it to mimic the adverse effects of mineralocorticoid / salt imbalance on blood vessels and the heart. Endogenous corticosterone plus salt, in the presence of the 11 $\beta$ -HSD2 inhibitor

carbenoxolone produced similar vascular inflammatory responses and tissue remodelling via activation of MR (Young et al., 2003). Mechanisms postulated to mediate these actions centre around reduced intracellular levels of NADH. When 11 $\beta$ -HSD2 is inhibited and NADH levels decrease (NADH is produced when cortisol is converted to cortisone), cortisol acts in the same manner as aldosterone (Mihailidou and Funder, 2005). Tissue damage and ROS generation also reduce cellular levels of NADH by the induction of NADPH oxidases, even though 11 $\beta$ -HSD2 may still be operational. Both mechanisms might serve to activate the glucocorticoids-MR complexes (Wilson et al., 2009). In patients with CKD, 11 $\beta$ -HSD2 activity (N'Gankam et al., 2002) and expression (Quinkler et al., 2005) decline with progressively impaired renal function suggesting that in these patients blood pressure and cardiovascular injury might be partly driven by glucocorticoid-induced MR activation.

#### **1.12.2.4 Genomic and non-genomic actions of aldosterone**

The classic genomic model of aldosterone action on epithelial cells is well established (ref). However, the changes in gene expression and protein production result in a 1-2 hour lag period before changes in target cell activity. More recently it has been demonstrated that aldosterone activation also induces rapid (10-15 minutes) non-genomic cellular responses (independent of gene transcription and translation) by modulating intracellular secondary messenger systems including calcium and cyclic adenosine monophosphate (cAMP), Na / H exchange activity and phosphorylation of signalling molecules (Wehling, 1997).

The non-genomic mechanisms have been demonstrated predominantly within non-epithelial tissue but also epithelial cells of the colon. Whether they are mediated through the classical MR remains uncertain. Early studies using human mononuclear leucocytes and vascular smooth muscle cells identified aldosterone-selective responses that were not

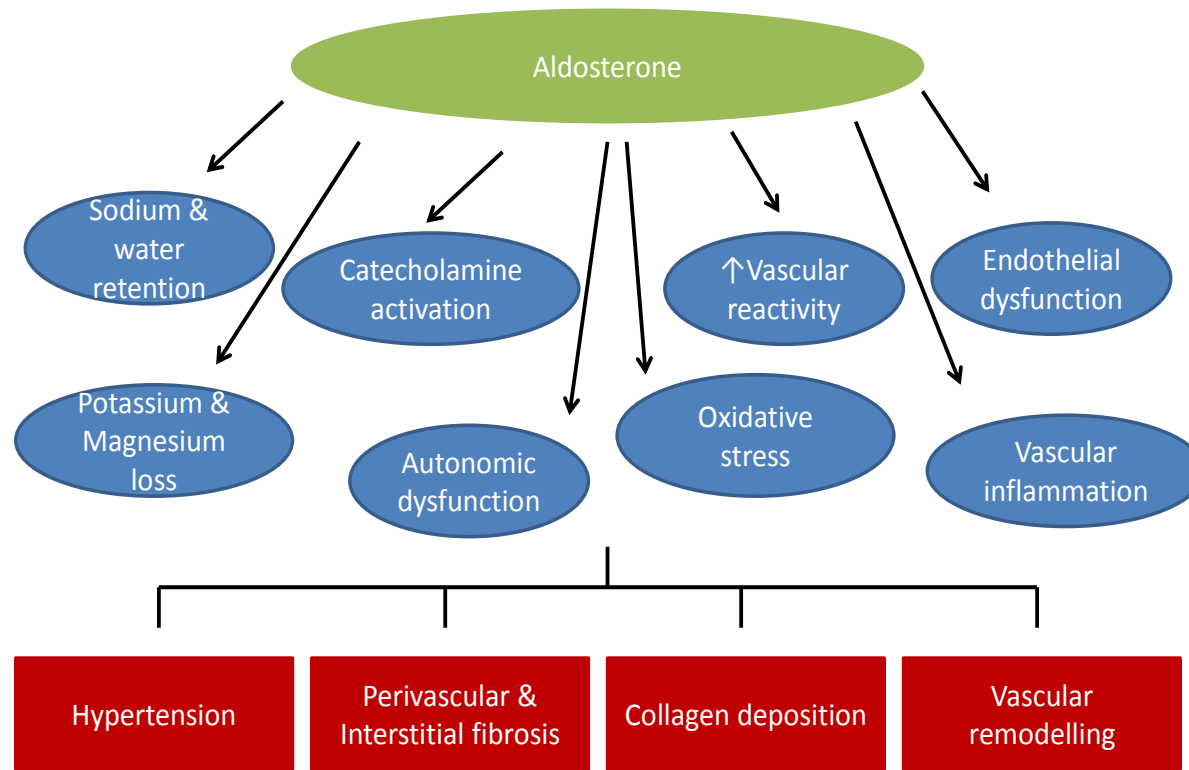
mimicked by cortisol (Mihailidou & Funder, 2005). In rat arterial smooth muscle cells, rapid sodium efflux mediated by aldosterone was insensitive to actinomycin (implying it is independent of gene transcription) but sensitive to inhibition by spironolactone and the selective MR antagonist RU-28318, implying the involvement of the classical MR (Connell & Davies, 2005). Rapid MR-mediated responses have now been described for an increasing number of vascular and cardiac targets raising the possibility that they are mediated via a closely related protein receptor different from the MR but associated with the cell membrane and which has a high affinity for aldosterone and not glucocorticoids (Connell & Davies, 2005).

#### **1.12.2.5 Potential mechanisms of aldosterone induced injury in the cardiovascular system**

In a number of experimental studies Rocha et al. eloquently demonstrated the adverse effects of aldosterone independent to those of angiotensin II. For example, infusing uninephrectomised rats drinking 0.9% saline solution with angiotensin II for 3 weeks progressively increased blood pressure and induced widespread perivascular and interstitial inflammatory cell infiltration and coronary wall thickening. In the same model but with adrenalectomised rats, blood pressure remained elevated (indicating an angiotensin II effect) but vessel walls were normal and the intense inflammatory response absent. Furthermore, infusion of aldosterone to adrenalectomised rats produced comparable vascular and interstitial changes to those observed with angiotensin II (Rocha et al., 2002a).

The detailed mechanisms by which aldosterone induces cellular injury in the cardiovascular system (Figure 1.8) were not specifically addressed by this thesis. In the following section, an overview of the experimental evidence to date is provided but reader is referred to two excellent detailed reviews which address the mechanisms in further detail (Briet and Schiffrin, 2010;Brown et al., 2000).

**Figure 1.8** Potential mechanisms by which aldosterone mediates cardiovascular injury





#### 1.12.2.6 Aldosterone and vascular injury – aldosterone induced vasculopathy

Aldosterone mediates vascular damage by:

1. **Inflammation and oxidative stress** – Aldosterone increases the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the production of ROS leading to increased oxidative stress in vascular smooth cells (VSMC), monocytes, macrophages and endothelium. Subsequent activation of inflammatory transcription factors induce a cascade of inflammatory mediators including the recruitment of monocytes and macrophages which promote proliferation of fibroblasts in the perivascular space and cytokines such as interleukin (IL)-6 which stimulate the expression of pro-fibrotic factors such as transforming growth factor (TGF) and plasminogen activator inhibitor (PAI-1). The net effect is cellular transformation of fibroblasts, synthesis of matrix proteins and increased MMPs which increase collagen production and promote fibrosis within the arterial wall (Brown, 2008).

Rocha et al (2002) demonstrated that the initial pathogenic event in an animal model of mineralocorticoid hypertension (uninephrectomised rats treated with exogenous aldosterone and high salt diet) was inflammatory cell infiltration and expression of pro-inflammatory molecules after about 1 week of treatment. Focal changes within the arterial wall characterised by thickening and disorganisation of smooth muscle cells within the medial arterial layer and perivascular and coronary inflammatory lesions with focal necrotic and ischaemic changes were evident after two weeks of treatment. Furthermore, concomitant treatment with the selective aldosterone blocker eplerenone reduced expression of pro-inflammatory molecules, attenuated vascular and cardiac histopathological changes with only a partial reduction in blood pressure. These data suggested that a vascular inflammatory

phenotype is the prerequisite step for the subsequent development of aldosterone / salt-induced vascular and myocardial injury (Rocha et al., 2002b).

2. **Effects on the vascular smooth muscle cells (VSMC)** – Early studies with the mineralocorticoid deoxycorticosterone acetate (DOCA - a precursor of aldosterone with mineralocorticoid properties) in-vivo and later using aldosterone in-vitro and in-vivo have demonstrated an up-regulation of components of the RAAS in VSMC (Briet & Schiffrin, 2010). Increases in the angiotensin receptor, angiotensin converting enzyme and a resultant increase in the local generation of angiotensin II activate a series of signalling cascades which promote the growth and proliferation of the VSMC and subsequently promote vascular remodelling and inflammation (Schiffrin, 2006). Recent studies have also shown synergistic cross-talk between angiotensin II and aldosterone. Infusion of low doses of angiotensin II and aldosterone (at doses ineffective individually) increased VSMC proliferation in adult rats. These effects were blocked by an angiotensin I receptor antagonist and spironolactone leading the authors to postulate that the synergistic interaction is mediated through both genomic and non-genomic signalling of the MR and AT 1 receptor. Thus blockade of both receptors might provide enhanced protection from vascular remodelling (Min et al., 2005).

3. **Effects on the endothelium** – Aldosterone has been shown to cause endothelial dysfunction (Farquharson and Struthers, 2000). Tissue culture studies showed that aldosterone inhibited inducible nitric oxide release in response to cytokine stimulation (Ikeda et al., 1995). These data were extended in-vivo by examining the effect of acute short-term systemic administration of aldosterone on flow-mediated dilatation of the brachial artery in healthy volunteers. Aldosterone adversely attenuated endothelial dependent vasodilatation function

induced by cholinergic agents but had no effect on endothelial independent vasodilatation with sodium nitroprusside. These acute changes were postulated to reflect non-genomic effects of aldosterone on NO bioavailability (Farquharson and Struthers, 2002).

As described in section 1.8.3, endothelial dysfunction promotes arterial stiffness (Wilkinson et al., 2004) and stiff arteries perpetuate a vicious circle by reducing endothelial expression of NO synthase (Peng et al., 2003). Chronic exposure to aldosterone has been shown to reduce endothelial relaxation in aortic segments of spontaneously hypertensive rats (the best animal analogue of human hypertension) (Blanco-Rivero et al., 2005). Furthermore, infusion of DOCA to salt loaded hypertensive rats increased levels of the vasoconstrictor peptide endothelin I (ET-1) in the aorta and large and small mesenteric arteries (Lariviere et al., 1993). Endothelin dependent relaxation was down-regulated through reduced bioavailability of NO and resultant oxidative stress in the wall (Pu et al., 2003). In-vitro, aldosterone induced endothelial cell growth and stiffening with concentrations in the low pathophysiological range (Hillebrand et al., 2007).

Mineralocorticoid receptor blockers used in experimental and human studies have consistently been shown to improve endothelial function through a reduction in the generation of ROS and oxidative stress (Farquharson & Struthers, 2000; Farquharson & Struthers, 2002). The key mediators of this response are increased NO bioavailability and reduced NADPH. One of the first studies to demonstrate this effect was in chronic heart failure where infusion of NO synthase inhibitor (L-NMMA) to patients treated with spironolactone increased vasoconstriction, indicative of an increase in basal NO following aldosterone blockade (Farquharson & Struthers, 2000). Other possible mechanisms by which MRBs improve endothelial function include; up-regulation of other endothelial dependent vasodilators such as vascular prostacyclin or endothelium-derived hyperpolarising factor (EDHF), attenuation

of angiotensin I-mediated vasoconstriction and improved endothelial signalling pathways leading to NO synthase synthesis (Struthers, 2004).

#### **1.12.2.7 Aldosterone and cardiac injury**

Chronic treatment (8 weeks) of exogenous aldosterone with a high salt diet induces structural remodelling within the heart. Seminal animal work by Brilla et al. (1992) demonstrated characteristic changes of LVH, accumulation of collagen within the interstitial space and adventitia of intramyocardial coronary arteries in the absence of myocyte necrosis (known as interstitial and perivascular fibrosis) and myocardial scarring irrespective of the presence of myocardial hypertrophy. These pathological changes were prevented with concurrent treatment with low dose spironolactone without reducing hypertension (Brilla & Weber, 1992). These data were postulated to reflect a direct humoral response to aldosterone rather than systolic hypertension as fibrillar collagen was increased in both right and left ventricles irrespective of the presence of hypertrophy and was subsequently confirmed by the finding of equivalent cardiac fibrosis in animals infused with aldosterone but made normotensive (Young et al., 1995).

These studies identified aldosterone as a key modulator of cardiac fibrosis and have led to the hypothesis that aldosterone changes the mechanical and electrical properties of the ventricle by increasing fibrillar collagen content thereby affecting subsequent structural remodelling and reparative scar formation of the ventricle.

Although cardiac fibroblasts are the principal source of collagen in the heart they are devoid of high affinity MR binding sites and most in vitro studies have failed to show direct effects of aldosterone on collagen synthesis. This suggests that aldosterone exerts its effects primarily on cardiomyocytes which in turn induce collagen synthesis of adjacent fibroblasts in

a paracrine fashion (Fullerton and Funder, 1994). The MR has a central role in mediating cardiac fibrosis via mineralocorticoids and possibly glucocorticoids, independent of hypertension. In a recent study, infusion of DOCA and salt treatment for 8 weeks to macrophage-specific MR knockout mice increased the numbers of infiltrating macrophages without inducing cardiac fibrosis or hypertension (Rickard et al., 2009).

Histologically, cardiac fibrosis is characterised by proliferation of cardiac myocytes and fibroblasts with intense perivascular inflammation and fibrosis. It remains to be determined whether or not aldosterone acts directly on cardiac fibroblasts. Some studies have shown aldosterone to increase collagen I synthesis in cardiac fibroblasts (Robert et al., 1994) and in-vivo data of cultured cardiac fibroblasts showed a direct cellular anabolic effect comparable to those of angiotensin II (Brilla et al., 1994). However, not all studies have been able to replicate such findings. Other mechanisms by which the aldosterone / salt model might increase cardiac fibrosis include cross-talk signalling pathways with angiotensin II, up-regulation of angiotensin II type 1 receptor mRNA expression and angiotensin II type 1 receptor binding in the heart, endothelin-1 receptor density and ACE in the heart and vascular walls (Briet & Schiffrin, 2010).

The evidence for cardiac biosynthesis of aldosterone remains controversial. Early studies reported human genes encoding aldosterone synthase (CYP11 $\beta$ 2) were present in adrenal and cardiac tissue (Silvestre et al., 1998). Levels were shown to be raised in heart failure and after myocardial infarction and also correlated with collagen volume (Satoh et al., 2002). Subsequent studies have failed to replicate such findings. Levels of genes in cardiac tissue are several magnitudes lower than in the adrenal cortex and adrenalectomy in angiotensin II / salt animal models abolished cardiac fibrosis suggesting that systemic effects

might still be mediated from aldosterone derived from the adrenal cortex (Rocha et al., 2002a).

#### **1.12.2.8 Aldosterone and renal injury**

Kidney injury following exogenous infusion of aldosterone in normotensive animal models is characterised by mesangial cell proliferation, glomerular tuft expansion, sclerotic changes, podocyte injury, arteriolar hyalinosis, interstitial inflammation and fibrosis. The pathological mechanisms of injury mediated by aldosterone mirror those observed in the vasculature with increased expression of inflammatory mediators and ROS. Stimulation of pro-fibrotic factors including TGF- $\beta$  and PAI<sub>1</sub> within mesangial cells increase collagen synthesis, decrease extracellular matrix degradation and promote fibroblast proliferation (Briet & Schiffrin, 2010)

Initial studies in stroke-prone spontaneously hypertensive rats (SHRSP) identified angiotensin II as the major mediator of renal injury (Stier, Jr. et al., 1989). Treatment with ACE inhibitors, ARBs and the mineralocorticoid receptor blocker spironolactone were all shown to be effective at reducing proteinuria and preventing development of renal vascular and glomerular injury (Camargo et al., 1993; Rocha et al., 1998; Stier, Jr. et al., 1992). These benefits were initially attributed to reductions in the vascular actions of angiotensin II and endogenous inhibition of aldosterone synthesis. Subsequent studies extended this work and confirmed a central role for endogenous aldosterone as a humoral mediator of renal injury. Concurrent administration of aldosterone with an ACE inhibitor to SHRSP rats abolished the renoprotective effects provided by ACE inhibition despite the persistence of hypertension. These data support a major role of aldosterone in the kidney possibly independent of other components of the RAAS (Rocha et al., 1999).

### **1.13 The role of mineralocorticoid receptor blockers in preventing aldosterone mediated end-organ damage in clinical studies**

As described in section 1.9.2, there are extensive data from animal models of hyperaldosteronism demonstrating on the adverse actions of aldosterone within the cardiovascular and renal systems. Further indirect evidence has been provided by the use of mineralocorticoid receptor blockers (MRBs) such as spironolactone and eplerenone. Both agents inhibit aldosterone by binding to the MR rendering it transcriptionally inactive and are effective at reducing end-organ damage in the heart, brain and kidneys without significant reductions in blood pressure (Stier, Jr. et al., 2002). There are some limitations of such experimental models including: the dependence on a high salt diet, use of un-physiological dosages of exogenous infusions of mineralocorticoids and profound hypertensive responses, which might limit their applicability to clinical studies.

In the following section, the phenomenon of aldosterone escape and aldosterone breakthrough in man is discussed and the current literature regarding the role and efficacy of MRBs in human studies of heart failure and CKD are summarised.

#### **1.13.1 Aldosterone breakthrough and aldosterone escape**

Plasma aldosterone levels are primarily regulated by potassium and angiotensin II in response to salt balance and plasma volume. In diseases such as heart failure, primary hyperaldosteronism and CKD circulating aldosterone levels can be inappropriately raised independently of these traditional feedback mechanisms. The terms aldosterone breakthrough and aldosterone escape are often used interchangeably to describe this phenomenon but actually refer to two different patho-physiological mechanisms as outlined below:

1. **Aldosterone breakthrough** – describes increased plasma aldosterone levels despite chronic inhibition of the RAAS with ACE inhibitors and ARBs. One proposed definition is; “a serum aldosterone level that exceeds a baseline (pre-ACE inhibitor and / or ARB therapy) value 6 – 12 months after initiation of RAAS-blocking therapy” (Bomback & Klemmer, 2007). This phenomenon has been reported in up to 50% of patients with heart failure and CKD (Bomback & Klemmer, 2007).

Mechanisms proposed to contribute to aldosterone breakthrough include:

i) Non-angiotensin converting enzyme (ACE) dependent generation of angiotensin II – Non-ACE enzymes capable of cleaving angiotensin I to angiotensin II are present in coronary arteries and medium small resistance arteries of healthy volunteers, patients with coronary heart disease and patients with heart failure treated with ACE inhibitors (Petrie et al., 2001). This pathway converts angiotensin I to angiotensin II using serine proteases such as chymase and can be blocked in coronary arteries in-vitro by chymase inhibitors such as chymostatin. It was postulated that dual blockade of ACE and non-ACE enzymes might produce more complete suppression of angiotensin II and aldosterone. However, this is unlikely to be the primary mechanism of aldosterone breakthrough as it would be expected to occur less often in patients treated with ARBs which has not been demonstrated to date.

ii) Serum potassium levels – Increased serum potassium levels directly stimulates aldosterone secretion, independent of any effect on the circulating RAAS (Williams, 2005). An acute increase in serum potassium of 0.1 mmol/L produces as much as a 25% increase in serum aldosterone levels (Himathongkam et al., 1975). This raises the possibility that an increase in plasma potassium levels during ACE inhibitor and / or ARB therapy could



promote aldosterone breakthrough. However, available evidence to date does not support this hypothesis. There was no significant change in pre and post treatment levels of potassium following chronic AT-1 receptor blockade or healthy volunteers receiving ACE inhibitors and ARBs (Bakris et al., 2000). In CKD mean potassium levels are not significantly changed with ARBs (Schjoedt et al., 2004) but Bakris et al. (2000) did demonstrate an increase in mean potassium levels (0.3 mmol/L) in a sub-group of patients with an eGFR  $\leq 60$  ml/min/1.73m<sup>2</sup> treated with ACE inhibitors.

iii) Loss of the normal feedback mechanism between aldosterone and salt / fluid balance- In a recent small study of patient with ESKD, progressive expansion of extracellular volume during dialysis failed to suppress aldosterone levels (Bomback et al., 2009a). Thus in disease states characterised by RAAS activation such as heart failure and CKD, the normal negative feedback response appears to be lost.

iv) Unopposed activation of angiotensin II type 2 receptor (AT-2) - The AT-2 receptors account for about 20% of all AT receptors but are not blocked by ARBs. In SHRSP rats treated with 24 weeks of AT-1 receptor blockade (candestartan) levels of angiotensin II were elevated throughout (secondary to negative feedback) while aldosterone levels decreased over the first 4 weeks but subsequently increased above pre-treatment levels. Concurrent administration of an AT-2 receptor blocker decreased aldosterone suggesting that aldosterone breakthrough in the presence of chronic treatment with ARBs might reflect unopposed stimulation of AT-2 receptor (Naruse et al., 2002).

2. **Aldosterone escape** - occurs in disease states characterised by sodium retention and fluid overload such as heart failure, liver failure and ESKD. The reduction in intravascular volume and expanded extracellular volume impairs the normal feedback mechanisms for

aldosterone homeostasis. As a result aldosterone levels are inappropriately increased relative to sodium and fluid balance (Schrier, 2010). Contributory mechanisms include; i) reduced renal perfusion pressure and filtration rate, ii) chronic inappropriate stimulation of the RAAS and iii) sympathetic nervous system, iv) increased sodium resorption in the proximal tubule, distal tubule by aldosterone and collecting duct by natriuretic hormones.

Aldosterone breakthrough and escape can co-exist in heart failure and are associated with increased aortic and arterial stiffness (Duprez et al., 1998). Observational studies of patients after myocardial infarction have demonstrated a graded relationship between plasma aldosterone levels and cardiovascular mortality which is particularly strong with sudden cardiac death (Palmer et al., 2008; Tomaschitz et al., 2010). This relationship is present even when plasma aldosterone levels are within the normal physiological range suggesting that local production of aldosterone or glucocorticoid within the myocardium might mediate cardiac injury via the MR. This paracrine action may not be reflected by plasma aldosterone levels. It is perhaps not surprising therefore, that the therapeutic strategy of adding MRBs to ACE inhibitors and ARBs has proved highly efficacious in reducing cardiac mortality in the populations in which it has been studied to date. Chronic kidney disease is also characterised by aldosterone breakthrough and escape, with an inverse graded relationship between plasma aldosterone levels and eGFR (Tomaschitz et al., 2011). This raises the question as to whether similar efficacy and clinical benefits might be achievable with MRBs in this high-risk population.

### **1.13.2 The use of mineralocorticoid receptor antagonist in heart failure**

There has been a major increase in the use of MRBs over the past decade following the demonstration of their efficacy in the treatment of heart failure, left ventricular dysfunction following myocardial infarction and primary hyperaldosteronism (Catena et al., 2007;Pitt et al., 1999;Pitt et al., 2003b;Zannad et al., 2011). In the Randomized Aldactone Evaluation Study (RALES) of patients with severe heart failure (New York Heart Association [NYHA] class III and IV) and an LV ejection fraction (EF)  $\leq$  35% treated with an ACE inhibitor and loop diuretic, randomisation to 25mg daily of spironolactone reduced the risk of cardiac death by 31% and rates of hospitalisation for a cardiac cause by 30% compared to standard treatment and placebo (Pitt et al., 1999). In the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) treatment with 25-50mg of the selective MR blocker eplerenone in patients 3-14 days after acute myocardial infarction complicated by LV dysfunction (EF  $\leq$  40%), reduced the relative risk of death from a cardiovascular cause by 17% and hospitalisation from heart failure by 15% compared to placebo (Pitt et al., 2003b). The mortality benefits in both studies were independent of reductions in blood pressure and were driven by significant reductions in rates of progressive heart failure and sudden cardiac death. More recently, the benefits of MRBs were shown to extend to patients with mild heart failure (LV EF  $\leq$  35% and NYHA class II symptoms). The Eplerenone in Mild Patients Hospitalisation and Survival Study in Heart Failure (EMPHASIS-HF) demonstrated that treatment with eplerenone 50mg daily in addition to standard therapies such as ACE inhibitor or ARB reduced cardiac mortality by 24% and hospitalisation from heart failure by 42% compared to placebo (Zannad et al., 2011).

Improvements in mortality in these studies were postulated to reflect reductions in aldosterone mediated LV remodelling secondary to reduced pro-inflammatory and pro-

fibrotic processes within the injured myocardium (Hayashi et al., 2003). This view was subsequently supported by a sub-analysis of serological markers of cardiac collagen turnover and fibrosis from patients in RALES. Treatment with spironolactone reduced levels of collagen turnover biomarkers compared to increased levels over the 6 month follow-up in patients on placebo. Furthermore, the mortality benefits with spironolactone were most apparent in patients with the highest baseline levels of biomarkers indicating that aldosterone mediated cardiac fibrosis is an “active” process in patients with heart failure and after myocardial infarction which can be reduced with aldosterone blockade (Zannad et al., 2000).

The immediate introduction of MRBs following revascularisation for myocardial infarction, reduced echo features (LV EF and dilatation) of adverse LV remodelling and was associated with a reduction in cardiac tissue levels of aldosterone. These data suggest that high aldosterone levels within myocardium after acute injury stimulate acute adverse LV remodelling (Hayashi et al., 2003). Increased levels of aldosterone synthase (which catalyses the final step of aldosterone production) were also present in endomyocardial biopsies of patients with chronic heart failure but are barely detectable in healthy volunteers. Levels were associated with increased myocardial collagen volume, increased LV volumes and decreased LV systolic function and were decreased by treatment with spironolactone and ACE inhibitors (Satoh et al., 2002).

Evaluation of plasma aldosterone early after myocardial infarction is also predictive of an adverse prognosis in both the short and long-term (Palmer et al., 2008; Tomaschitz et al., 2010). However, it is important to note that in these studies, the plasma level of aldosterone is rarely ( $\leq 1\%$ ) above the upper limit of the normal range, reflecting a poor correlation with potentially higher aldosterone levels within tissue. An alternative hypothesis for the efficacy of MRBs in heart failure when aldosterone plasma levels and salt-status are normal, might

relate to blockade of glucocorticoids within cardiomyocytes and vascular smooth muscle cells. Inactivation of 11 $\beta$ -HSD2 or generation of reactive oxygen species in damaged tissue (secondary to inflammation and oxidative stress) are postulated to activate MR-glucocorticoid complexes, allowing physiological levels of glucocorticoids to mimic the activity of aldosterone at the MR. Thus, the MRBs might provide dual protection of the MR in many clinical conditions rather than just states of aldosterone / salt excess (Funder, 2005).

### **1.13.3 The use of mineralocorticoid receptor blockers in chronic kidney disease**

Aldosterone breakthrough is present in about 50% of patients with CKD after a year of treatment with ACE inhibitors, ARBs or combination therapy, with no differences between groups (Horita et al., 2006). Thus, augmentation of RAAS blockade with MRBs might provide an important therapeutic strategy in patients with CKD.

Prior to the work described in this thesis, there were no published prospective clinical studies investigating the effect of MRBs in combination with an ACE inhibitor or ARB in CKD on markers of cardiovascular function. Data were limited to animal models and a few small clinical studies investigating the role of MRBs in reducing proteinuria (Bomback et al., 2008a). These data supported a beneficial action of MRBs in reducing the progression of renal disease (Bianchi et al., 2010; Chrysostomou and Becker, 2001). A systematic review of 15 clinical studies involving 436 patients confirmed reductions in baseline proteinuria of between 15% and 54%, with most estimates in the 30% to 40% range. Half of these studies reported a reduction in blood pressure with additive MRB treatment (Bomback et al., 2008a).

Despite these apparent clinical benefits, MRBs are rarely used in CKD because of the potential risks of hyperkalaemia and further deteriorations in renal function (Bomback et al.,

2008a). These side-effects are well reported in the heart failure population although reported hyperkalaemic complication rates remain variable. In RALES there was a mean increase in serum potassium levels of 0.3-0.4 mmol/L with spironolactone and rates of serious hyperkalaemia of 2%. However, following the publication of RALES a number of groups reported higher rates of hyperkalaemia with some fatal events (Juurlink et al., 2004). Analyses identified poor patient selection as a major contributory factor for the adverse events observed in the “real world”; over a third of patients prescribed spironolactone after RALES did not meet the study inclusion criteria at the time of discharge from hospital. Specifically, 17% of patients had severe renal impairment (eGFR < 30 ml/min/1.73m<sup>2</sup>), 23% had discharge potassium of > 5 mmol/L and 17% were still receiving potassium supplements (Juurlink et al., 2004).

Data from a meta-analysis of MRB use in proteinuric CKD reported a serum potassium  $\geq 5.5$  mmol/L in 5.5% of patients (Bomback et al., 2008a). Further analyses have suggested that patient-related factors including baseline potassium  $\geq 4.5$  mmol/L and eGFR  $\leq 45$  ml/min/1.73m<sup>2</sup> might help predict an increased risk of hyperkalaemia in CKD (Khosla et al., 2009). Overall, the true rates of hyperkalaemia and associated morbidity with MRBs in CKD remain uncertain. Mild hyperkalaemia (defined as serum potassium between 5.5-5.9 mmol/L) is common with in all patient groups using MRBs. A recent prospective observational study involving over 800 patients with CKD stage 3-5, reported a mild hyperkalaemia rate of 8% but with no associated increase in mortality or progression to end-stage CKD (Korgaonkar et al., 2010). This data serves to emphasise the need for large-scale, prospective, randomised studies to evaluate the many unanswered questions when using MRBs in CKD.

## **1.14 Summary**

End-stage kidney disease is associated with high rates of cardiovascular mortality particularly sudden cardiac death. Characteristic structural and functional changes in the vasculature (arterial stiffness) and heart (left ventricular hypertrophy and fibrosis) might be central to these adverse outcomes. However, the natural history of cardiovascular abnormalities in CKD remains poorly understood and reflects a paucity of data in early stage CKD.

Aldosterone levels are increased in CKD and might be an important cause of vascular and cardiac damage. To date, there have been no studies examining the effects of mineralocorticoid receptor blockers on cardiovascular function in CKD. Whether these agents are effective or safely tolerated in CKD is yet to be fully elucidated.

## 1.15 AIMS AND HYPOTHESES

The three principle aims of this thesis were:

1. Determine whether early stage CKD is associated with abnormalities of arterial and ventricular function.
2. Determine whether blocking the action of aldosterone with a mineralocorticoid receptor blocker – spironolactone, is effective at improving markers of cardiovascular disease.
3. Examine the safety and tolerability of spironolactone in early stage.

The following hypotheses were addressed:

1. Patients with early stage CKD without known cardiovascular disease have evidence of large artery stiffness and adverse changes in systolic and diastolic ventricular function compared to healthy volunteers. (Chapter 3).
2. Abnormalities of myocardial deformation precede overt abnormalities detected with standard parameters of ventricular function in early stage CKD (Chapter 4).
3. In patients with early stage CKD, the addition of spironolactone to concomitant treatment with ACE inhibitors and angiotensin receptor blockers will improve prognostically significant markers of arterial stiffness and left ventricular mass (Chapter 5).
4. In patients with early stage CKD, spironolactone will improve markers of LV systolic and diastolic function and reduce serological markers of inflammation, ventricular stress, and collagen turnover (Chapter 6).
5. Spironolactone is well tolerated and can be used safely in patients with early stage CKD (Chapter 7).



## **CHAPTER 2**

### **METHODS – ASSESSMENT OF HAEMODYNAMIC, BIOCHEMICAL AND CARDIOVASCULAR STRUCTURE AND FUNCTION**

## 2.1 GENERAL

### 2.1.1 Subjects

Supported by a study research nurse, I screened all renal out-patient clinics at University Hospital Birmingham for potential subjects between March 2005 and March 2007. Using a database of all renal out-patients, we identified patients with a stable estimated GFR (eGFR) in the range specified in the inclusion criteria. Electronic letters were then screened for these patients to identify the aetiology of renal disease, associated co-morbidities and drug history. Inclusion criteria were;

1. Aged 18-80 years
2. Stage 2 (eGFR 60-89 ml/min/1.73m<sup>2</sup> and evidence of kidney damage for  $\geq 3$  months) or stage 3 CKD (GFR 30-59 ml/min1.73m<sup>2</sup>). GFR was estimated (eGFR) using the 4-variable Modification of Diet in Renal Disease formula (Levey et al., 1999)
3. Treatment with an ACE inhibitor and / or angiotensin II receptor blocker for  $\geq 6$  months at maximally tolerated dosage
4. Controlled blood pressure (defined as a mean daytime 24 hour ambulatory blood pressure monitoring  $\geq 130/85$  mmHg)

Exclusion criteria were;

1. History of angina, myocardial infarction, heart failure, valvular heart disease
2. History of cerebral vascular disease
3. History of peripheral vascular disease
4. Permanent atrial fibrillation
5. A history of uncontrolled hypertension

6. Diabetes mellitus
7. History of anaemia (defined as a haemoglobin  $\leq$  12 g/dL)
8. Previous hyperkalaemia (defined as a serum potassium  $\geq$  5.5 mmol/L)

Patients identified from screening who fulfilled the study inclusion criteria were spoken to by me or a study research nurse during their out-patient clinic appointment. If the patient expressed an interest about possible participation, a detailed patient information sheet was provided. All patients were advised to discuss with appropriate family members and asked to return a reply slip with contact details if they were agreeable to participation. Contact details of a consultant nephrologist, independent from the study were provided for further unbiased advice. Details of the study and contact details of the research team were formally provided for each patient's general practitioner after written consent was obtained.

### **Healthy Volunteers**

Forty healthy volunteers (aged 18-80 years) were recruited from staff at our institution by way of an advertisement. Detailed information about the study was provided. Volunteers were unable to participate if they had evidence of CKD (using eGFR and urine analysis) or met any of the study exclusion criteria.

#### **2.1.2 Ethical Considerations**

The study protocol was approved by the South Birmingham Research and Ethics committee (04/Q2707/294), University Hospital Birmingham Research and Development committee (RRK2749) and was undertaken in accordance with the Declaration of Helsinki of the World Medical Association. All patients and volunteers gave written informed consent before entry into the study.

## 2.2 METHODS OF CLINICAL ASSESSMENT

### 2.2.1 Estimated glomerular filtration rate

Glomerular filtration rate was estimated using the 4-variable Modification of Diet in Renal Disease Equation (4-v MDRD) Study equation (Levey et al., 1999):

$$\text{eGFR (ml/min/1.73m}^2\text{)} = \frac{175 \times [\text{serum creatinine (mg/dl)}]^{-1.154} \times [\text{age}]^{-2.03}}{[\text{x 0.742 for women}] [\text{x 1.210 for blacks}]}$$

This formula is recommended by the K/DOQI guidelines and has been validated against the urinary clearance of  $^{125}\text{I}$ iothalamate in multiple populations.

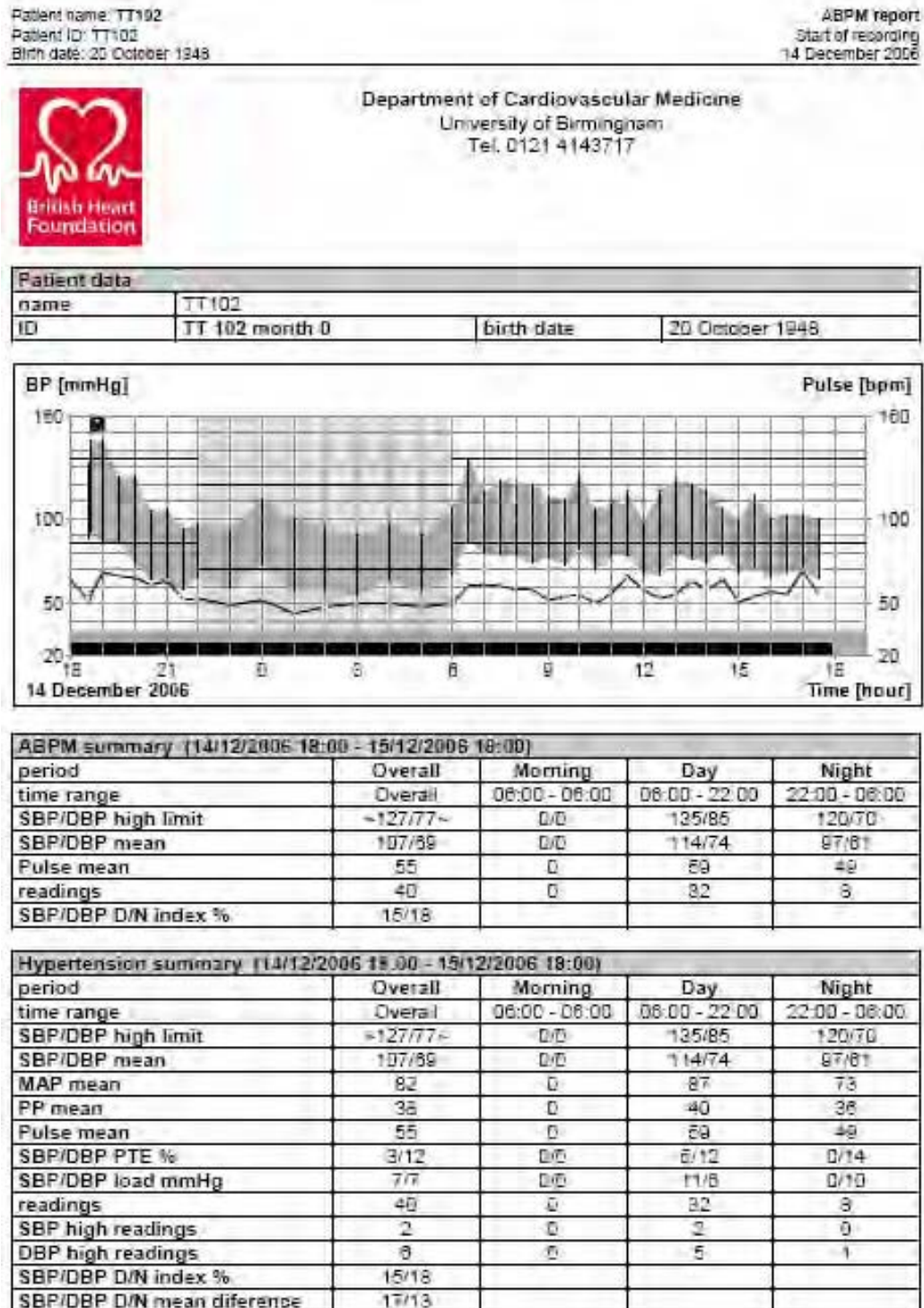
### 2.2.2 Blood pressure measurement

Resting “office” brachial blood pressure was assessed using the Dinamap Procare oscillometric sphygmomanometer (GE Healthcare, Hatfield, United Kingdom) which has been independently validated by the British Hypertension Society (Reinders et al., 2006). Three readings were taken in the non-dominant arm after 10, 20 and 30 minutes of supine rest, with the last two reading averaged and used for assessment of arterial stiffness.

Patients underwent 24 hour ambulatory blood pressure monitoring (ABPM) using a Meditech ABPM-04 monitor (PMS Instruments, Maidenhead, United Kingdom). This model was validated by the British Hypertension Society (Barna et al., 1998). An appropriate sized cuff was fitted to the non-dominant arm; adult cuff 24-32cm circumference or large adult cuff 32-42cm circumference. Patients were told to engage in normal activities during the recording period and educated about the procedure specifically; keeping the arm straight and steady

during measurements, the frequency of inflations, methods to deflate the cuff if required and how to remove the device after 24 hours. Monitors were programmed to record blood pressure every 30 minutes in “daytime hours” 06.00 and 22.00 and hourly over “night” between 23.00 and 05.00. Data from the recordings was analysed by Cardio Visions dedicated software (PMS Instruments, Maidenhead, United Kingdom) according to the British Hypertension Society guidelines (O'Brien et al., 2000). Valid data was considered  $\geq 14$  systolic and diastolic recordings in “daytime” and 7 over “night”. Nocturnal dipping was defined as  $\geq 10\%$  reduction in mean nocturnal blood pressure compared to mean daytime blood pressure. An example of ABPM-04 software is provided in Figure 2.1.

**Figure 2.1** Example of 24 hour ambulatory blood pressure recording using Cardio Visions dedicated software (PMS Instruments, Maidenhead, UK)



### **2.2.3 Non-invasive assessment of large artery stiffness**

Patients were asked to refrain from caffeine containing products and smoking for 4 hours prior to assessment. Measurements of pulse wave velocity (PWV) and pulse wave analysis (PWA) were performed using the SphygmoCor® system (AtCor, Sydney, Australia) after 30 minutes of quiet supine rest with brachial blood pressure recorded at 10 minute intervals. I performed all studies to minimize operator variability and was blinded to treatment group and preceding measurements. Measurements made using the SphygmoCor® system are highly reproducible in adults and well validated in patients with CKD (Laurent et al., 2006).

#### **2.2.3.1 Pulse wave velocity**

The SphygmoCor® system (AtCor, Sydney, Australia) was used to measure carotid-femoral pulse wave velocity (Ca-Fem PWV). This device consists of a Millar piezo-resistive pressure tonometer (SPC-301; Millar Instruments, TX, USA) coupled to a SphygmoCor® electronic console (AtCor, Sydney, Australia), attached to a computer with specialised SphygmoCor® software (SCOR 2000, Version 7.0). The micromanometer recorded sequential pressure waveforms from the carotid and femoral pulses which were then averaged over an 11 second recording window. The transit time between the two arterial sites was determined by an “anchor point” on the foot of the pressure waveform (end-diastole) and the peak of the R-wave on the simultaneously measured ECG (Figure 2.2). The time between the R-wave and the proximal pulse is subtracted from the time between R-wave and distal pulse to obtain the pulse transit time. The distance between the arterial sites is measured in a direct line from the sternal notch to the femoral pulse and the distance from the sternal notch to the carotid pulse subtracted from the total path length. This method takes account of parallel transmission in the aortic arch branch vessels, providing a more accurate calculation of PWV.

PWV is calculated as:

$$PWV = D \text{ (meters)} / \Delta t \text{ (seconds)}.$$

Where; D was the distance between the carotid and femoral pulse sites and  $\Delta t$  is the transit time between the foot of arterial wave between the two arterial sites. The average of three stable readings was calculated for analysis. At typical carotid and femoral applanation tonometer recording is shown in Figure 2.2.



**Figure 2.2** Carotid-femoral pulse wave velocity using applanation tonometry



Typical tonometry recording at the carotid and femoral arteries measured using the SphygmoCor® system.

Pulse Transit time = [Time between peak R-wave to foot of femoral pulse (b)] – [Time from peak R-wave to foot of carotid pulse (a)]

Arterial distance is measured from sternal notch-to-femoral pulse minus the distance from the sternal notch-to-carotid pulse in order to take account of flow into the aortic arch branches.

### 2.2.3.2 Pulse wave analysis

Applanation tonometry using the SphygmoCor® system (AtCor, Sydney, Australia) was used to assess pulse wave analysis (PWA). The radial artery was gently compressed against underlying bone using a micromanometer pressure transducer (SPC-301; Millar Instruments, TX, USA) thereby equalizing circumferential pressures and allowing a high-fidelity pressure waveform to be recorded (Figure 2.3). The radial waveform was calibrated using the brachial systolic and diastolic pressures measured using a validated blood pressure cuff. This data was transmitted to the SphygmoCor® electronic console and a validated transfer function algorithm (between the aorta and radial artery) applied to calculate an average central aortic waveform from sequential contiguous pulses (Chen et al., 1997). The data displayed were; radial and reconstructed aortic arterial wave forms and peripheral and calculated central aortic systolic and diastolic pressures (Figure 2.4). Using the reconstructed central aortic wave form further parameters were extracted relating to the pressure load in the aorta:

1. Augmented pressure (AP) - the difference between the forward pressure wave (usually the shoulder / inflection on the ascending limb of the ascending wave, P1) and resultant peak from the composite of forward and reflected waves, P2. Thus  $AP = \Delta P$ , where  $\Delta P$  is the difference between the later systolic shoulder / peak pressure P2 and early systolic shoulder P1) (Figure 2.4).
2. Augmentation index (AIx) - represents the difference between the second and first peaks of the central pressure waveform in systole, expressed as a percentage of the pulse pressure. Thus the AP is quantified in terms of the relative change over the whole pulse;  $AIx = [\Delta P / \text{pulse pressure}] \times 100$ , where  $\Delta P$  is the difference between the early systolic shoulder and later systolic shoulder).

3. Augmentation Index standardised for a heart rate of 75 beats per minute (AIx 75) - the known effects of heart rate on wave reflection were adjusted for by calculating AIx for at a reference heart rate of 75bpm using the SphygmoCor® electronic console.

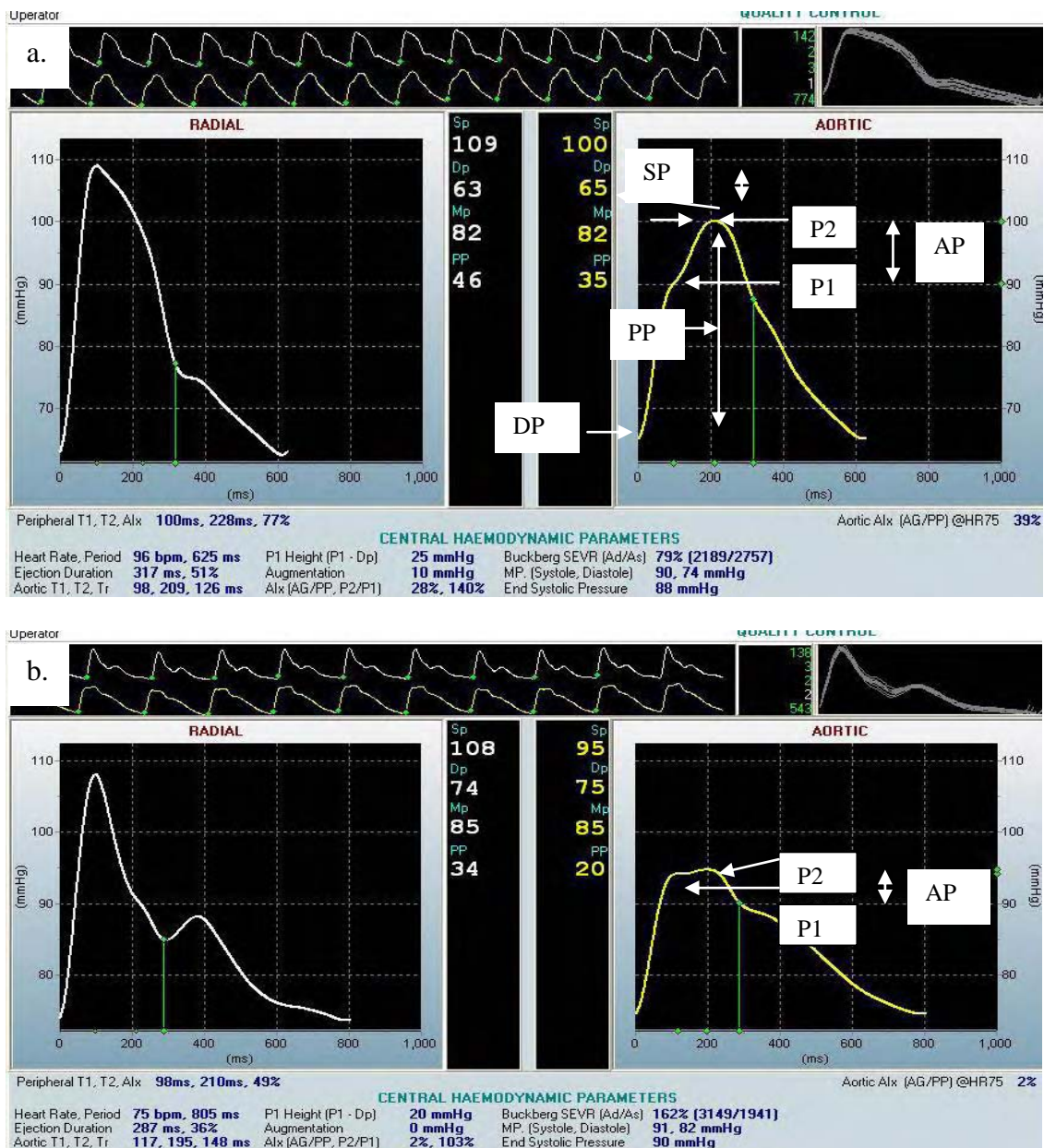
**Figure 2.3** An example of radial artery applanation tonometry (SphygmoCor® system, AtCor, Sydney, Australia)



The radial artery pressure wave form was sampled over 10 seconds using the micromanometer (SPC-301; Millar Instruments) and calibrated with the average blood pressure measured non-invasively at the brachial artery. Waveforms were processed with dedicated software (SphygmoCor® version 7, AtCor) to calculate an averaged radial artery waveform and to derive a corresponding central aortic pressure waveform using a validated generalised transfer function.



**Figure 2.4** Pulse wave analysis using applanation tonometry in: a) patient with end-stage kidney disease and b) age and gender matched healthy volunteer



Haemodynamic parameters derived by pulse wave analysis of the central aortic waveform; P1; first peak / shoulder from out-going pressure wave, P2; late peak from the reflected wave, SP; central systolic pressure, DP; central diastolic pressure, PP; pulse pressure, AP; augmentation pressure (P2-P1). In ESKD (example a), P2 occurs earlier in systole with significant augmentation of central pressure. The height of the late P2 above the inflection (P1) defines the augmentation pressure. In the healthy volunteer (example b), P2 occurs late in systole with little augmentation of central pressure.

## **2.2.4 Transthoracic echocardiography**

Transthoracic echocardiography uses ultrasound waves to provide detailed imaging of the heart. It is non-invasive, portable and rapidly available making it the primary imaging modality for the assessment of cardiac anatomy and function.

### **2.2.4.1 Basic principles**

Ultrasound waves are inaudible mechanical vibrations with a frequency greater than 20,000 cycles per second (20 KHz) that induce alternate refractions and compressions of the physical medium through which they pass. They are generated from the piezoelectric crystals within the transducer of the echo machine. An alternating current expands and compresses polarized particles within the crystal generating a burst or pulse of ultrasound wave which is transmitted (propagates) through tissue (medium) as a straight beam. The acoustic properties of the media determine how much of the beam is reflected, refracted (changes the direction of the wave) and attenuated (absorption of ultrasound energy) and are the basis of ultrasound imaging. The reflected ultrasound energy is received by the piezoelectric crystal in the transducer and converted to low amplitude voltage signals. The signal undergoes complex manipulation to form an image (Armstron WF, 2010).

### **2.2.4.2 Imaging modalities**

Advances in echo technology now allow multiple image displays. I have provided a brief description of the techniques employed in my research programme.

1. Motion (m) mode - allows a single dimension of anatomy to be graphically presented against time. It allows a high repetition frequency of the pulse transmission and receive phase of the transducer. The high sampling rate is valuable for evaluation of

rapid intra-cardiac motion of continuously moving structures but is disadvantaged by a single 1D “line of sight”.

2. Two-dimensional imaging (2D) – generated by sweeping of the ultrasound beam made up of multiple scan lines across the tomographic plane. Reflected ultrasound signals from each scan line are received by the transducer to generate a cross-sectional image depicting structure and function in real time. Tissue harmonic imaging was employed in all studies. Harmonic frequency energy is generated as the signal penetrates through tissue and differs from fundamental frequency which attenuates constantly during propagation. The advantages include; increased depth of propagation, reduced artefact and better endocardial definition.
3. Doppler imaging- based on the change in frequency of the backscattered signal from small moving structures. It is frequently used to assess the velocity, direction and pattern of intra-cardiac blood flow.

#### **2.2.4.3 Imaging Protocol**

All echocardiograms were performed at University Hospital Birmingham using second harmonic imaging on a Vivid 7 (GE Vingmed Ultrasound, Horten, Norway) machine with a M3S multi-frequency phased-array transducer (1.5 MHz). Patients were positioned in a left decubitus positioning with ECG and brachial blood pressure monitoring. Standard parameters for chamber quantification, left ventricular mass and left ventricular systolic and diastolic function are outlined in Table 2.1. All measurements were made in triplicate and averaged according to the recommendations of the American Society of Echocardiography (Lang et al., 2005;Nagueh et al., 2009)

**Table 2.1** Standard echo parameters measured using American Society of Echocardiography guidelines (Lang et al., 2005)

|                                               |                                |
|-----------------------------------------------|--------------------------------|
| Chamber dimensions & Mass                     |                                |
| Left ventricular dimensions & volumes         | M-mode, 2D measurements        |
| LV mass                                       | M-mode , 2D area-length method |
| Ventricular systolic function                 |                                |
| Ejection fraction                             | Biplane method of discs        |
| Tissue Doppler myocardial systolic velocities | Pulse wave Doppler             |
| Myocardial deformation                        | 1D strain & 2D strain imaging  |
| Ventricular diastolic function                |                                |
| Trans-mitral inflow                           | Pulse wave Doppler             |
| Pulmonary venous velocities                   | Pulse wave Doppler             |
| Colour m-mode propagation velocity            | M-mode                         |
| LA volume                                     | 2D imaging                     |
| Isovolumic relaxation time                    | Pulse wave Doppler             |
| Tissue Doppler early myocardial velocities    | Pulse wave Doppler             |
| Ventricular systolic & diastolic function     |                                |
| Tei Index                                     | Pulse wave Doppler             |

Examples of echo images demonstrating these measurement are shown in Figure 2.6



#### 2.2.4.4 Conventional measurements of left ventricular mass, systolic and diastolic function

**LV mass** – I used the American Society of Echocardiography (ASE) guidelines to calculate LV mass. Recommended methods include:

- i) Two-dimensional (2D)-targeted m-mode LV measurements (Figure 2.5a). Mass is estimated using the recommended cube formula proposed by Devereux et al. for leading edge to leading edge measurements (Devereux et al., 1986):

$$\text{LV mass (m-mode)} = 0.8 \times (1.04 (\text{LVIDd} + \text{PWTd} + \text{SWTd})^3 - (\text{LVIDd})^3) + 0.6\text{g}$$

Where; LVIDd; left ventricular internal diameter at end-diastole, PWTd; posterior wall thickness at end diastole and SWTd; septal wall thickness at end diastole.

This formula is limited to patients with normal LV geometry as cubing used in the formula, magnifies small errors in the measurements and increases the variability in serial assessments.

- ii) Two-dimensional imaging (Figure 2.5b). Myocardial area was assessed at a mid-papillary level in the parasternal short axis view and LV length in the apical 4 chamber (4C) view (Lang et al., 2005). Measurements were applied to the area-length (A/L) formula:

$$\text{LV mass (A/L method)} = 1.05 \left[ \left\{ \frac{5}{6} \times A1 \times (a+d+t) \right\} - \left[ \frac{5}{6} \times A2 \times (a+d) \right] \right]$$

Where;  $A_1$  = total LV area (epicardial contour),  $A_2$  = LV cavity area (endocardial contour),  $a$  = LV long axis length,  $d$  = widest short-axis diameter at mitral annular plane,  $LV$ ,  $t$  = mean wall thickness derived from epicardial and endocardial areas.

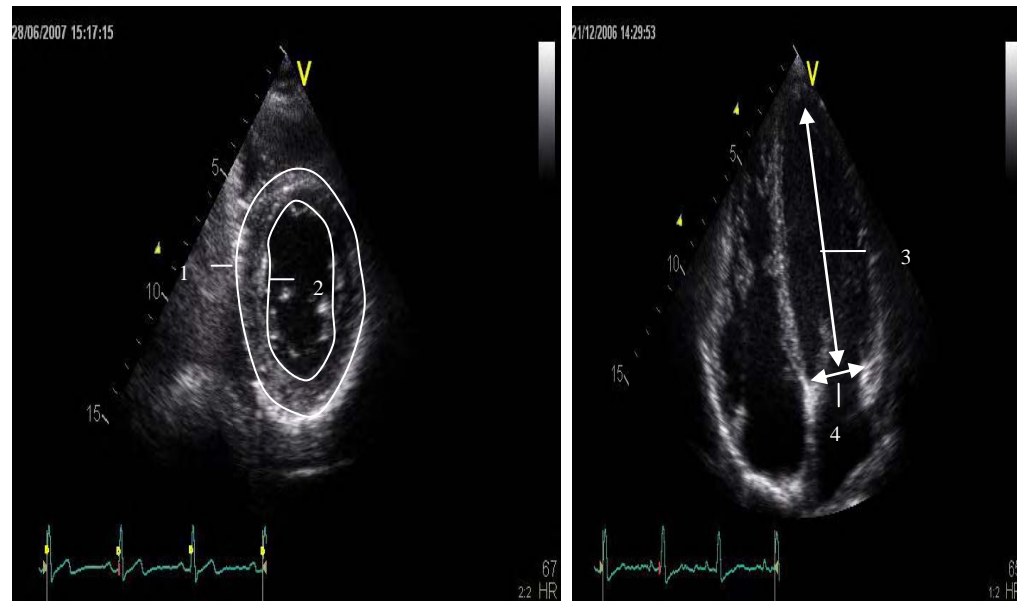
**Figure 2.5** Estimation of left ventricular mass on echo using American Society of Echocardiography guidelines

a. 2D-target m-mode method



1. Septal wall thickness at end-diastole
2. LV internal diameter at end-diastole
3. Posterior wall thickness at end-diastole

b. 2D area-length method



1. Epicardial contour
2. Endocardial contour
3. LV long-axis length
4. Widest short-axis diameter at the mitral annular plane

**Mitral inflow velocities** – measured using pulse wave Doppler with the sample volume at the tips of the mitral valve during diastole (Figure 2.6b). Measurements include the peak early filling (E-wave) and late diastolic filling (A-wave) velocities, the E/A ratio and deceleration time (DT) of early filling velocity. Traditionally E/A has been used to assess diastolic function as a marker of the LA-LV pressure gradient, however both are effected by increasing age ( E velocity and E/A ratio decrease and DT and A velocity increase), heart rate and rhythm disturbance.

**Colour m-mode propagation velocity ( $V_p$ )** – measured in an A4C view using a colour m-mode Doppler through the centre of the LV with measurement of the slope of the first aliasing velocity during early filling (Figure 2.6c). It is a marker of LV diastolic function which measures the mitral-apical flow rate. In the normal ventricle there is rapid early filling of the LV (LV suction) driven by the LA-LV pressure gradient. A  $V_p \leq 50$  cm/s is considered abnormal.

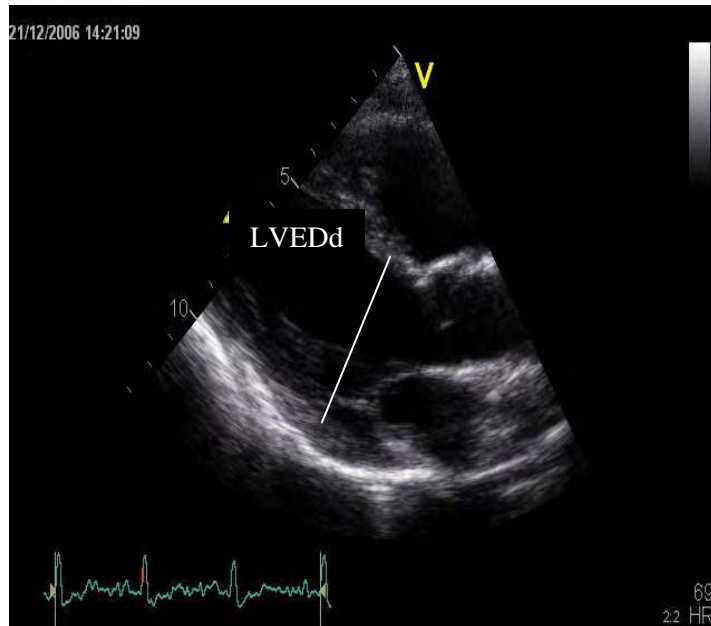
**Isovolumic relaxation time** – measured by continuous wave Doppler positioned in the LVOT outflow, simultaneously showing the onset of mitral inflow and end of aortic ejection (Figure 2.6d). Impairment of myocardial relaxation reduces the LA-LV pressure gradient. The isovolumic relaxation period is therefore increased until LV pressure is  $<$  LA pressure and the mitral valve opens.

**Myocardial performance Index (MPI) / Tei index** – A Doppler-derived index of combined systolic and diastolic myocardial performance. It compares the total systolic time from mitral valve closure to mitral valve opening with systolic time involved in actual aortic flow (Figure

2.6d).  $MPI = (\text{isovolumic contraction time} + \text{isovolumic relaxation time}) / \text{aortic ejection time}$ . The normal LV MPI is  $\leq 0.4$  and progressively higher values imply worsening ventricular function and correlates with adverse cardiac outcome in ischaemic and non-ischaemic heart disease.

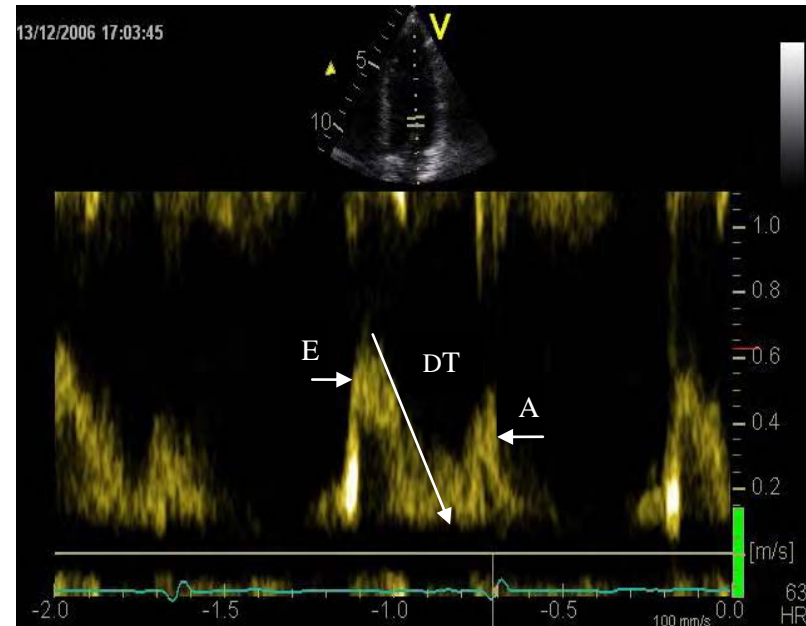
**Figure 2.6** Conventional measures of left ventricular systolic and diastolic function

a. Left ventricular dimensions in diastole using by 2D imaging in the parasternal long axis view



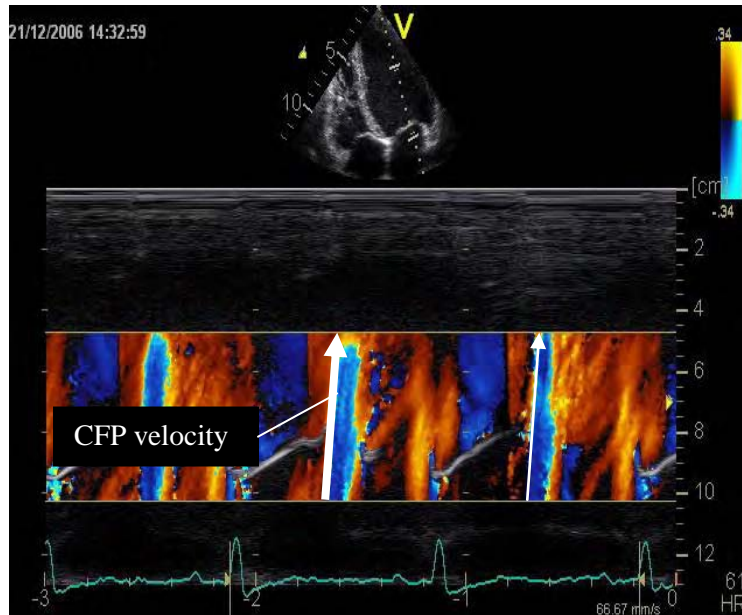
LVEDd –end-diastolic diameter

b. Trans-mitral inflow velocities (m/s) and deceleration time (ms) measured by pulse wave Doppler at the tips of the mitral valve in an apical 4 chamber view



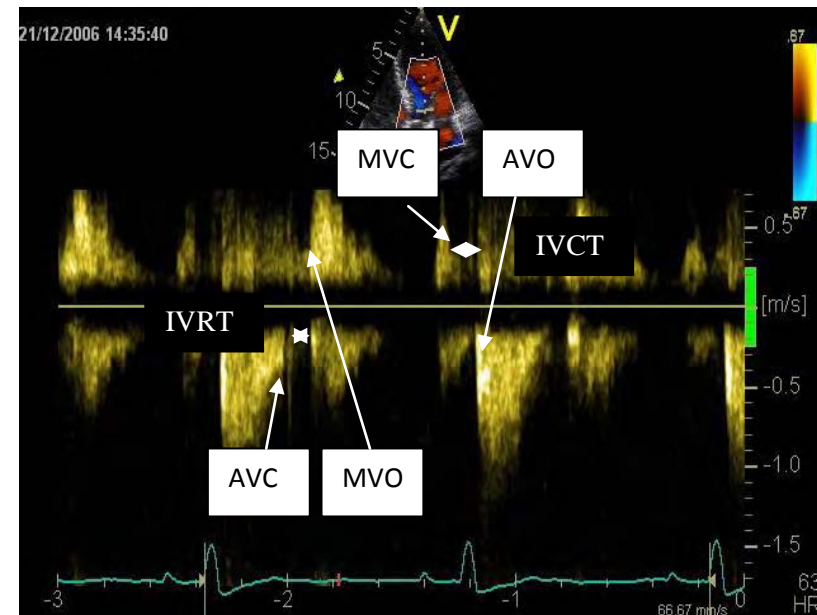
E; peak early filling velocity (m/s), A; late diastolic filling velocity (m/s), DT; deceleration time of early filling velocity (ms)

c. Colour flow propagation velocity (cm/s) using colour m-mode Doppler in an apical 4 chamber view



CFP; colour flow propagation velocity

d. Isovolumic relaxation and contraction times (ms) measured using continuous wave Doppler in an apical 4 chamber view



IVRT  $\blacktriangleright$  ; isovolumic relaxation time = time between aortic valve closure (AVC) and mitral valve opening (MVO)

IVCT  $\blacklozenge$  ; isovolumic contraction time = time between MV closure (MVC) and AV opening (AVO)

#### **2.2.4.5 Assessment of longitudinal ventricular function**

The LV myocardial wall has a complex arrangement of myofibres which are responsible for the twisting motion of the ventricles. As I described in chapter 1, subendocardial fibres are longitudinally orientated. Following electromechanical activation the fibres shorten, moving LV base towards the apex (Mor-Avi et al., 2011). Assessment of this motion is known as longitudinal or long-axis systolic function. The subendocardial fibres also move inward toward the LV cavity and contribute approximately 40% of LV thickening along with the circumferential mid-wall fibres. This is known as radial systolic function. The subepicardial fibres have an oblique orientation and are responsible for the rotational motion of the heart (Figure 2.7). A comprehensive assessment of longitudinal systolic and diastolic function was made in work described in this thesis using a number of complimentary echo techniques outlined in sections 2.2.4.4.1 and 2.2.4.4.2.

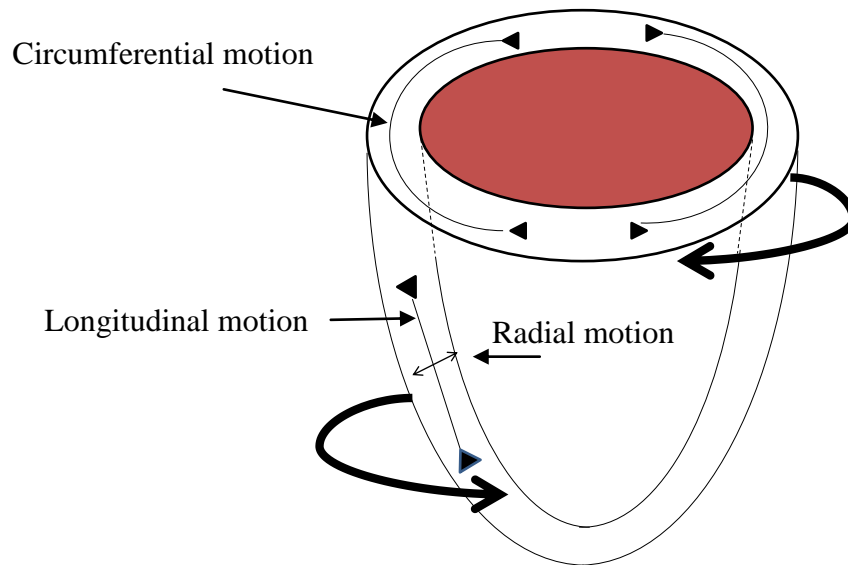
##### **2.2.4.5.1 Tissue Doppler myocardial velocities**

Tissue Doppler imaging (TDI) is a variation of conventional Doppler flow techniques, allowing quantification of the Doppler shift of myocardial tissue motion rather than blood. Assessment of longitudinal function using TDI provides quantitative data for the assessment of ventricular systolic and diastolic function and ventricular haemodynamics (Yu et al., 2007) and is prognostic indicator in ESKD (Rakhit et al., 2007).

Tissue Doppler imaging was used to assess the long-axis function of the LV in the basal and mid myocardial segments of the septal, lateral, anterior, inferior, antero-septal posterior walls using standard apical 4 chamber (4C), 2 chamber (2C) and 3 chamber (3C) views. A schematic representation of the method used to acquire TDI is presented in Figure 2.8.



**Figure 2.7** Schematic representation of left ventricular myofibre orientation



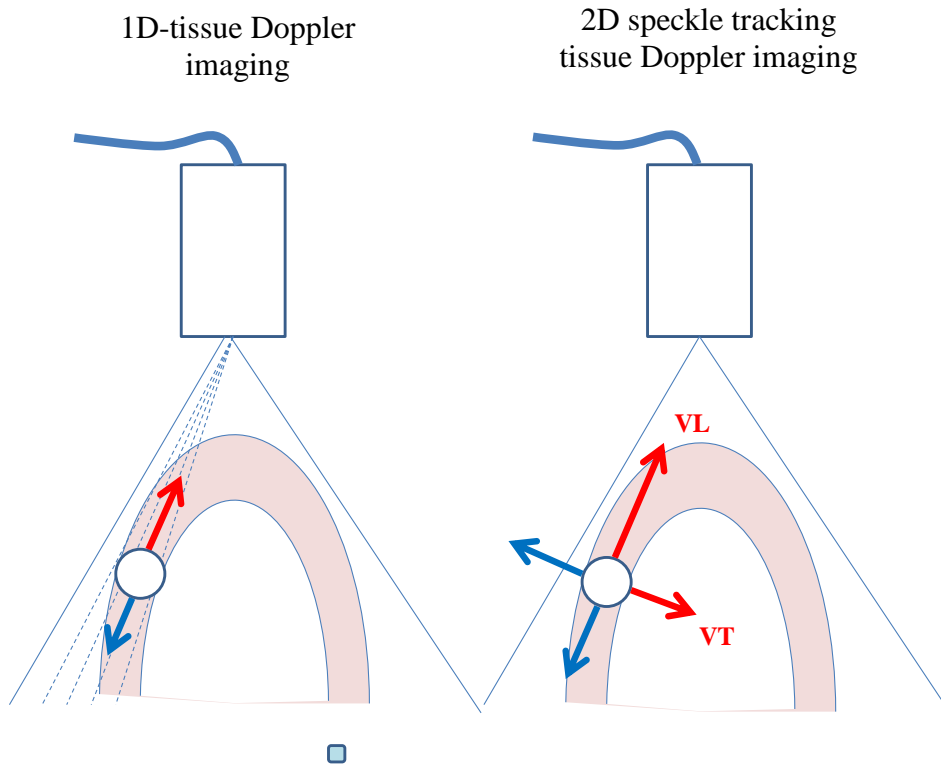
Adapted from Armstrong WF & Ryan T (2010)

Longitudinal movement of the base toward the apex (long-axis systolic function) is directed by shortening of the subendocardial fibres

Left ventricular myocardial thickening (radial systolic function) is directed by the subendocardial and circumferential fibres

The twisting motion (torsion) of the left ventricle is directed by the subepicardial fibres

**Figure 2.8** A schematic representation of the method for obtaining myocardial velocities using tissue Doppler imaging



Adapted from Armstrong WF & Ryan T (2010)

The longitudinal velocity of the myocardium is assessed by placing a sample volume within the myocardium at a region of interest. One dimensional tissue Doppler imaging only measures the velocity along the scan lines aligned with the direction of motion. The myocardium moves toward the transducer in systole and away in diastole.

Two-dimensional speckle tracking derived tissue Doppler is (Doppler) angle independent and measures the vector velocities in the any direction within the imaging plane relative to the direction of muscle contraction (VL – longitudinal, VR – radial).

Data are presented graphically as velocity of the myocardium relative to time. Examples are presented in Figure 2.9a and 2.12a.

Parameters were measured using two techniques; spectral pulse wave TDI (PW-TDI) with a sample volume placed within the myocardium at the level of the mitral and tricuspid annulae. The low Doppler shift frequencies are recorded as the myocardium moves through the sample volume in a single cardiac cycle (Figure 2.9a). The major strengths of this technique include; measurement of an instantaneous peak velocity in the region of interest (ROI), high temporal resolution and reproducible for serial evaluations. The disadvantages include; the inability to simultaneously perform measurements in different walls within the same cardiac cycle and need for parallel align of the ultrasound beam with the ROI (angle dependency) as with any Doppler technique which excludes some myocardial segments. In later studies I used 2D colour TDI. A colour velocity map was fitted over the wall of interest or completely over the apical 4-chamber image. Each pixel encoded a mean myocardial velocity relative to the transducer. Data was analysed off-line generating a graphical representation of myocardial velocities against time in multiple segments within the same cardiac cycle (Figure 2.9b). The advantages of this method were; post-processing adjustment of the sample volumes to track myocardial motion, thus staying in the same ROI throughout the cardiac cycle and sampling of multiple myocardial regions in the same cardiac cycle.

The following parameters were measured:

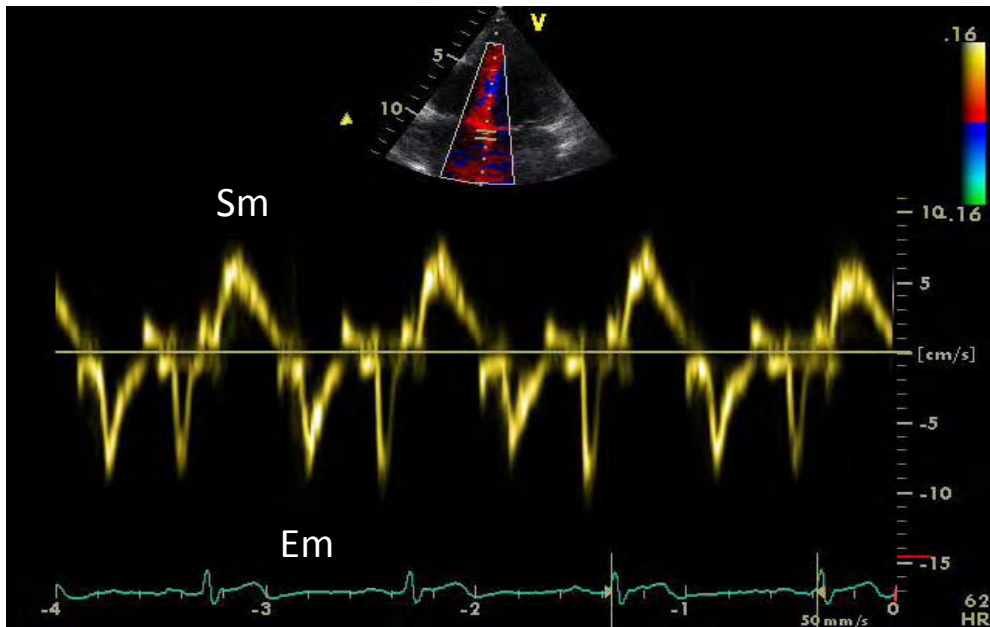
1. Systolic velocity (Sm)- the velocity of myocardium contraction. On spectral TDI this was measured at the level of the valve annulae and on colour TDI in the basal and mid segments. It is a sensitive marker of mildly impaired systolic even with a normal EF. A reduction in Sm (usually  $\leq 8$  cm/s) signifies impaired contraction.
2. Early myocardial diastolic velocity (Em)- a marker of LA-LV pressure gradient and hence myocardial relaxation. Values decrease with age but in general lateral Em

$\geq 10$  and septal  $E_m \geq 8$  are usually observed in the normal subject and are reduced with impaired LV relaxation and increase LV filling pressure. A reduction in  $E_m$  (usually  $\leq 8$  cm/s) is one of the earliest markers of diastolic dysfunction. Unlike mitral inflow velocities,  $E_m$  is less influenced by changes in pre-load and remains low with more advanced left ventricular impairment.

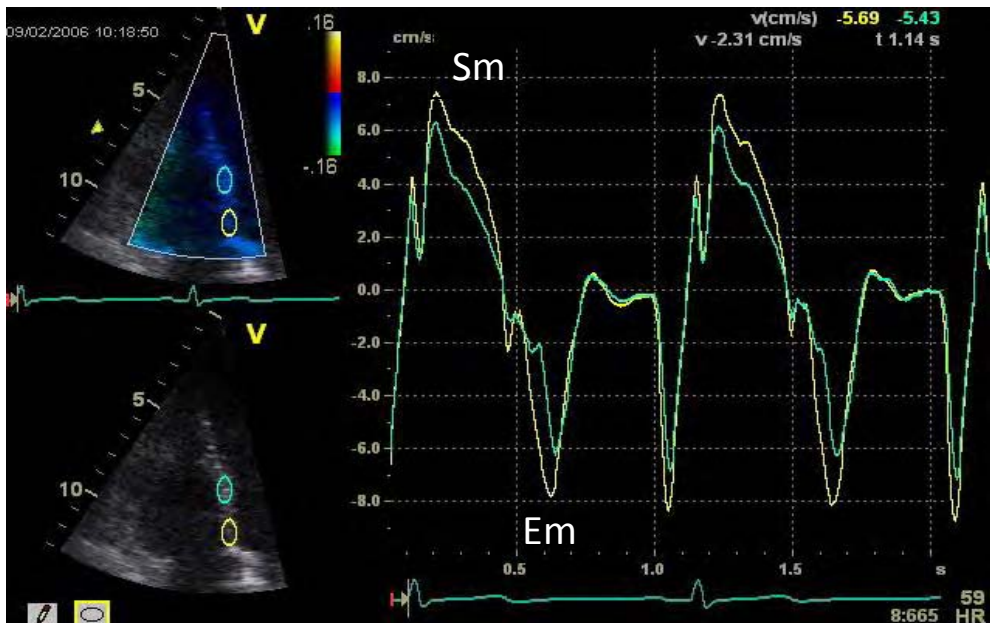
3. Ratio of mitral inflow velocity to  $E_m$  ( $E/E_m$ ) - an estimation of LV filling pressure which correlates well with invasive assessment at cardiac catheterization (Ommen et al., 2000). An  $E/E_m$  lateral ratio  $>12$  and septal  $\geq 15$  are correlated with elevated LV early diastolic pressure.  $E/E_m$  lateral and septal  $< 8$  is correlated with normal LV early diastolic pressure (Figure 2.10).

Normal values for TDI measurements decrease with age, differ between different walls and across the wall, being higher in the subendocardium than epicardium. There is also a base to apex gradient with velocities in the base greater than at the apex. Colour TDI measurements are typically 20-30% lower than spectral TDI reflecting measurement of the mean velocity of the region of interest and positioning in the basal segment rather than the higher velocity annular region.

**Figure 2.9a** An example of spectral pulse wave tissue Doppler Imaging

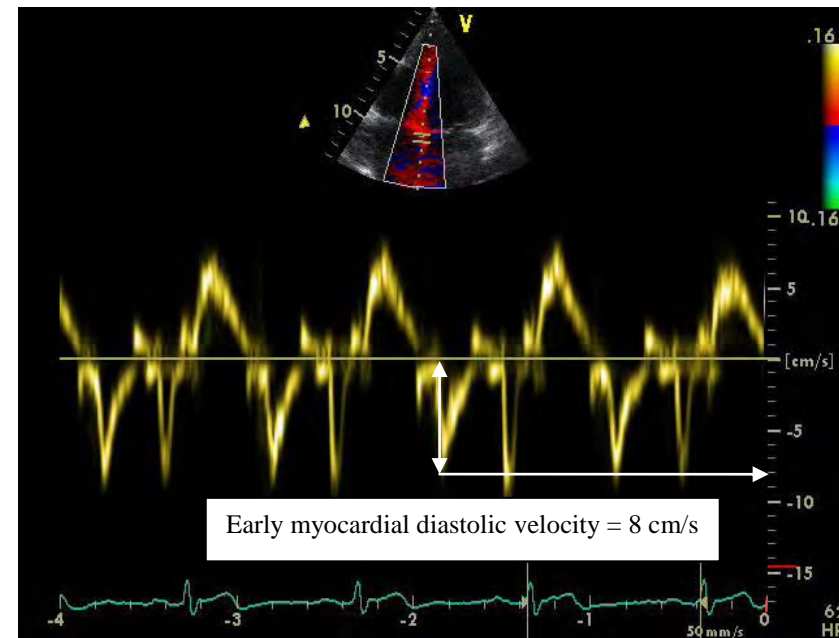
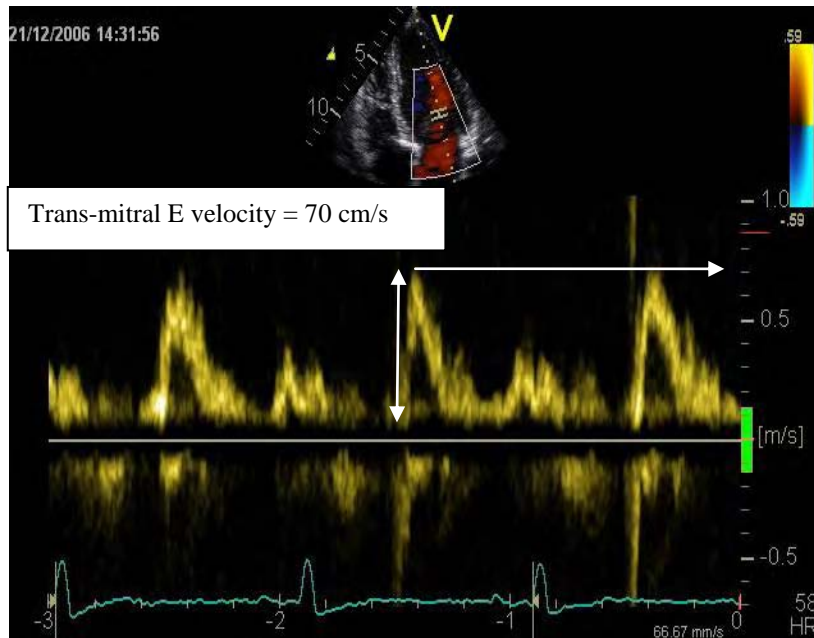


**Figure 2.9b** An example of colour tissue Doppler Imaging



Analysis of basal and mid segments of the LV septum performed off line (EchoPac software PC version 7.0.1; GE Vingmed Ultrasound AS). Sm; systolic velocity, Em; early myocardial diastolic velocity.

**Figure 2.10** Calculation of early filling velocity / early myocardial velocity ratio (E/Em) using pulse wave and tissue Doppler imaging



Early trans-mitral inflow velocity = 70 cm/s

Early myocardial diastolic relaxation velocity 8cm/s

Basal septal annular E/Em = 9

#### 2.2.4.5.2 Myocardial deformation

The echocardiographic measurement of myocardial deformation provides a series of regional and global quantitative measures of myocardial function and contractility. Deformation indices include strain, strain rate and torsion which provide a more direct assessment of intrinsic myocardial contractility than EF or wall motion analysis (Mor-Avi et al., 2011).

Strain (S) is a measure of deformation (%) and refers to the change in myocardial length from an applied force. Strain rate (SR) is the time course of the deformation ( $s^{-1}$ ) and is derived from the difference of two velocities normalized to the distance between them. By convention, ventricular contraction shortens longitudinal and circumferential fibres giving a negative strain and radial fibres lengthen and thicken giving positive strain. As such, normal longitudinal contraction is defined by negative systolic strain followed by biphasic positive diastolic strain corresponding to early and late diastolic filling (Figure 2.11).

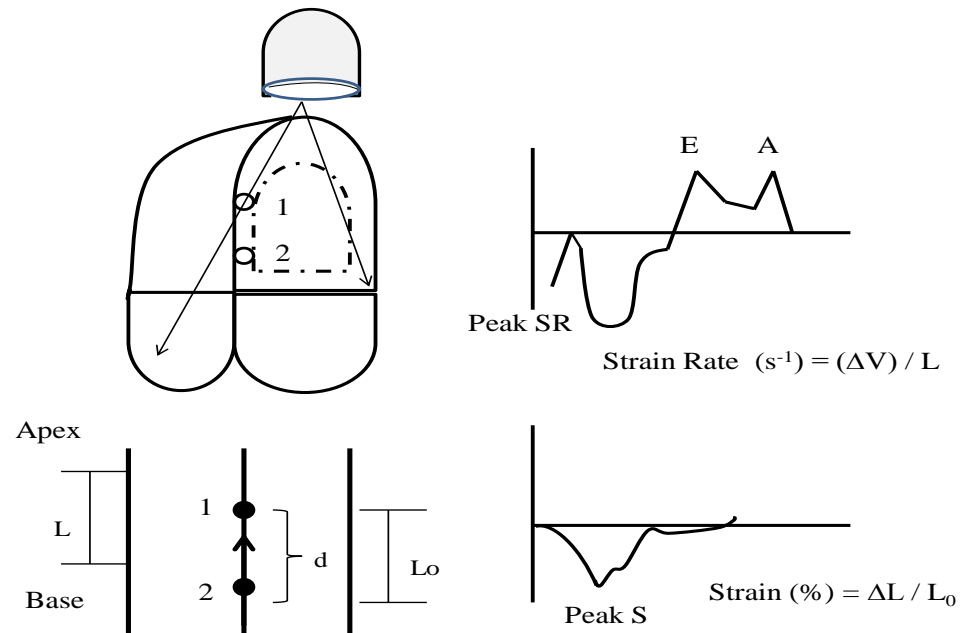
Indices can be calculated using 1D-TDI or from 2D (Doppler independent) speckle tracking techniques. In my early studies, I used the first reported methodology known as 1D-strain which is derived from TDI. The selected wall of interest was positioned in-line with the ultrasound beam and a narrow colour velocity map is placed over it to achieve frame rates of 180-220 frames per second. A 12 x 8 mm sample volume was positioned in each of the basal, mid and apical segments. Three cardiac cycle loops triggered to the electrocardiogram were acquired for each image in end-expiration and stored digitally for off-line analysis. Using commercially available software (EchoPac, GE Health Care, Norway) an instantaneous gradient of velocity along a sample length is quantified by performing a regression calculation between the velocity data from adjacent sites along the scan line. These instantaneous data may then be combined to generate a SR curve and subsequent integration of SR curves

provides data on strain (Figure 2.11). These data therefore reflect the movement of one tissue site relative to another within the sample volume, in contrast to TDI velocity data which reflect movements of one site relative to the transducer and can be influenced by contraction of adjacent segments. This method is technically challenging (signal noise, angle dependence) and time consuming (analysis of individual walls) but has been well validated using microcrystals and later cardiac MRI (Edvardsen et al., 2002;Urheim et al., 2000).

Newer 2D speckle tracking (Doppler independent) semi-automated techniques for assessing deformation are accurate, well validated and not effected by 1D methodology limitations (Amundsen et al., 2006). This technique analyzes motion by tracking speckles (natural acoustic markers) generated from structures smaller than the ultrasound wavelength. Blocks of speckles in a discrete location are tracked from frame to frame (simultaneously in multiple regions) and tissue displacement data is derived from which strain and strain rates can be calculated. Two dimensional grey scale images from the apical four and two chamber views with sector depth and width optimised, were acquired at end-expiration at a frame rate  $\geq 80$  / second. The endocardial border was manually tracked at end-systole using commercially available software (2D-strain EchoPac PC v.7.0.1, GE Healthcare, Horten, Norway). The software automatically defined the epicardial and mid myocardial line within the region of interest and calculates the frame-to-frame displacements of the speckle-pattern throughout the cardiac cycle (Figure 2.12). The advantage of speckle tracking include: quantification of deformation indices in any direction within the imaging plane and in simultaneous ROIs, less angle dependence and less time consuming semi-automated off-line analysis packages.



**Figure 2.11** A schematic representation of the methodology for obtaining and calculation of myocardial deformation for the septal wall of the left ventricle

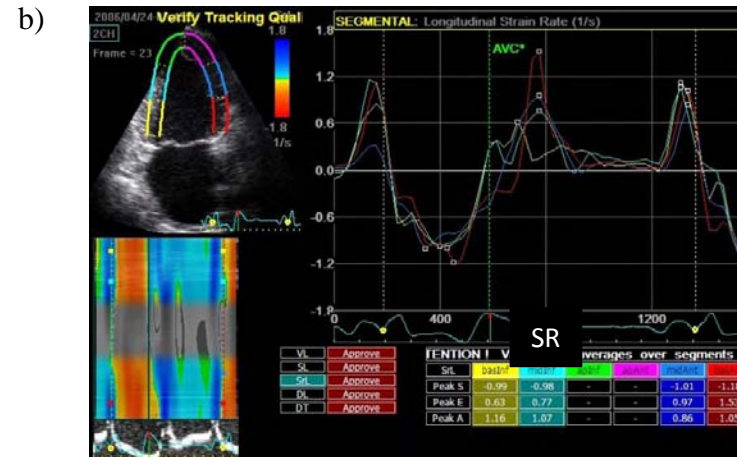
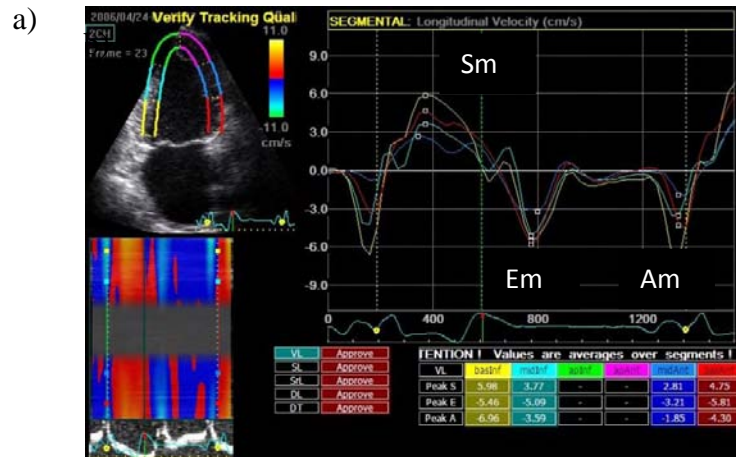


As the ventricle contracts, muscle shortens in the longitudinal dimension. The change in distance between two discrete points within the region of interest is used to calculate strain and change in velocity to calculate strain rate.

A; late myocardial strain, d; displacement, E; early myocardial strain,  $\Delta L$ ; change in length in segment studied,  $L_0$ ; baseline length, peak SR; peak systolic strain rate, peak S; peak systolic strain,  $\Delta V$ ; change in velocity gradient in segment studied

**Figure 2.12** Examples of 2D speckle tracking using (EchoPac PC v.7.0.1 software (GE Healthcare, Horten, Norway) for calculation of :

a) myocardial tissue velocities (Sm, Em, Am), b) strain rate (SR), c) strain (S)



See text for further explanation of the parameters measured.

#### **2.2.4.5.3. Assessment of arterial-ventricular interaction**

The interaction of the left ventricle with the arterial system, termed arterial-ventricular interaction or coupling, was assessed on echo. The parameters of “elastance” provide a non-invasive estimate of stiffness of the aorta (arterial elastance), left ventricle in systole (end-systolic elastance) and left ventricle in diastole (diastolic elastance). Each parameter of elastance was mathematically derived using echo methodologies previously validated against “gold standard” invasively measured using conductance catheters (Table 2.2).

Effective arterial elastance represents the net arterial load that is imposed on the left ventricle. It is not a measure of a specific arterial property; rather it is an integrative index that incorporates the principal elements of arterial load, including peripheral vascular resistance, total arterial compliance, pulsatile load, and systolic and diastolic time intervals (Kelly et al., 1992a).

Left ventricular end-systolic elastance represents an index that integrates intrinsic LV contractility as well as the modulating effects of the geometric, structural and functional properties of the left ventricle. It is considered a load independent integrated measure of LV chamber performance that can be related to an integrated measure of arterial load ( $E_a$ ) (Chen et al., 2001).

Diastolic elastance is an index of diastolic stiffness. The ratio of  $E/E_m$  is well validated as a measure of LV end-diastolic pressure. The use of stroke volume (a substitute for the volume of filling in diastole assuming no aortic regurgitation) provides a means of adjustment for changes related to increasing age and gender (Redfield et al., 2005).

The arterial-ventricular coupling ratio is measure of interaction between the left ventricle and arterial system and thereby provides an index of cardiac performance. A normal

coupling ratio of 0.3-1.3 matches properties so that an optimal transfer of blood from the left ventricle to the periphery without excessive changes in pressure; an optimal or near-optimal stroke work and energetic efficiency are all achieved, while maintaining blood pressures and cardiac outputs within in a physiological range (Kass, 2002).

**Table 2.2** Data acquired during echo to calculate the parameters of elastance

| Parameter of elastance                       | Required echo measurements                                   | Formula to calculate parameter of elastance            |
|----------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------|
| Arterial Elastance (Ea)                      | SBP, LVOT diameter, LVOT TVI                                 | $Ea = Pes / SV$                                        |
| Arterial Elastance (Ea) indexed to BSA (EaI) | BSA                                                          | $EaI = Ea / BSA$                                       |
| End-systolic elastance (Ees)                 | SBP, DBP, LVOT diameter, LVOT TVI,<br>CW Doppler LVOT, LV EF | $Ees = DBP - (E_{Nd} \times Pes) / (SV \times E_{Nd})$ |
| Diastolic elastance (Ed)                     | E-wave, Em, LVOT diameter, LVOT TVI                          | $(E/Em) / SV$                                          |
| Arterial-ventricular coupling ratio (Ea/Ees) |                                                              | $Ea / EeS$                                             |

End-systolic pressure (Pes) = SBP x 0.9

Stroke volume (SV); LVOT area x LVOT time velocity integral

Pre-ejection period measured from onset of R-wave on ECG to start of aortic ejection on continuous wave Doppler

Total ejection period measure from onset of R-wave on ECG to end of aortic ejection on continuous wave Doppler

Left ventricular ejection fraction measured by Biplane method of disc (modified Simpsons)

Estimated  $E_{Nd}$  is the normalized elastance value at the onset of ejection

It is calculated by;  $= ((DBP / (LV \text{ volume at end-diastole}) - SBP)) / (Pes / (LV \text{ volume at end-systole} - SBP))$

BSA; body surface area, DBP; brachial diastolic blood pressure, E; early transmitral inflow velocity, Em; Lateral annular early myocardial velocity, LVOT; left ventricular outflow tract, SBP; brachial systolic blood pressure measured at time of echo, TVI; time velocity integral measured from pulse wave Doppler.

## **2.2.5 Cardiac magnetic resonance imaging**

Cardiac magnetic resonance (cardiac MRI) has become an important imaging modality capable of comprehensive non-invasive assessment of cardiac structure, function and tissue characterisation without a need for ionizing radiation. At present most cardiac MRIs are performed using magnetic field strengths of 1.5 Tesla (T) and 3T which are 30,000 and 60,000 times greater than the 0.05mT field strength on the earth's surface. The high magnetic field strengths are generated by electromagnets built using coils of superconducting wire. These allow electrical current with no resistance to circulate indefinitely but must be cooled to cryogenic temperatures (-273.16 °C) most frequently using liquid helium (McRobbie DW, 2007).

### **2.2.5.1 Basic principles**

MRI is based on the phenomenon of nuclear magnetic resonance (NMR). This is the principle by which an energy signal is emitted by atomic nuclei of molecules in response to the application radiofrequency (RF) energy. Current clinical MRI techniques are based on the detection of additive signal from the atomic nuclei (single proton) of hydrogen atoms which are in abundance in water and fat within the human body.

Application of a magnetic field aligns the protons in either a low energy state parallel to the main magnetic field (longitudinal magnetization,  $B_0$ ). To create a signal for image derivation, a transient RF energy pulse is applied perpendicular to the main magnetic field (transverse magnetization,  $B_1$ ) which excites protons from the lower energy state to the higher energy "unstable" state and tilts the longitudinal magnetization into the transverse plane. The degree of proton excitation is proportional to the amplitude and duration (milliseconds) of the

pulse while higher magnetic field strengths increase the proton spins in the low energy state. After discontinuation of the RF pulse, protons relax and fall back to equilibrium in the low energy state. Rapid dissipation of RF energy occurs which is detected as current by antennae (receiver coils) embedded within the bore of the MRI machine. The raw data signals (K-space) are converted to 2D or 3D image by the process of Fast Fourier transformation which separates out the individual spatial frequency components in the detected signal allowing localisation to discrete anatomical locations (McRobbie DW, 2007).

### **2.2.5.2 Imaging protocol**

All cardiac MRI studies were performed at University Hospital Birmingham using a 1.5 Tesla Symphony MRI scanner (Siemens, Erlangen, Germany) (Figure 2.13). All pulse sequences were run in accordance with standard methodologies (Finn et al., 2006). The research protocol took approximately 45 minutes per patient.

#### **2.2.5.2.1 Patient preparation**

Meticulous standard safety procedures were performed before subjects entered the scan room to exclude any contraindication to MRI. An emergency buzzer was provided in case of patient discomfort or possible side-effects during the scan. Four ECG electrodes were applied to the chest wall and a flexible surface radiofrequency coil was placed over the top for signal reception. To minimize cardiac motion artefact, scans were timed with respect to the R-R interval of an ECG (ECG-gating) and to minimize respiratory drift, breath held images were acquired on end-expiration.

**Figure 2.13** Siemens Symphony 1.5T MRI machine at University Hospital Birmingham





#### **2.2.5.2.2 Multi-plane localisers and transaxial “stack” of the thorax**

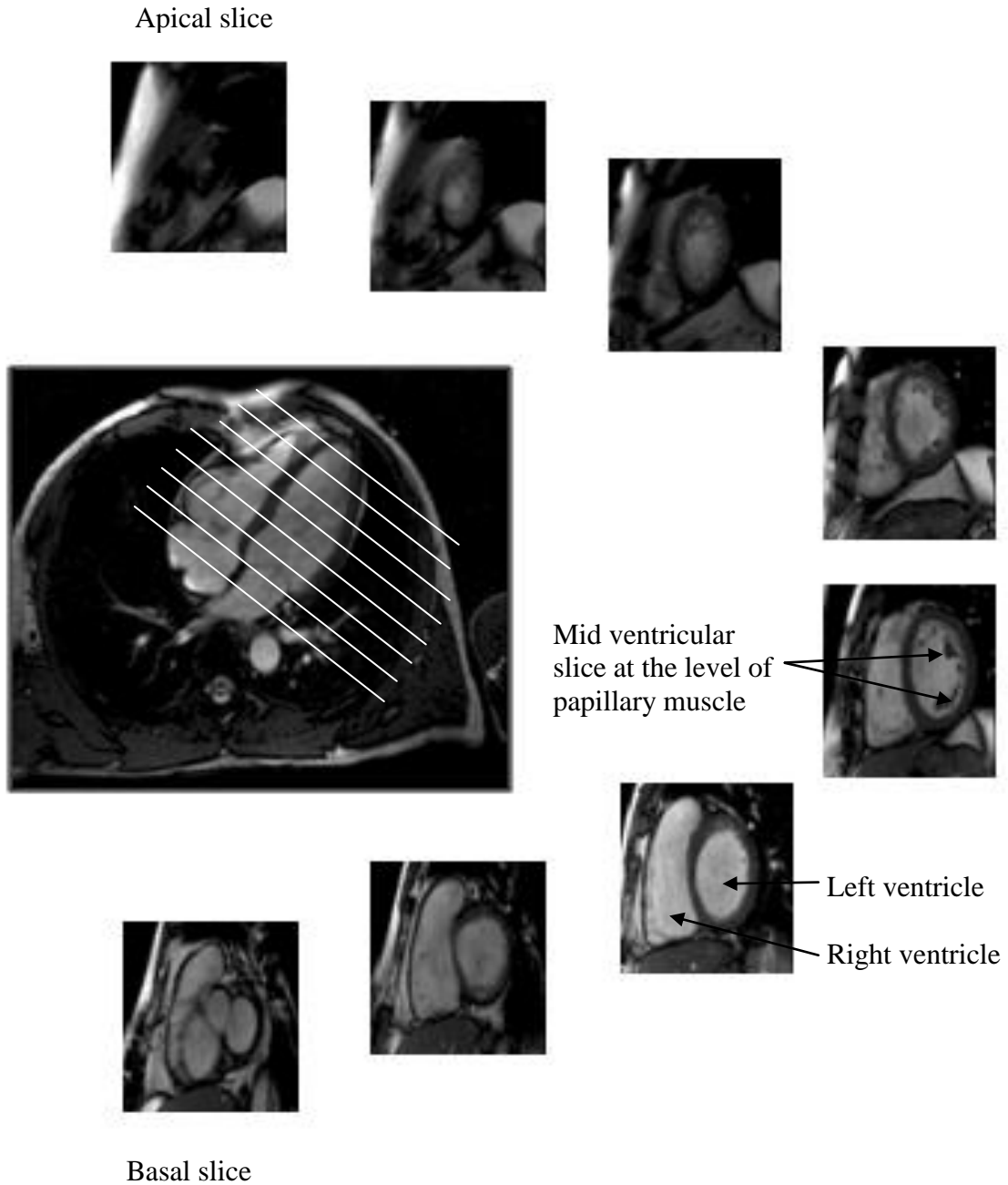
Standard pilot images in the three orthogonal planes (axial, coronal and sagittal) were acquired with a short breath hold (approximately 10 seconds). In addition, low resolution anatomical imaging of the thorax was performed using an axial black blood T2 Half Fourier Acquisition Single Shot Turbo Spin Echo (HASTE) non-breath-hold sequence with prospective ECG-gating. These initial scans optimize subject placement within the magnetic field, provide a useful reference for planning subsequent scans and provides an opportunity to examine cardiac connections and extra-cardiac pathology.

#### **2.2.5.2.3 Left ventricular volume, mass and function**

The scout and axial images were used to plan more accurate breath-held vertical long axis (VLA / 2 chamber), horizontal long axis (HLA / 4 chamber) and short axis (SAX) views to ensure the true long axis of the heart identified. A series of 7mm thick short axis images (Figure 2.14, 2.15a) separated by a 3mm gap were piloted from long-axis views, starting at the atrio-ventricular groove and continuing the entire length of the ventricle (approximately 8-10 images commonly referred to as the short axis stack) These images are acquired using steady state free precession (SSFP) sequence which is characterised by high intrinsic contrast between blood pool and surrounding structures and excellent signal to noise ratio. The image is optimized by breath holding (approximately 8-10 seconds) and ECG gating over several cardiac cycles (retrospective) which provides a temporal resolution of approximately 30ms and an in-plane spatial resolution of approximately 1-2mm. When the images are displayed in rapid succession, a 2D movie-like cine image made up of 20-40 frames (phases) at progressively advancing points of the cardiac cycle.

Data was analyzed off-line using Argus software (Siemens, Erlangen, Germany). The short-axis stack was used to calculate left ventricular volumes, ejection fraction and mass (Table 2.3). The epicardial and endocardial borders were manually traced in end-diastole (defines as the first slice or phase) and end-systole (visually defined as the smallest LV volume). The basal slice was considered to be within the left ventricle if the blood volume was surrounded by 50% or more of ventricular myocardium (Maceira et al., 2006). Papillary muscles were excluded from ventricular volume calculations. LV mass was calculated using the end-diastolic myocardial volume (including papillary muscle) multiplied by the specific density of myocardium (taken as  $1.05\text{g/cm}^3$ ).

**Figure 2.14** End-diastolic short axis images of the left and right ventricles



A series of 7mm thick short axis images from the atrio-ventricular groove to the apex of the heart. Each “slice” is separated by a 3mm gap. The LV epicardial and endocardial borders were manually traced in end-diastole and end-systole to calculate LV volumes, ejection fraction and mass.

**Table 2.3** Left ventricular indices calculated using cardiac MRI

| Parameter                       | Methods                                                                                                      |
|---------------------------------|--------------------------------------------------------------------------------------------------------------|
| LV end-diastolic volume (LVEDV) | Largest LV cavity volume at first phase                                                                      |
| LV end-systolic volume (LVESV)  | Smallest cavity volume                                                                                       |
| LV stroke volume(SV)            | LVEDV - LVESV                                                                                                |
| LV ejection fraction (LVEF)     | SV / LVEDV x100                                                                                              |
| LV mass                         | Multiplication of ED tissue volume multiplied<br>by specific density of myocardium (1.05 g/cm <sup>3</sup> ) |

ED; end-diastole, ES; end-systole, SV; stroke volume

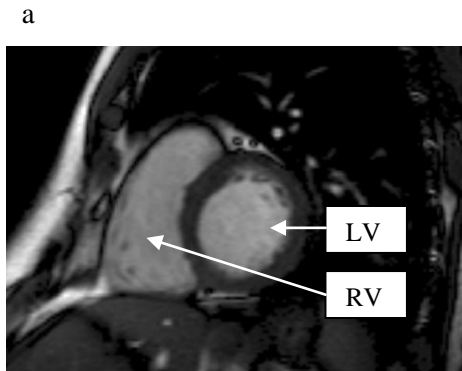
#### 2.2.6.2.4 Aortic distensibility in the ascending aorta

A steady state free precession cine image was acquired through the mid ascending aorta at the level of the right pulmonary artery (Figure 2.15b) with simultaneous measurement of brachial blood pressure. Aortic areas were analysed off-line and aortic distensibility ( $\times 10^{-3}$  mmHg) calculated using standard formulae (Groenink et al., 1998).

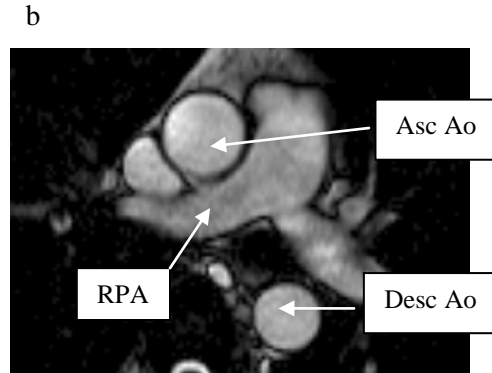
$$\frac{\Delta \text{Ao area}}{\text{Ao area min} \times \text{pulse pressure.}}$$

$\Delta$  Ao area = maximum (systolic) aortic area ( $\text{mm}^2$ ) – minimum (diastolic) aortic area ( $\text{mm}^2$ ), Ao area min = aortic area in diastole ( $\text{mm}^2$ ).

**Figure 2.15** Standard cardiac MRI images using steady state free precession (a & b), tagging (c), and inversion recovery (d) sequences



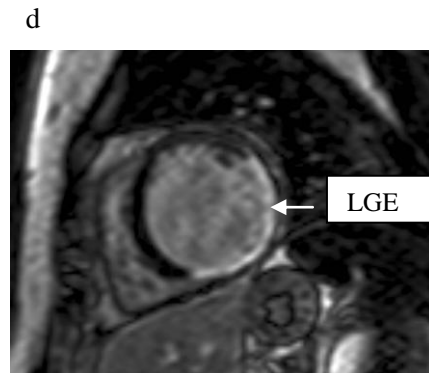
Steady State Free Precession image of the basal left and right ventricles in short axis used in assessment of ventricular volumes, systolic function and mass (LV; left ventricle, RV; right ventricle).



Steady State Free Precession image of the mid ascending aorta for measurement of distensibility (Asc Ao; ascending aorta, Desc Ao; descending aorta, RPA; right pulmonary artery).



Myocardial tagging image of the left ventricle at the basal short-axis.



Inversion recovery image of the left ventricle in short axis showing late gadolinium enhancement (LGE) indicative of full thickness myocardial infarction in the lateral and inferior walls at a basal-mid level.

#### **2.2.5.2.5 Myocardial tagging**

Myocardial tagging using MRI allows accurate quantification of left ventricular regional systolic myocardial function (strain, strain rate, torsion) and diastolic function. In contrast to echo, MRI tagging involves the layout of a saturation grid which moves with the myocardium during the cardiac cycle (Finn et al., 2006). Deformation of these lines due to myocardial contraction can be quantitatively assessed with commercially available software.

Prior to image acquisition, the myocardium was magnetically labelled or “tagged” with non-invasive markers by spatially modulating the degree of magnetization (SPAMM) in two orthogonal directions yielding a grid pattern (Axel and Dougherty, 1989). Short-axis cine images at the LV base (mitral valve), mid (papillary muscle) and apex and a horizontal long axis image (4C) were then acquired using prospective ECG gating. Reduced signal was obtained from the saturated tissue which appeared as a hypodense (black) gridline on the images (Figure 2.15c). The tag lines move with the tissue to which they are magnetized and hence deform with the myocardium during systole and diastole allowing intramural myocardial deformation to be assessed.

Quantitative analysis was performed by the Auckland MRI Research Group, New Zealand using in-house 2D-Tagg software (Young, 1999). This software is well validated with good inter-operator variability. Left ventricular longitudinal shortening (%), apical and basal rotation and LV torsion (difference in apical and basal rotation multiplied by the average radius and divided by the distance between apical and basal slices) were calculated.

MRI tagging offers some methodological advantages over measurements derived using echo. To quantify lengthening and shortening an initial measurement of length is required. MRI uses “Lagrangian strain” where the reference unstressed initial length is derived using the end-

diastolic dimension. In echo “Eulerian or natural strain” is measured. This reflects an instantaneous length at a moment in time and is less reproducible. Other advantages include measurement of deformation and torsion in all three planes (longitudinal, circumferential and radial) which is important given the complex myocardial fibre architecture. Furthermore, superior spatial resolution and image quality allow greater reproducibility for serial studies.

#### **2.2.5.2.6 Delayed enhancement inversion recovery imaging**

Delayed enhancement MRI (DE-MRI) is a well validated technique for the detection of irreversibly damaged myocardium after acute or chronic myocardial infarction (Kim et al., 2000). In addition, it characterizes interstitial fibrosis associated with infiltrative disorders such as hypertrophic cardiomyopathy, sarcoid and chronic myocarditis (Finn et al., 2006).

An inversion recovery sequence was used to image the left ventricle at the base, mid and apical short axis and horizontal long axis. This sequence is run 10-15 minutes after administration of the intravenous paramagnetic contrast agent gadolinium diethylenetriamine pentaacetic acid (Gd-DPTA) commercially known as Magnevist® (Bayer Schering Pharma) at a dose of 0.2 ml/Kg. Patterns of late gadolinium enhancement were qualitatively assessed.

These highly diffusible small molecules, readily distribute in the interstitial space (extracellular, extravascular) where they are limited by the bound chelate. An expansion of the interstitial space following infarction, fibrosis or from other infiltrative processes allows gadolinium to accumulate in high relative concentrations and is increased further through the slower distribution kinetics of the abnormal myocardium. Signal is increased where gadolinium accumulates appearing as bright white areas (hyper-enhancement) compared to unaffected myocardium which appears dark (nulled) (Figure 2.15d). The pattern of infarcted myocardium



may involve the subendocardium or extend to variable degrees of transmural extent depending on the degree of ischaemia. Infiltrative processes typically promote inflammatory and eventually fibrotic change within the myocardium, producing a diffuse patchy mid-wall appearance of hyper-enhancement.

## **2.2.6 VENOUS SAMPLING AND LABORATORY ASSAYS**

### **2.2.6.1 Venous blood sampling**

Following 30 minutes of supine rest, 40 ml of venous blood was collected using a Vacutainer system® (Becton Dickinson Company) from the subcutaneous veins of the antecubital fossa.

Blood was collected into Vacutainer® (Becton Dickinson Company, Oxford, UK) blood tubes with the following additives;

1. Liquid potassium ethylene diamine tetra-acetic acid (EDTA) – full blood count, renin, parathyroid hormone, stored plasma for N-terminal pro-B-type natriuretic peptide (NT-BNP).
2. Clot activator and gel for serum separation – renal function, liver function, calcium, phosphate, total cholesterol, high density lipoprotein,
3. Potassium oxalate / sodium fluoride –glucose
4. Silicone coated – stored serum for High Sensitivity C-Reactive Protein (hsCRP), Intact amino-terminal propeptide of type III procollagen (PIIINP), Carboxyterminal telopeptide of type I collagen (Ctx)
5. Lithium heparin – Plasma aldosterone
6. EDTA / phenanthroline – Angiotensin II

### **2.2.6.2 Haematology and clinical biochemistry**

Routine haematological and biochemical assays were undertaken on fresh venous samples in the Departments of Haematology and Clinical Biochemistry, University Hospital Birmingham, UK.

### **2.2.6.3 Renin-angiotensin II, aldosterone**

Samples were immediately placed on ice for 15 minutes and then centrifuged at 3000 rpm for 15 minutes at 4°C. Platelet free plasma was removed and stored at -80°C. Samples were transported on dry ice to Glasgow for analysis (Medical Research Council Cardiovascular Research Group, Glasgow, Scotland). Plasma renin concentration (PRC) was determined by an in-house antibody trapping technique in the presence of added excess renin substrate. The intra and inter assay coefficient of variation (CoV) were < 5 and < 10% respectively. Angiotensin II, was determined by an in-house radioimmunoassay (RIA) with Angiotensin II pre-extracted from plasma before assay (Cardiovascular Assays, Glasgow, UK). Intra and inter-assay CoV were 6.4% and 9%. Plasma aldosterone concentration (PAC) was determined by RIA measured with a solid-phase coated tube kit (Diagnostic Products Corporation, Siemens, UK). Intra and inter-assay CoV were 6% and 16%, respectively.

### **2.2.6.4 N-terminal pro-B-type natriuretic peptide**

Plasma NT-BNP: measured by an in-house non-competitive immunoluminometric assay on unextracted plasma in Leicester (University of Leicester, Leicester, UK). There was no significant cross-reactivity with other hormones (N-ANP (N-terminal ANP), BNP or CNP (C-type natriuretic peptide)). The lower limit of detection was 0.3 pmol/l. Intra and inter assay CoV were 2.3% and 4.8%, respectively.

### **2.2.6.5 High sensitivity C-reactive protein**

High sensitivity C reactive protein was measured using a Tina-quant® Cardiac C-reactive Protein Latex High Sensitive (CRPHS) immunoturbidimetric assay (Roche Diagnostics, Burgess Hill,

UK) on a Roche Modular P analyser by The Binding Site (The Binding Site Group Ltd, Birmingham, UK). Functional sensitivity of assay was 0.11mg/L (analytical sensitivity 0.03mg/L). Intra and inter assay CoV were 0.28-1.34% and 2.51-5.70%, respectively.

#### **2.2.6.6 Markers of collagen turnover**

Intact amino-terminal propeptide of type III procollagen (PIIINP) concentration was measured by RIA (Orion Diagnostica, Finland). The lower limit of detection was 0.3µg/L. Intra and inter-assay CoV were 7%. Carboxyterminal telopeptide of type I collagen (Ctx) was measured by electrochemiluminescence immunoassay (Roche E module, Roche Diagnostics, Burgess Hill, UK). The lower limit of detection (sensitivity) was 0.07ng/mL. Intra and inter-assay CoV were 6% and 8% respectively. Both markers were analysed in the Department of Clinical Biochemistry, University Hospital Birmingham, UK.

#### **2.2.7.7 Urinary albumin-creatinine ratio**

A 20 ml early morning urine sample was collected for assessment of the albumin-creatinine ratio (ACR mg/mmol). Urinary creatinine and albumin were measured by the rate-blanked Jaffe method and immunoturbidimetry respectively (Roche Modular P analyser, Roche Diagnostics, Burgess Hill, UK) in the Department of Clinical Biochemistry, University Hospital Birmingham, UK.

## **2.2 DATA ANALYSIS AND STATISTICAL METHODS**

The following methods were employed to examine data and statistical analysis using SPSS version 16 software (SPSS Inc, Chicago, Illinois, USA).

### **2.3.1 General data handling**

Continuous data are expressed as mean  $\pm$  standard deviation (SD) or absolute change  $\pm$  standard error of the mean (SEM) unless otherwise stated. Categorical data are expressed as frequency with percentage (%). Visual examination with histograms and the Kolmogorov–Smirnov test was used to test for normal distribution of data ( $\leq 0.1$ ), where appropriate non-normally distributed data was log transformed and expressed as median as  $\pm$  inter-quartile range (IQR).

### **2.3.2 Statistical methods**

Categorical variables were compared by the Pearson Chi-square test. Continuous data were compared as appropriate using; two-tailed Student's t-test for independent samples and analysis of variance (ANOVA) with a Tukey post-hoc comparison test. Between weeks 0-40 groups were compared using repeated measure of variance (for changes over time). Pearson correlation coefficients (R) were used to measure associations between continuous variables. Trend was assessed by the use of the Jonckheere-Terpstra test. Statistical significance was taken as the p value of  $< 0.05$  or  $< 0.01$  as stated in each study.

### **2.3.3 Regression models**

Regression models employed were used to predict relationships and do not infer causal mechanisms. Co-linearity between explanatory variables was assessed by examining the variable inflation factor.

#### **2.3.3.1 Multiple linear regression**

Multiple linear regression models were used to assess independent variables which predicted the dependent variable being investigated. Variables included in the model were limited to variables known to influence the dependent variable in previous studies and variables that were significant at the level of  $p < 0.1$  based on simple linear regression. Data for the model fit are presented as coefficients of determination ( $r^2$ ). The associations found in the analyses are presented as unstandardised  $\beta$ -coefficient ( $\beta$ ) + Standard Error (SE). Assumptions include; linearity between variables, the residuals (dependent variable values predicted from the regression model minus the observed values) are normally distributed, categorical variables had only two categories (yes/no).

#### **2.3.3.2 Logistic regression**

This model was used when the dependent variable was categorical variable (two categories yes / no) and the independent variables were continuous, categorical, or both. As per multiple linear regression, the relationship between independent variables and dependent variable is estimated, but in logistic regression the probability that the dependent variable assumes a certain value (yes or no) is estimated, rather than estimating the value itself. Data are presented as Odds Ratios (OR) associated with each independent (predictor) variable with 95% confidence intervals. The odds ratio for a variable is defined as the relative amount by which the odds of the outcome

increase ( $OR > 1$ ) or decrease ( $OR < 1$ ) when the value of the predictor variable is increased by 1 units.

#### **2.3.4 Assessment of reproducibility**

Variability studies were performed on variables constituting the pre-specified primary and secondary end-points. Analysis was performed using; i) Intra-class correlation coefficients (ICC): an index of reliability for a single operator analysing the same data more than once and for two or more operators when analysing the same set of variables (Bland and Altman, 1986). Data are presented as ICC (r) and 95% confidence intervals (95% CI). ii) Bland-Altman Analysis; an assessment of the agreement between two methods of measurement. Data are presented as a scatter plot with the difference between each measurement (d) plotted against the mean for the 2 measurements (Bland & Altman, 1986). The limit of agreement between both measurements was determined by 95% confidence limits, calculated as  $d - 2SD$  and  $d + 2SD$ . The limit of agreement for each variable was compared to previously published guidelines to ensure acceptable comparison.

## CHAPTER 3

### **AORTIC DISTENSIBILITY AND ARTERIAL-VENTRICULAR COUPLING IN EARLY CHRONIC KIDNEY DISEASE: A PATTERN RESEMBLING HEART FAILURE WITH PRESERVED EJECTION FRACTION**

Contributory publications:

NC Edwards, CJ Ferro, JN Townend, RP Steeds Aortic Distensibility and Arterial-Ventricular Coupling in Early Chronic Kidney Disease: a Pattern Resembling Heart Failure with Preserved Ejection Fraction. *Heart*. 2008. Aug; 94(8): 1038-43.

NC Edwards, CJ Ferro, JN Townend, RP Steeds. Myocardial disease in systemic vasculitis and autoimmune disease detected by cardiovascular magnetic resonance. *Rheumatology (Oxford)*. 2007 Jul;46(7):1208-9.



### 3.1 SUMMARY

The high cardiovascular event rate in chronic kidney disease (CKD) is due in large part to sudden death and heart failure as a result of abnormalities of ventricular structure and function (ureamic cardiomyopathy). The hypothesis of this study was that arterial stiffness might be increased in early stage CKD and would be associated with abnormalities of both arterial and ventricular function compared to healthy controls with normal renal function.

Arterial and ventricular function and ventricular-vascular interaction were examined in 117 patients with stage 2 (60-89 ml/min/1.73m<sup>2</sup>) or stage 3 (30-59 ml/min/1.73m<sup>2</sup>) non-diabetic CKD without overt cardiovascular disease. These subjects were compared with 40 age and sex matched healthy controls. Aortic distensibility ( $\times 10^{-3}$  mmHg) was assessed using cardiac magnetic resonance imaging (cardiac MRI). Systolic and diastolic ventricular function and vascular-ventricular elastance (stiffness) were assessed by transthoracic echocardiography.

Compared to controls, patients with CKD had reduced aortic distensibility ( $p < 0.01$ ), increased arterial elastance and increased ventricular end systolic and diastolic elastance ( $p < 0.01$ ). Aortic distensibility was positively correlated with estimated GFR ( $p < 0.01$ ) and indices of elastance were inversely correlated ( $p < 0.05$ ). There was no difference in conventional markers of systolic function with early CKD compared to controls but markers of diastolic function were impaired including; a reduction in early myocardial diastolic filling velocities ( $E_m$ ) and an increase in left atrial pressure ( $E/E_m$ ) and end-diastolic elastance ( $E_d$ ) ( $p < 0.01$ ).

Early stage CKD is characterised by reduced aortic distensibility and increases in arterial, ventricular systolic and diastolic stiffness; arterial-ventricular coupling is preserved. This pattern of patho-physiological abnormalities resembles that seen in heart failure with preserved ejection

fraction and might account for the high levels of cardiovascular morbidity and mortality in patients at all stages of CKD.

### **3.2 INTRODUCTION**

End-stage kidney disease (ESKD) is characterised by major abnormalities of vascular and myocardial structure and function including increased arterial stiffness, left ventricular (LV) hypertrophy, dilatation, fibrosis and impaired systolic and diastolic function. There are few data on such abnormalities in early stage chronic kidney disease (CKD) when preventive treatment might be most effective.

The hypothesis of this study was that arterial stiffness might be increased and detectable early in the natural history of CKD. Secondly, that increased stiffness of the large arteries would be associated with adverse changes in ventricular structure and systolic and diastolic function. Cardiac MRI and echocardiography were used to examine ventricular and arterial function in patients with early CKD and compared to age and sex-matched healthy controls.

### **3.3 METHODS**

#### **3.3.1 Study design**

This was a cross-sectional observational study. The protocol was approved by South Birmingham Local Research Ethics Committee.

#### **3.3.2 Subjects**

Using the inclusion and exclusion criteria described in section 2.1.1, 117 patients were recruited from renal clinics at University Hospital Birmingham. ACE inhibitors were used in 67% patients, ARBs in 30% and dual therapy in 3%. Other treatments included beta blockers 22%, calcium channel blockers 27%, diuretics 34% and statins 40%. Patients were matched for age and sex with 40 healthy controls with no evidence of CKD as defined by K/DOQI guidelines (National Kidney Foundation, 2002).

#### **3.3.3 Data collection**

All subjects underwent:

1. Twelve-lead electrocardiogram
2. Resting brachial blood pressure as described in section 2.2.2.1
3. Transthoracic echocardiogram for assessment of:
  - i) LV systolic and LV diastolic function described in sections 2.2.4-5
  - ii) Arterial-ventricular interaction described in sections 2.2.4.5.3

4. Cardiac MRI for assessment of: LV volumes, systolic function, mass, aortic distensibility and myocardial scarring as described in section 2.2.6
5. Twenty four hour ambulatory blood pressure monitoring as described in section 2.2.2.2
6. Venous blood sampling as described in section 2.2.7
7. Urine analysis for albumin-creatinine ration as described in section 2.2.7.7

### 3.3.4 Statistical analysis

Statistical analyses were performed as described in Chapter 2.

An intra-operator variability study was performed. This assessed the variability of a single observer (blinded to all clinical data) in performing measurements on 10 patients, on two separate occasions (at least 6 weeks apart) for echo and cardiac MRI. The method of coefficient of variation was used in this study in keeping with previous published data (Grothues et al., 2002). It is calculated as follows:

$$\text{Coefficient of variation} = \frac{\text{Mean standard deviation of the two measurements}}{\text{Mean of the two measurements}} \times 100$$

(CoV %)

Coefficient of variation expresses the standard deviation as a percentage of the sample mean and is useful when interest is in the size of variation relative to the size of the observation. For

example, a CoV of 3% means the standard deviation is equal to 3% of the average. It is useful when comparing between data sets with different units or widely different means.

## **3.4 RESULTS**

### **3.4.1 Patient characteristics**

We identified 2,196 patients attending renal clinics who had non-diabetic stage 2 or 3 CKD. In total, 1,911 were not eligible for recruitment for the following reasons: 46% not on treatment with an ACE inhibitor or ARB, 29% had evidence of renovascular disease or a history of uncontrolled blood pressure, 15% had previous cardiovascular events, 2% had atrial fibrillation and 8% other contraindications including anaemia or a past history of hyperkalaemia. Thus 287 patients met the inclusion / exclusion criteria; 170 patients declined to participate; therefore 117 patients were formally consented. Data from two patients was excluded from analysis; one patient had evidence of uncontrolled blood pressure on 24 hour ambulatory assessment and one patient had a baseline serum potassium level of 5.6 mmol/L. Therefore 115 patients were included in the observational study.

There were no significant differences in demographic data between patients with CKD and controls (Table 3.1). The most common causes of CKD were; glomerulonephritis 55%, vasculitis 13%, polycystic kidney disease 8% and systemic lupus erythematosus 7%. Seventy percent of patients had a history of hypertension controlled on an average of 2.1 antihypertensive agents. There was no difference in blood pressure between the groups.

### **3.4.2 Cardiac magnetic resonance imaging**

There was no difference between patients and controls in mean LV volumes or ventricular ejection fraction measured using cardiac MRI (Table 3.2). Mean LV mass was greater in CKD

than in controls. A third of patients with CKD had LV hypertrophy (LVH) as defined by an LV mass greater than age, gender and body surface area corrected limits (male  $\geq 89$ -93 g/m<sup>2</sup> depending, female  $\geq 77$ -78 g/m<sup>2</sup> depending on decade) (Maceira et al., 2006). The mean LV mass and proportion of patients with LVH did not differ significantly between stage 2 and 3 CKD.

Aortic distensibility (Figure 3.1) was reduced in both stage 2 and stage 3 CKD compared to controls ( $p < 0.001$ ). There was a significant positive correlation between aortic distensibility and eGFR ( $r = 0.349$ ,  $p < 0.01$ ) (Figure 3.2a). LV mass was inversely correlated with aortic distensibility ( $r = -0.284$ ,  $p < 0.01$ ) (Figure 3.2b). In a multivariate regression model including age, systolic blood pressure, aortic distensibility, eGFR, and concomitant medications, LV mass index (LVMI) was predicted by systolic blood pressure, aortic distensibility and eGFR ( $r^2 = 0.18$ ,  $p < 0.05$ ). Aortic distensibility contributed 6%, eGFR 2% and systolic blood pressure 10% of the variance in LVMI.

Myocardial scarring was present in 8 patients (7%) and no controls. Six patients had late gadolinium enhancement (LGE) in the mid-wall with a diffuse distribution not specific to a coronary artery territory. This pattern is indicative of myocardial fibrosis. One patient had an area of confluent LGE in the subepicardium consistent with previous myocarditis and one patient had subendocardial LGE in a distribution typical of previous myocardial infarction. In all patients LV volumes, function and mass were within normal limits for gender and age. The non-ischaemic patterns of scarring were present in six patients with an inflammatory autoimmune or vasculitic aetiology (typically systemic lupus erythematosus (SLE) and Wegener's granulomatosis (WG)), proven on biopsy as a cause of CKD. This represents a prevalence of over 50% in patients recruited with these diagnoses.



**Table 3.1** Population characteristics

|                                        | Controls<br>(n=40) | CKD Stage 2<br>(n=28) | CKD Stage 3<br>(n=87) |
|----------------------------------------|--------------------|-----------------------|-----------------------|
| Male sex (%)                           | 15 (50)            | 11 (38)               | 60 (68) †             |
| Age                                    | 50.3 ±9.2          | 55.9 ±11.6            | 53.8 ±11.8            |
| BSA (g/m <sup>2</sup> )                | 1.84 ±0.17         | 1.80 ±0.22            | 1.93 ±0.19            |
| Office systolic BP (mmHg)              | 126 ±17            | 128 ±19               | 132 ±17               |
| Office diastolic BP (mmHg)             | 80 ±8              | 75 ±10                | 78 ±10                |
| Pulse pressure (mmHg)                  | 46 ±12             | 52 ±14                | 54 ±12*               |
| 24 hour SBP (mmHg)                     |                    | 126 ±12               | 124 ±11               |
| 24 hour DBP (mmHg)                     |                    | 77 ±8                 | 77 ±9                 |
| Heart rate (bpm)                       | 64 ±12             | 69 ±12                | 65 ±12                |
| Mean eGFR (ml/min/1.73m <sup>2</sup> ) | 70.1 ±5.2          | 68.7 ±6.3             | 45.4 ±9.1**††         |
| Serum creatinine (µmol/L)              | 95±10              | 98 ± 23               | 149 ±39**††           |
| Albumin-creatinine ratio (mg/mmol)     | 0.11 ±0.2          | 47.9 ±87.5**          | 55.4 ±132.3**         |
| Haemoglobin (g/dL)                     | 14.2 ±1.6          | 13.3 ±1.8*            | 13.6 ±1.4             |
| Number of patients on treatment        |                    |                       |                       |
| ACE inhibitor (ACE I)                  | 0                  | 18                    | 61                    |
| Angiotensin receptor blockers (ARB)    | 0                  | 9                     | 26                    |
| ACE I + ARB                            | 0                  | 0                     | 3                     |
| Beta blockers                          | 0                  | 6                     | 20                    |
| Calcium blockers                       | 0                  | 6                     | 26                    |
| Diuretics                              | 0                  | 8                     | 32                    |
| Statins                                | 0                  | 14                    | 33                    |

Values are mean ± standard deviation P-values derive from one-way analysis of variance for continuous variables. \* p < 0.05 \*\* p < 0.01 vs. controls. † < 0.05 †† < 0.01 CKD 2 vs. CKD 3 BSA; body surface area, DBP; diastolic blood pressure, SBP; systolic blood pressure

**Table 3.2** Vascular and cardiac structure and function measured on cardiac MRI

|                                                  | Controls<br>(n=40) | CKD Stage 2<br>(n=25) | CKD Stage 3<br>(n=82) |
|--------------------------------------------------|--------------------|-----------------------|-----------------------|
| Aortic Distensibility ( $\times 10^{-3}$ mmHg)   | 4.12 $\pm$ 1.3     | 2.94 $\pm$ 1.8*       | 2.18 $\pm$ 1.8**†     |
| LV Ejection Fraction (%)                         | 71.6 $\pm$ 5.9     | 69.9 $\pm$ 6.2        | 69.1 $\pm$ 8.4        |
| LV Mass/BSA ( $\text{g}/\text{m}^2$ )            | 55.1 $\pm$ 9.4     | 64.2 $\pm$ 15.2*      | 63.7 $\pm$ 12.7*      |
| LVH (%)                                          | 0                  | 9 (36)                | 23 (28)               |
| LVEDV/BSA ( $\text{ml}/\text{m}^2$ )             | 60.1 $\pm$ 17.6    | 63.9 $\pm$ 13.0       | 57.9 $\pm$ 11.5       |
| LV stroke volume /BSA ( $\text{ml}/\text{m}^2$ ) | 45.6 $\pm$ 8.7     | 44.5 $\pm$ 7.8        | 39.7 $\pm$ 7.3**†     |
| RV Ejection Fraction (%)                         | 60.8 $\pm$ 6.3     | 58.4 $\pm$ 9.5        | 58.1 $\pm$ 7.1        |
| RVEDV/BSA ( $\text{ml}/\text{m}^2$ )             | 74.3 $\pm$ 13.9    | 75.4 $\pm$ 18.6       | 68.6 $\pm$ 12.0       |

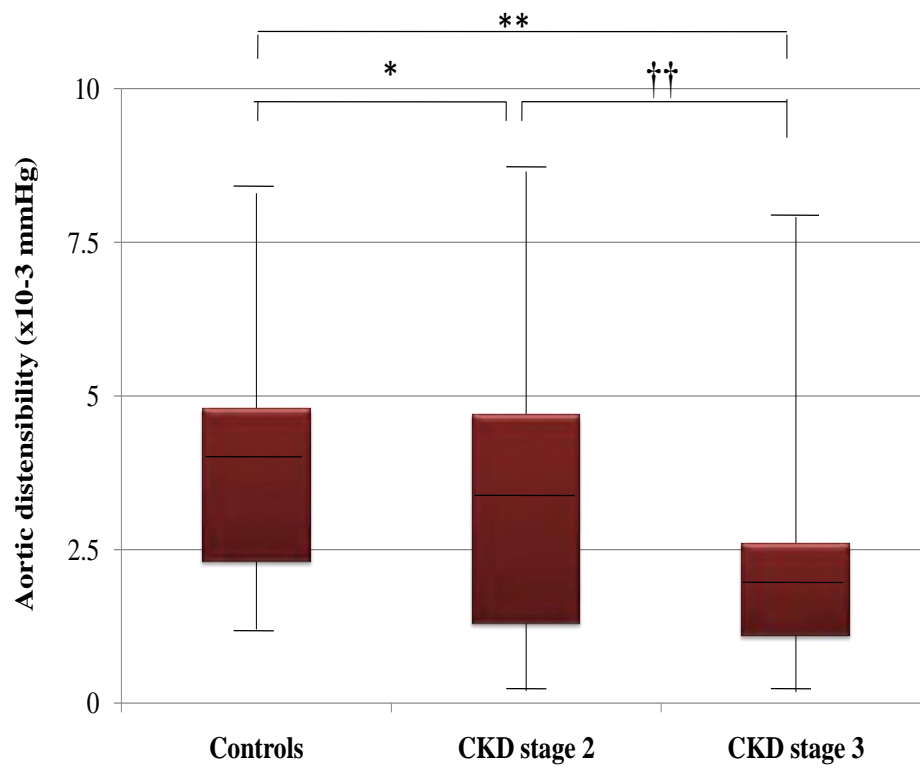
Values are mean  $\pm$  standard deviation

P-values derive from one-way analysis of variance for continuous variables.

\*  $p < 0.05$  \*\*  $p < 0.01$  vs. controls. †  $p < 0.05$  ††  $p < 0.01$  CKD 2 vs. CKD 3

BSA; body surface area, EDV; end-diastolic volume, LV; left ventricle, LVH; left ventricular hypertrophy, RV, right ventricle

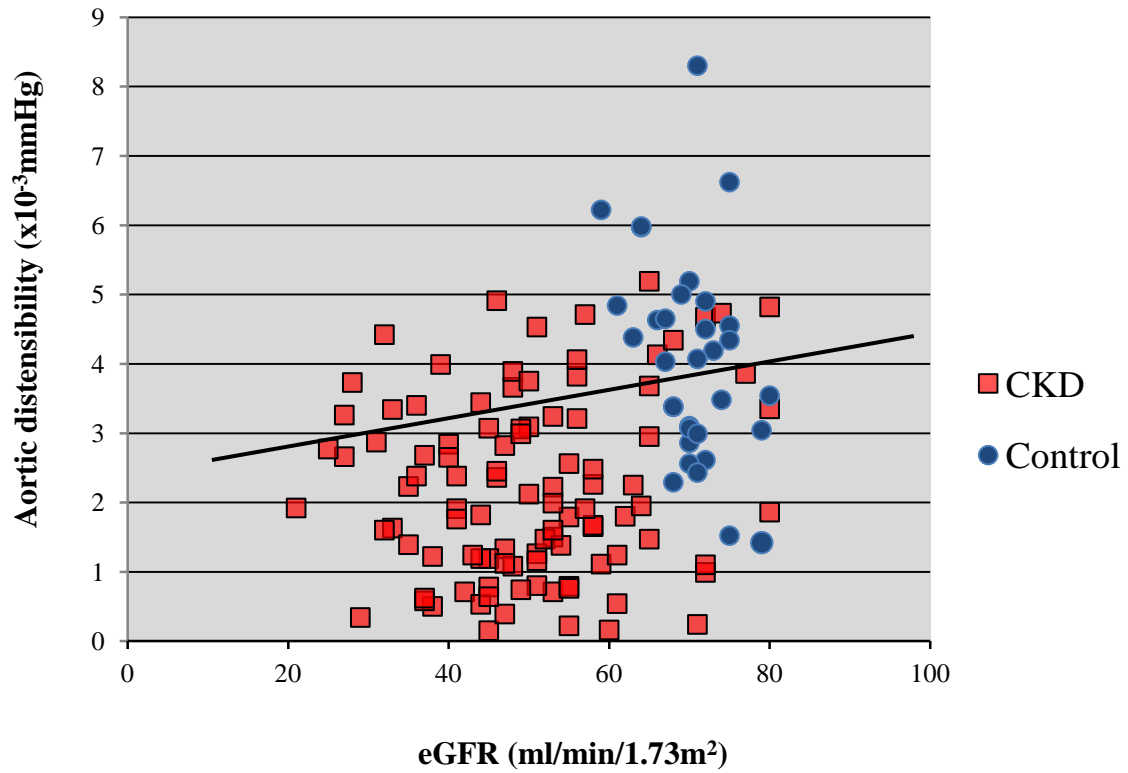
**Figure 3.1** Aortic distensibility measured on cardiac MRI in the ascending aorta in healthy controls and patients with chronic kidney disease



Values are median (horizontal bar), upper and lower quartiles (box) and range (error bars)

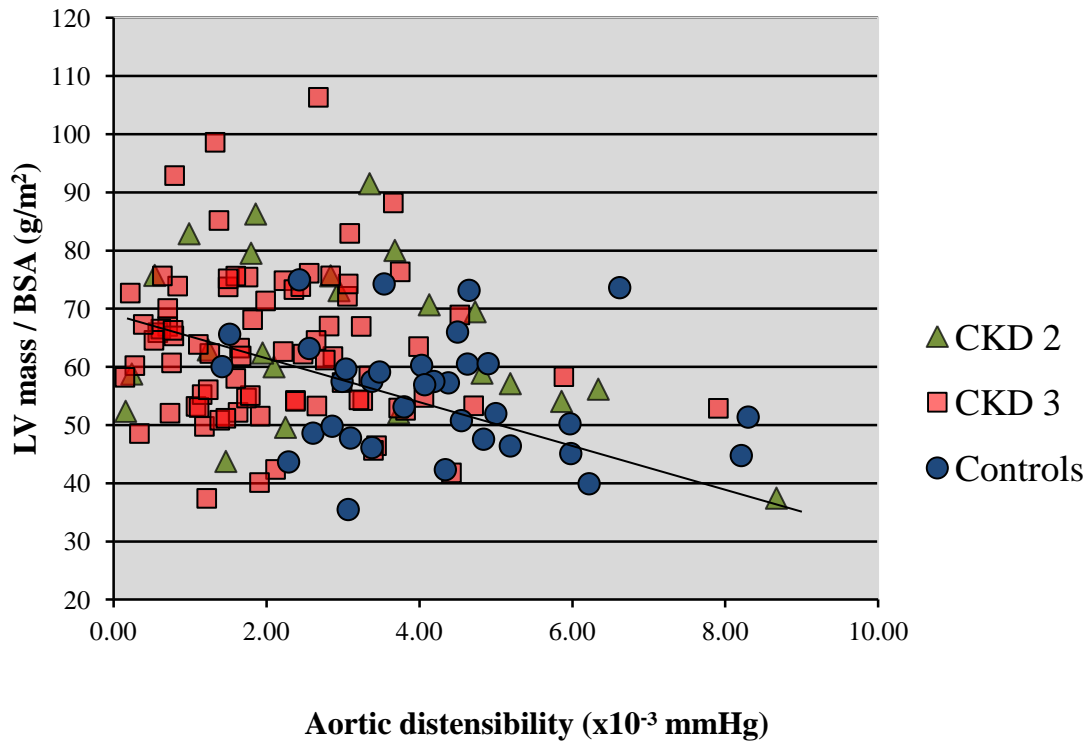
\*  $p < 0.05$  \*\*  $p < 0.01$  vs. controls. †  $p < 0.05$  ††  $p < 0.01$  CKD 2 vs. CKD 3.

**Figure 3.2a** The association between estimated glomerular filtration rate and aortic distensibility in patients with early stage chronic kidney disease and healthy volunteers



Pearson correlation coefficient  $r = 0.349$ ,  $p < 0.01$

**Figure 3.2b** The association between aortic distensibility and left ventricular mass index in patients with early stage chronic kidney disease and healthy volunteers



Pearson correlation coefficient  $r = -0.284$ ,  $p < 0.01$

### **3.4.3 Echocardiography**

#### **3.4.3.1 Conventional measurement of left ventricular systolic and diastolic function**

There was no difference in conventional measures of systolic function between patients with CKD and controls. Ejection fraction and longitudinal systolic tissue Doppler velocities in CKD patients were within normal limits (Table 3.3 & Figure 3.3a). Diastolic parameters including early myocardial relaxation velocities (basal Em, Figure 3.3b), E/Em ratio, M-mode colour flow propagation, and pulmonary venous a-wave duration were abnormal in CKD compared with controls (Table 3.3). Left atrial volumes were greater in patients with CKD than controls. Right and left ventricular Tei indices were greater in CKD than in controls (Table 3.3).

#### **3.4.3.2 Arterial-ventricular interaction**

Arterial elastance (Ea), arterial elastance index (EaI), LV end-systolic elastance (Ees), LV end-diastolic elastance (Eed) were all greater in patients with CKD than in controls (Table 3.4). In patients with CKD stage 3, systemic vascular resistance index (SVRI) and pulse pressure were increased compared to controls indicating that both mean resistive and pulsatile load were increased. There were no differences between SVRI, PP and heart rate between patients with stage 2 CKD and controls indicating that differences in EaI between these subjects principally reflected altered pulsatile load and hence the oscillatory arterial properties (stiffness). Combining values for both patients and controls, EaI and Ees were significantly correlated (Pearson's  $r = 0.692$ ,  $p < 0.01$ ) (Figure 3.4). The arterial-ventricular coupling ratio (Ees/Ea) was maintained in both patients and controls although the absolute values for both of Ea and Ees were increased.

Arterial elastance ( $r = -0.282$ ,  $p < 0.05$ ), Ees ( $r = -0.137$ ,  $p < 0.05$ ) and Eed ( $r = -0.347$ ,  $p < 0.01$ ) were all inversely correlated with eGFR.

**Table 3.3** Two dimensional echocardiography and pulse wave Doppler parameters in controls and patients with chronic kidney disease

|                                | Controls<br>(n=40) | CKD Stage 2<br>(n=28) | CKD Stage 3<br>(n=87) |
|--------------------------------|--------------------|-----------------------|-----------------------|
| Ejection Fraction (%)          | 65.4 ±5.6          | 63.3 ±4.7             | 62.7 ±6.8             |
| Lateral annular TDI Sm (cm/s)  | 8.8 ±1.5           | 8.4 ±2.4              | 7.9 ±1.9              |
| E/Em                           | 5.6 ±1.1           | 7.4 ±1.8**            | 8.0 ±2.4**            |
| E (cm/s)                       | 67.2 ±10.3         | 74.3 ±9.9             | 67.4 ±13.1            |
| A (cm/s)                       | 56.0 ±11.6         | 61.1 ±12.7            | 67.0 ±15.4**          |
| E/A                            | 1.3 ±0.4           | 1.2 ±0.3              | 1.1 ±0.3**            |
| DT (msec)                      | 207 ±22.3          | 212 ±39.3             | 225 ±55.7             |
| CFP (cm/s)                     | 63.8 ±11.1         | 54.4 ±15.0*           | 49.0 ±14.2**          |
| IVRT (ms)                      | 89.4 ±12.8         | 93.0 ±13.0            | 95.4 ±15.7            |
| Pulmonary vein A duration (ms) | 70.5 ±13.7         | 80.9 ±19.3            | 91.6 ±19.5**          |
| LA volumes (ml/BSA)            | 19.7 ±4.9          | 25.7 ±5.4*            | 27.4 ±6.3**           |
| EDP (mmHg)                     | 15.4 ±0.70         | 16.5 ±1.09**          | 16.7 ±1.48**          |
| RV Tei                         | 0.27 ±0.10         | 0.37 ±0.14*           | 0.49 ±0.15**          |
| LV Tei                         | 0.39 ±0.04         | 0.49 ±0.10**          | 0.51 ±0.12**          |

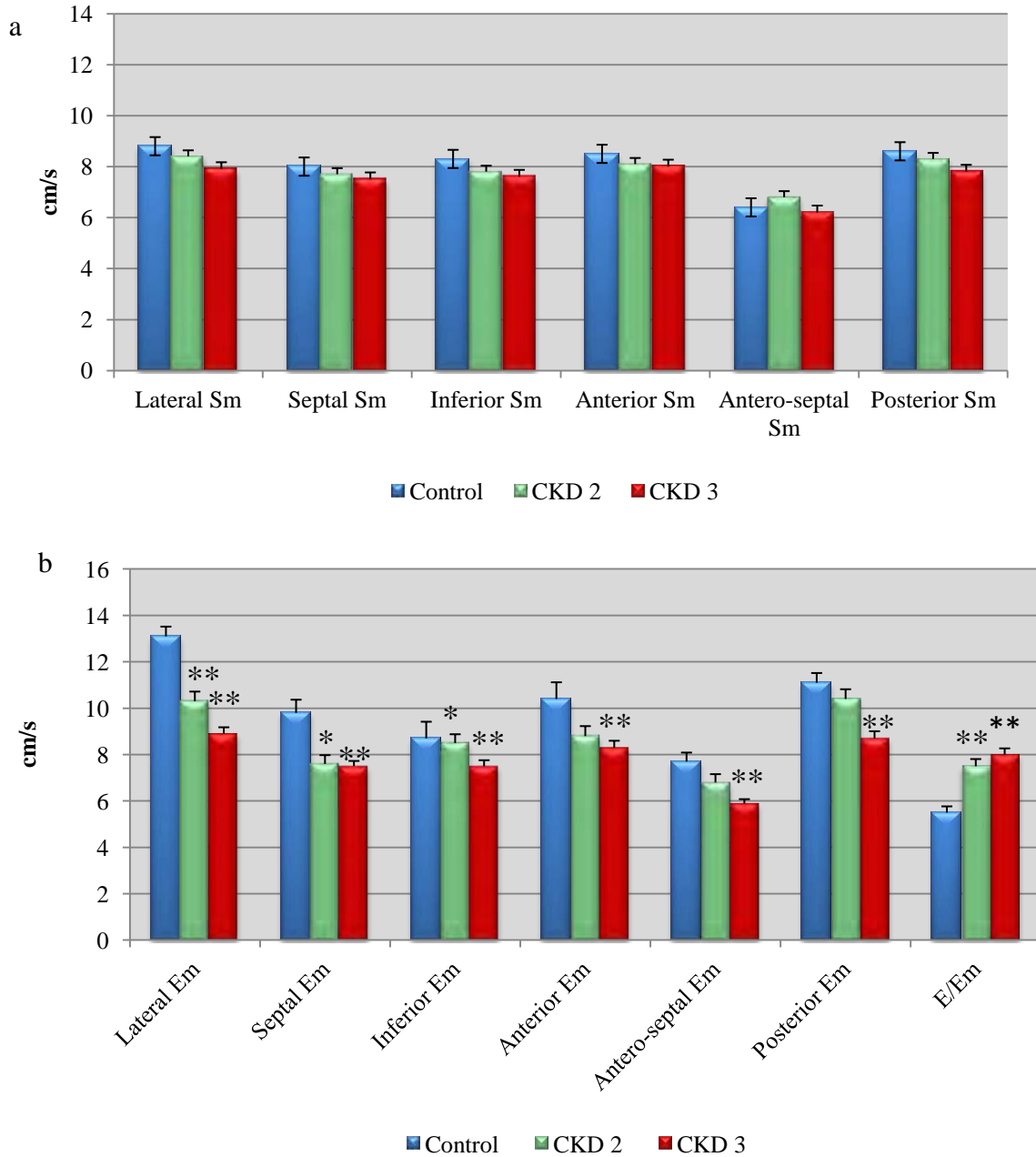
Values are mean ± standard deviation, p-values derive from one-way analysis of variance

\* p < 0.05 \*\* p < 0.01 vs. controls, † p < 0.05 †† p < 0.01 CKD 2 vs. CKD 3†

CFP; colour flow propagation velocity, DT; deceleration time, EDP; end-diastolic pressure, IVRT; isovolumic relaxation time



**Figure 3.3a** Bar graph of pulsed tissue Doppler a) systolic (Sm) velocities and b) early mitral filling (Em) myocardial velocities from each of the six basal segments of the left ventricle



Values are mean  $\pm$  standard error of the mean \*  $p < 0.05$  \*\*  $p < 0.01$  vs. controls  
 Sm, myocardial systolic velocity, Em, early myocardial diastolic filling velocity

**Table 3.4** Measures of vascular and ventricular structure and function and haemodynamics in controls and patients with chronic kidney disease

|                                                             | Controls<br>(n=40) | CKD Stage 2<br>(n=28) | CKD Stage 3<br>(n=87) |
|-------------------------------------------------------------|--------------------|-----------------------|-----------------------|
| Arterial Elastance (Ea) (mmHg)                              | 1.40 ±0.22         | 1.65 ±0.40*           | 1.74 ±0.48**          |
| Arterial Elastance Index (EaI)<br>(mmHg ml/m <sup>2</sup> ) | 0.77 ±0.17         | 0.94 ±0.30*           | 0.93 ±0.29**          |
| End-systolic elastance (Ees)<br>(mmHg/ml)                   | 1.88 ±0.48         | 2.43 ±0.83*           | 2.42 ±0.78**          |
| End-diastolic elastance (Eed)                               | 0.07 ±0.04         | 0.11 ±0.04**          | 0.12 ±0.04**          |
| Ea/Ees                                                      | 0.73 ±0.16         | 0.71 ±0.16            | 0.75 ±0.19            |
| SV/BSA (ml/m <sup>2</sup> )                                 | 43.9 ±6.1          | 39.7 ±7.9             | 37.5 ±7.8**           |
| SVRI (dynes/s/cm <sup>-5</sup> /m <sup>2</sup> )            | 2763 ±635          | 2826 ±692             | 3316 ±804**††         |
| Heart rate (bpm)                                            | 64 ±11             | 69 ±12                | 65 ±12                |
| Systolic BP (mmHg)                                          | 125 ±13            | 127 ±21               | 130 ±18               |

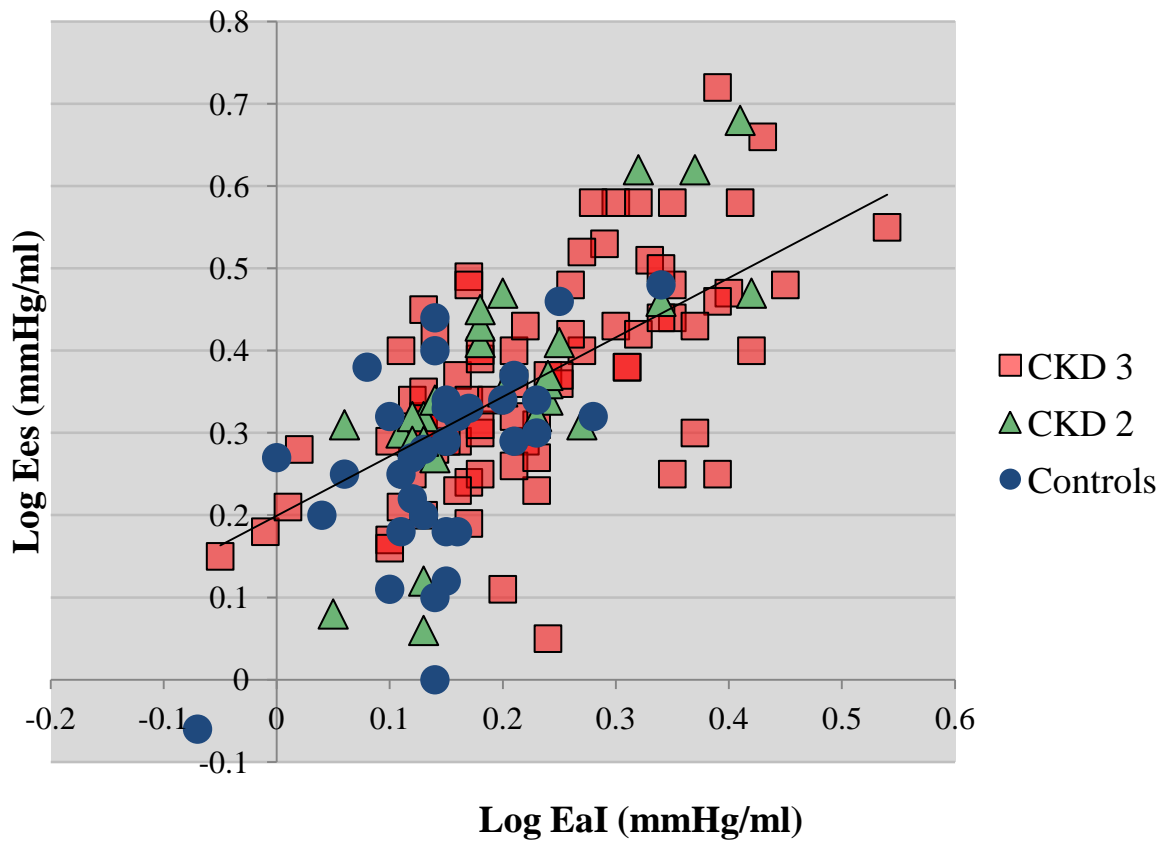
Values are mean ± standard error of the mean for continuous variables

P-values derive from one-way analysis of variance \* p < 0.05 \*\* p < 0.01 vs. controls † < 0.05

†† < 0.01 CKD 2 vs. CKD 3

BP; blood pressure, BSA; body surface area, SV; stroke volume

**Figure 3.4** The association of arterial elastance index with end-systolic elastance in controls and patients with chronic kidney disease, measured by echocardiography.



Data not normally distributed and was therefore log transformed for analysis

Ees is increased with EaI (Pearson correlation coefficient  $r = 0.685$ ). Ea is a significant predictor of Ees in a regression model ( $r^2 = 0.47$ ,  $p < 0.001$ )

EaI; arterial elastance indexed to body surface area, Ees; end-systolic elastance

#### **3.4.4 Effect of drugs and biochemical parameters**

Haemoglobin, calcium, phosphate and parathyroid hormone were within normal range in all subjects. There was no detectable association between cardiac and vascular stiffness measures on cardiac MRI and echocardiography or with biochemical parameters by univariate analysis. There was also no correlation between any marker of vascular function with any anti-hypertensive agent or with statin therapy.

#### **3.4.5 Intra-operator variability**

The repeated analysis of major parameters of arterial and cardiac structure function showed good reproducibility; cardiac MRI LV mass index 3.2%, cardiac MRI aortic distensibility 5.0 %, cardiac MRI ejection fraction 2%, echo ejection fraction 9%.

### **3.5 DISCUSSION**

Data presented in this study demonstrated i) increased aortic stiffness and LV stiffness, ii) major abnormalities of left diastolic ventricular function and an abnormal interaction with the arterial tree in patients with early stage CKD compared to healthy controls.

#### **3.5.1 Assessment of arterial and ventricular function in chronic kidney disease**

Most similar studies to date have examined patients with ESKD and used other measures of arterial stiffness such pulse wave analysis (PWA) and velocity (PWV) (Blacher et al., 1999b;Safar et al., 2002a). These provide valuable information on wave reflection and vascular stiffness in the aorta and iliac vessels (carotid-femoral PWV) but are an indirect measure of central aortic stiffness and are dependent upon the assumptions of a circulatory model (Blacher et al., 1999b). By using cardiac MRI, the change in ascending aortic dimensions in response to pressure fluctuation was assessed providing a direct measure of distensibility. The measurement of arterial and ventricular systolic elastance by echocardiography is an indirect but validated technique which provides information on both arterial and ventricular stiffness allowing the assessment of vascular ventricular coupling, a major determinant of cardiovascular performance (Chen et al., 1998).

Although we cannot exclude the influence of longstanding treated hypertension on the results, this was not a study of patients with hypertension and incidental renal dysfunction. All patients were recruited from a specialist renal clinic and had established renal disease, proven on

biopsy in 60% of cases. Blood pressure was optimally controlled prior to recruitment. Cardiovascular abnormalities, including reduced aortic compliance on cardiac MRI, have been demonstrated in patients with end stage CKD (Mark et al., 2006; Patrianakos et al., 2006; Zimmerli et al., 2007) but our study suggests that significant damage is already present in stage 2 disease often before a rise in serum creatinine has occurred. Perhaps most importantly, aortic distensibility was reduced and arterial elastance index, a measure of net ventricular afterload, increased in our patients compared to controls. Thus, it appears that an increased oscillatory load occurs early in the course of CKD at least in part as a result of changes in the aortic wall.

### **3.5.2 Arterial stiffness in chronic kidney disease**

It is established that arterial stiffness is a strong predictor of cardiovascular morbidity and mortality in end stage CKD and appears to be independent of other prognostic factors including age, LVH and blood pressure (Guerin et al., 2001). Our findings of increased arterial stiffness and reduced aortic distensibility in patients with early CKD are consistent with previous studies which have demonstrated a graded increase in arterial stiffness with advancing stage of CKD independent of the contribution of blood pressure and ageing (Briet et al., 2006; Mourad et al., 2001; Schillaci et al., 2006; Wang et al., 2005b).

A study of 1290 patients with mild CKD (serum creatinine  $\leq 130$   $\mu\text{mol/L}$ ) assessed carotid artery compliance using an echotracking ultrasound device and carotid-femoral PWV (Complior<sup>®</sup>). Patients in the lowest tertile of renal function (creatinine clearance  $\leq 68.5$   $\text{ml/min/1.73m}^2$ ) had an inverse association of creatinine clearance with carotid-femoral PWV and positive correlation with carotid artery compliance independent of blood pressure, age and

standard cardiovascular risk factors. These relationships were strongest in patients  $\leq 55$  years where renal function accounted for 20% of variance in carotid compliance indicating that increased arterial stiffness in CKD is driven by mechanisms other than ageing (Mourad et al., 2001).

In a study of 305 newly diagnosed and untreated hypertensive patients, the strong independent association of blood pressure with both central and peripheral arterial stiffness was confirmed using carotid-femoral PWV and carotid-radial PWV. However, an inverse relationship between aortic augmentation index and eGFR was also observed which was independent of confounding factors including office and 24 hour ambulatory blood pressure. These findings emphasised that alterations in the elastic properties of the arterial wall are present with mild reductions in renal function (Schillaci et al., 2006).

The influences of age and blood pressure were both addressed in a cross-sectional study which examined the structural and functional properties of the carotid artery and carotid femoral PWV in 95 patients with moderate CKD (mean GFR 36 ml/min/1.73m<sup>2</sup>), 121 hypertensive patients (mean brachial blood pressure 134/77 mmHg) and 57 normotensive healthy controls. Chronic kidney disease was associated with increased PWV and carotid artery diameter resulting in increased circumferential wall stress compared to both hypertension and controls. These findings are consistent with changes in ESKD and indicate outward remodelling of the artery probably due to quantitative and qualitative changes in the elastic fibres of the wall rather than a direct response to pulsatile stresses associated with hypertension and mechanical failure and fatigue associated with ageing (Briet et al., 2006). Alternative causes of aortic stiffness in early CKD include an increase in aortic wall calcium, endothelial dysfunction, elastin fragmentation and vascular inflammation (Zoccali et al., 2004b).

### **3.5.3 Left ventricular structure and function in early chronic kidney disease**

The results also show abnormalities of cardiac structure and function in early CKD. An increase in mean left ventricular mass and the prevalence of LVH in approximately a third of patients with stage 2 and stage 3 CKD suggests that a hypertrophic response occurs early although not invariably in the course of renal disease. Aortic distensibility was an independent predictor of LV mass index suggesting that it has an important role as a driver of increased LV mass in early renal disease.

Systolic ventricular function assessed using echocardiography and cardiac MRI, was preserved in early CKD while abnormalities of diastolic function were evident. Left ventricular relaxation was delayed and maximal ventricular end diastolic stiffness increased. Consistent with impaired diastolic function, left atrial volumes and left ventricular end-diastolic pressure were increased. The increase in Ees suggests that resting left ventricular contractility is enhanced in early CKD. This maintains the arterial-ventricular coupling ratio and cardiac performance. There is however good evidence that the “price” of this early compensatory response is a chronic increase in left ventricular stiffness and haemodynamic instability (Chen et al., 1998). Cardiac reserve is reduced and during exercise the increase in both Ees and Ea causes an excessive rise in systolic pressure increasing cardiac work and metabolic demand (Kelly et al., 1992b).

### **3.5.4 Left ventricular myocardial fibrosis**

Mid-wall diffuse myocardial scarring on cardiac MRI indicative of LV fibrosis was present in 7% of patients. No patients had LV hypertrophy or abnormal systolic function on conventional parameters such as ejection fraction. This pattern was present in 55% patients with inflammatory autoimmune and vasculitic disease and raises the possibility that myocardial damage might be a



combination of subclinical inflammatory and immunological processes rather than conventional coronary artery disease alone. The distribution of scarring supports post-mortem studies, which have shown patchy myocarditis and myocardial fibrosis in both SLE and WG (Doherty and Siegel, 1985; Goodfield et al., 1995). Acute presentation with myocarditis is rare and is thought to occur only in those with active disease, usually in association with pericardial change. These findings suggest that subclinical myocarditis with subsequent development of fibrosis in SLE and WG might be much more common than previously thought and may contribute to the high incidence of clinical cardiovascular events.

### **3.5.5 Heart Failure with preserved ejection fraction**

The abnormalities of both arterial and ventricular function are strikingly similar to those reported in patients with diabetes and heart failure with normal ejection fraction (HF<sub>n</sub>IEF) (Kawaguchi et al., 2003; Lam et al., 2007; Mottram et al., 2005; Redfield et al., 2005; van der Meer et al., 2007). Type II diabetics (mean age 55 years) with well controlled blood pressure (mean 133 / 79 mmHg) have been shown to have similar reductions in aortic distensibility and markers of diastolic function on cardiac MRI to those in early CKD (van der Meer et al., 2007). These findings were often present in the absence of LVH and reductions in conventional systolic markers function and support our hypothesis that increased arterial stiffness mediates subsequent changes in cardiac function. Kawaguchi et al. showed higher  $E_a$  and  $E_{es}$  in subjects with HF<sub>n</sub>IEF than controls and similar markers of impaired diastolic function (Kawaguchi et al., 2003). Patients with HF<sub>n</sub>IEF often have impaired renal function and it will be of great importance to determine whether this is a cause or effect of the HF<sub>n</sub>IEF disease process (Lam et al., 2007).

The ultrastructural changes responsible for these abnormalities of ventricular function in CKD have not been studied but we speculate that, as in HFnIEF, there may be abnormalities of myocardial collagen and perhaps changes in Titin isoform expression (Zile and Brutsaert, 2002). Further research in this area and on the impact of the increased left ventricular stiffness on effort tolerance and haemodynamic stability in CKD patients is required. Patients with HFnIEF have a poor prognosis and the implications for patients with early CKD are profound. It will also be of importance to determine the abnormalities we have described can be reversed by renal transplantation (Meier-Kriesche et al., 2004).

### **3.5.5 Limitations**

These are observational data and the relationships between eGFR and abnormalities of vascular and cardiac function cannot prove causality. The lack of a treated hypertensive control group means that we cannot exclude a major influence of hypertension on the functional abnormalities we have found in early CKD. The measurements of elastance were derived by non-invasive methodology rather than from invasive ventricular pressure-volume relations, although these methods are well validated against invasive conductance catheter techniques. Brachial artery pressure was used in the calculation of aortic distensibility and elastance as this is in accord with validated methods (Chen et al., 2001). It is accepted however, that central aortic pressure is the pressure 'seen' by the aorta and left ventricle. The use of derived central aortic pressure (applanation tonometry) in the calculation of aortic distensibility and in elastance values did not affect the significance of the results.

### **3.5.6 Conclusion**

Early stage CKD is characterised by increased arterial stiffness, left ventricular systolic stiffness, impaired early relaxation, and increased end diastolic stiffness. Arterial-ventricular coupling is preserved. These changes resemble closely those described in HF<sub>n</sub>IEF and may have implications for myocardial performance and prognosis. Measures to prevent or reverse these changes should be considered in patients with early stage CKD.

## **CHAPTER 4**

### **SUBCLINICAL ABNORMALITIES OF LEFT VENTRICULAR MYOCARDIAL DEFORMATION IN EARLY STAGE CHRONIC KIDNEY DISEASE- THE PRECURSOR OF UREAMIC CARDIOMYOPATHY**

Published by NC Edwards, A Hirth, CJ Ferro, JN Townend, RP Steeds. Subclinical abnormalities of left ventricular myocardial deformation in early stage chronic kidney disease- the precursor of uraemic cardiomyopathy? *J Am Soc Echocardiogr.* 2008. Dec; 21(12):1293-8.

## 4.1 SUMMARY

Abnormal left ventricular deformation is an independent predictor of poor cardiovascular outcome in end-stage renal disease. Studies in early stage chronic kidney disease (CKD) have not been performed despite the known graded inverse relationship between glomerular filtration rate and adverse cardiovascular events.

Forty patients with CKD stage 2 or 3, no history of cardiovascular disease or diabetes and 30 healthy controls underwent echocardiographic Doppler myocardial imaging for assessment of longitudinal deformation (strain/strain rate).

There were no differences in left ventricular (LV) ejection fraction or systolic tissue Doppler velocities between patients with CKD and controls. In CKD, mean global S ( $-15 \pm 4\%$  vs.  $-17 \pm 3\%$ ,  $p < 0.01$ ) and mean global SR were reduced compared to controls ( $-0.88 \pm 0.16 \text{ s}^{-1}$  vs.  $-1.06 \pm 0.31 \text{ s}^{-1}$ ,  $p < 0.05$ ). Peak systolic strain was reduced in the basal lateral ( $-13.9 \pm 0.9\%$  vs.  $-17.9 \pm 1.02\%$ ,  $p < 0.01$ ), basal septal ( $-17.1 \pm 0.8\%$  vs.  $-19.4 \pm 0.77\%$ ,  $p < 0.05$ ) and mid septal ( $-16.4 \pm 0.78\%$  vs.  $-18.9 \pm 0.88\%$ ,  $p < 0.05$ ) walls with more basal post-systolic shortening ( $p < 0.01$ ). Peak systolic strain rate was reduced in the basal lateral, mid lateral and mid septal segments ( $p < 0.05$ ).

Conventional measures of LV systolic function are preserved in early stage CKD but LV systolic deformation is abnormal, consistent with an adverse cardiovascular prognosis.

## 4.2 INTRODUCTION

In chapter 3, I reported that sub-clinical abnormalities of large artery function and left ventricular (LV) diastolic function are present in early chronic kidney disease (CKD) but found no evidence of reduced systolic function using conventional echocardiographic techniques.

Newer echo imaging techniques including the measurement of tissue Doppler velocities (TDI) and myocardial deformation (strain and strain rate) have identified that subclinical abnormalities in LV systolic function in end-stage kidney disease (ESKD) which precede changes in conventional indices of myocardial function such as ejection fraction (Rakhit et al., 2007). Strain and strain rate are of prognostic significance in patients with late and end stage CKD (Rakhit et al., 2007) but no data are available in early stage CKD.

The aim of this study was to determine whether changes in myocardial deformation in asymptomatic patients with stage 2 and 3 CKD are present using TDI and myocardial deformation imaging. It is hypothesised that such changes precede the overt abnormalities in ventricular volumes, mass and standard parameters of systolic function which are highly prevalent in patients with ESKD.

## **4.3 METHODS**

### **4.3.1 Study design**

A cross-sectional observational study.

### **4.3.2. Subjects**

The first fifty patients prospectively recruited for the cross-sectional observational study described in chapter 3, were studied in this pre-specified echocardiographic sub-study. Patients were compared with 30 healthy volunteers (recruited as outlined in chapter 2) matched for age and sex from University Hospital Birmingham.

Compared to the total cohort of CKD patients described in chapter 2, patients in the sub-study were younger ( $48 \pm 11$  years vs.  $57 \text{ years} \pm 12$ ,  $p < 0.01$ ) but did not significantly differ in other demographic or clinical characteristics (male 63% vs. 61%, systolic blood pressure  $130 \pm 13$  mmHg vs.  $131 \pm 16$  mmHg,  $p = 0.89$ , serum creatinine  $126 \pm 34$   $\mu\text{mol/L}$  vs.  $136 \pm 38$   $\mu\text{mol/L}$   $p = 0.15$ ).

### **4.3.3 Data collection**

All subjects underwent:

1. Twelve lead electrocardiogram
2. Resting brachial blood pressure as described in section 2.2.2.1
3. Transthoracic echocardiogram for assessment of:
  - i) LV systolic and diastolic function described in sections 2.2.4-5

- ii) LV mass was calculated according to Devereux's formula (Devereux et al., 1986) described in section 2.2.4.4
- iii) Relative wall thickness (RWT); calculated as the ratio of 2x posterior wall thickness to internal diastolic dimension. Calculation of RWT permits categorization of: normal LV mass with normal geometry ( $RWT \leq 0.42$ ), normal LV mass with concentric remodelling (normal LV mass +  $RWT \geq 0.42$ ), increased LV mass with concentric hypertrophy ( $RWT \geq 0.42$ ) and increased LV mass with eccentric hypertrophy ( $RWT \leq 0.42$ ) (Ganau et al., 1992).
- iv) Myocardial deformation using a 1D-TDI technique is described in section 2.2.4.5.2 (Figures 4.1-3).

The measurement of post-systolic shortening can be observed in strain analysis (Figure 4.2). It refers to a delay in active contraction of longitudinal LV after the aortic valve closure causing impairment of subsequent LV filling. The exact cause remains uncertain but is postulated to reflect delayed active contraction after reduction of regional wall stress and relaxation of other adjacent LV segments or delayed passive inward movement caused by adjacent normal contracting LV segments. Post-systolic shortening can be present in normal myocardium but is less than the shortening in systole. In abnormal myocardium such as ischaemic tissue, it exceeds contraction in systole (Figure 4.2). The timing of events within the cardiac cycle such as aortic and mitral valve opening and closure was obtained from pulse wave Doppler traces of the LV outflow tract and mitral inflow.



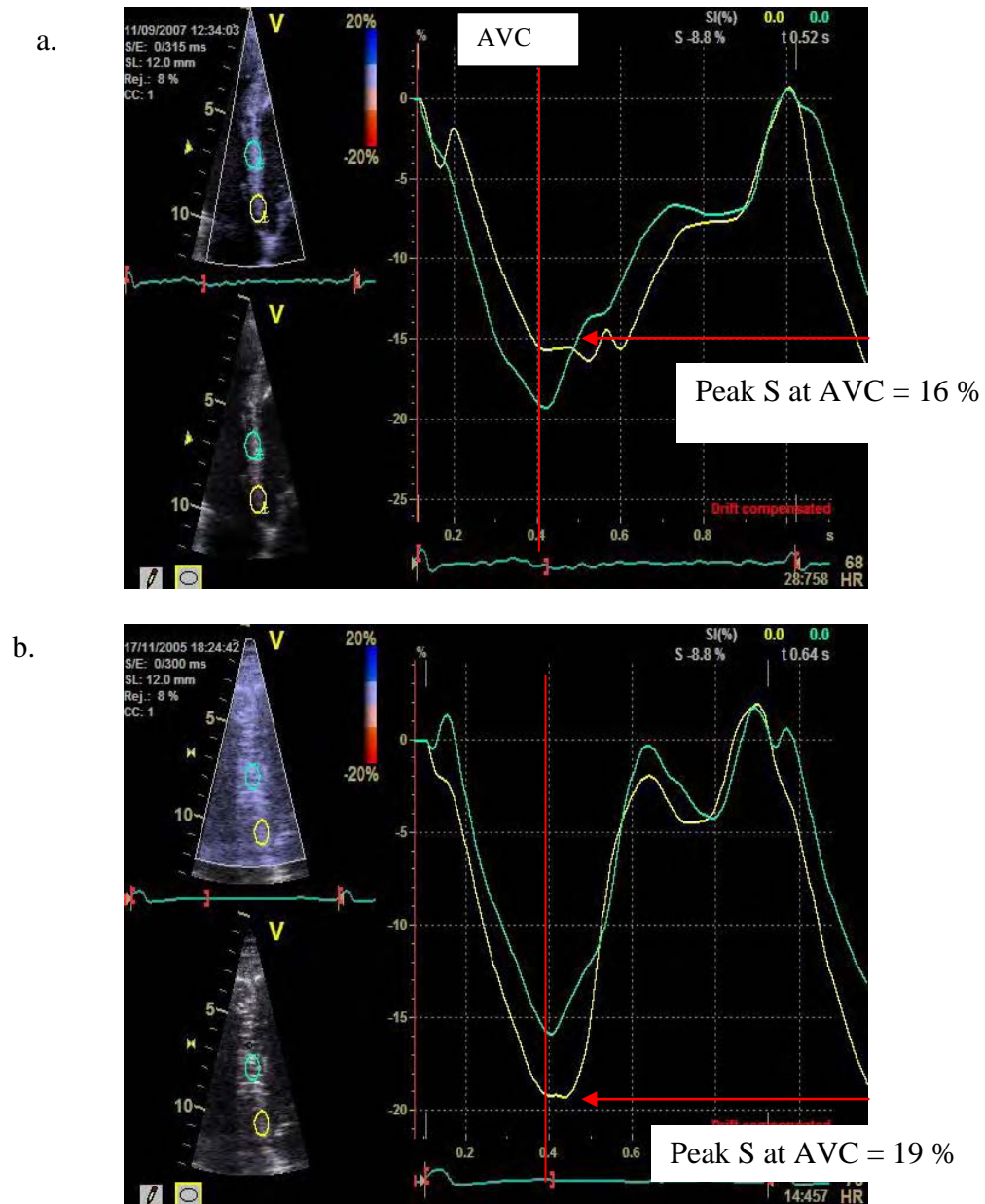
#### **4.3.4 Image analysis**

All analysis was performed off-line by two independent observers blinded to the clinical data (NE, AH) using an Echo-Pac (EchoPac, GE Health Care, Norway) work station and averaged over three consecutive measurements. Reproducibility between the two independent observers was checked by complete image analysis with position of the region of interest by each. The following parameters of LV long-axis function were recorded in both patients with CKD and healthy controls: peak systolic velocity (Sm), early diastolic velocity (Em), peak systolic strain (S) (Figure 4.1), post-systolic shortening (PSS) (Figure 4.2) and peak systolic strain rate (SRs) (Figure 4.3).

#### **4.3.5 Statistical analysis**

Statistical analyses were performed as described in Chapter 2. Intra and inter-observer variability were assessed in 10 randomly selected subjects using intra-class correlation coefficients and Bland-Altman limits of agreement (Bland & Altman, 1986) as described in section 2.3.4. The measurements assessed were: peak systolic tissue velocities, peak systolic basal strain and peak basal systolic strain rate.

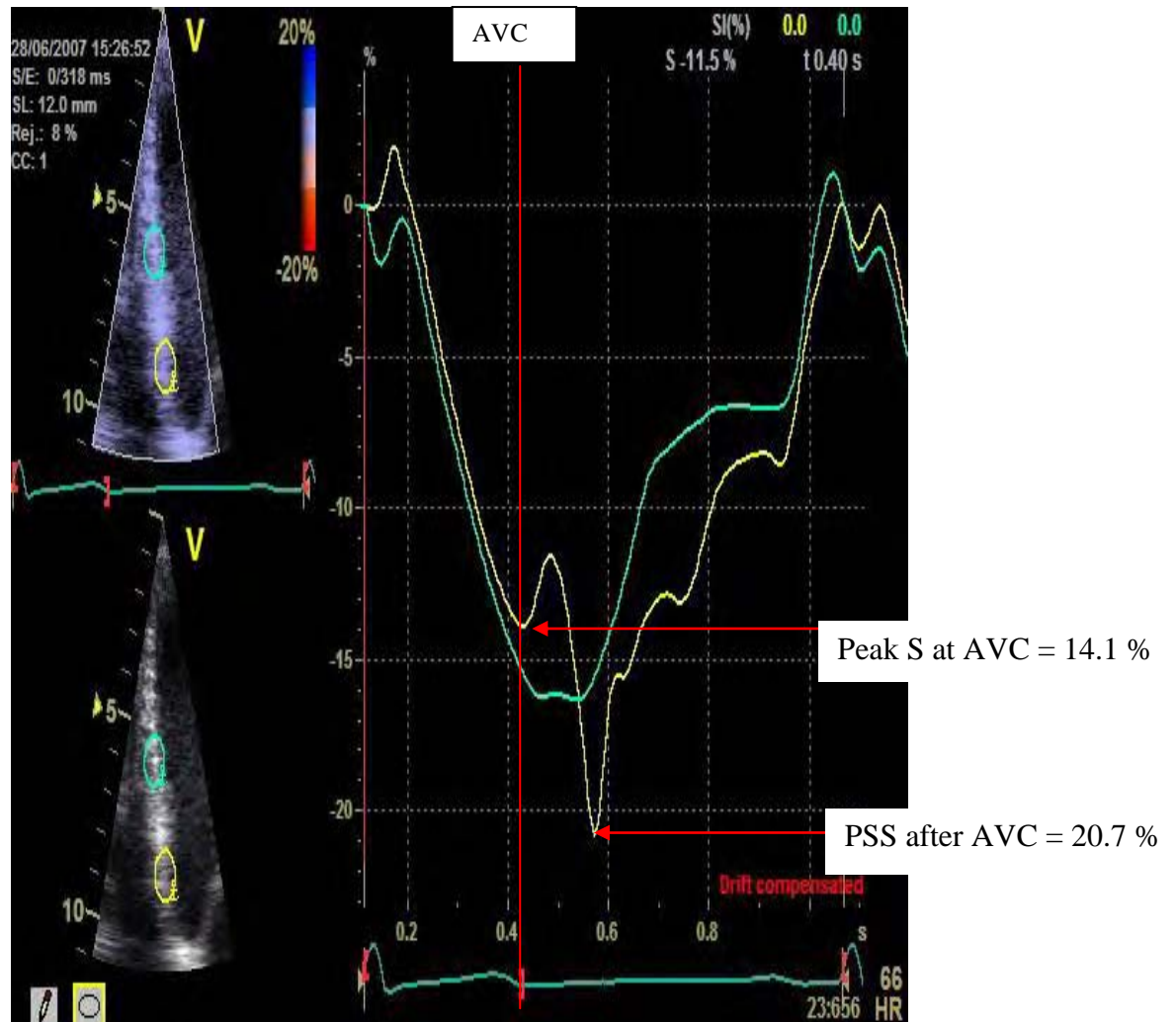
**Figure 4.1** Longitudinal systolic strain (S) curve over one heart cycle from the basal septal (yellow) and mid (blue) septal segments in a) patient with chronic kidney disease and b) healthy control



Peak systolic S is measured at AVC for timing of end systole.

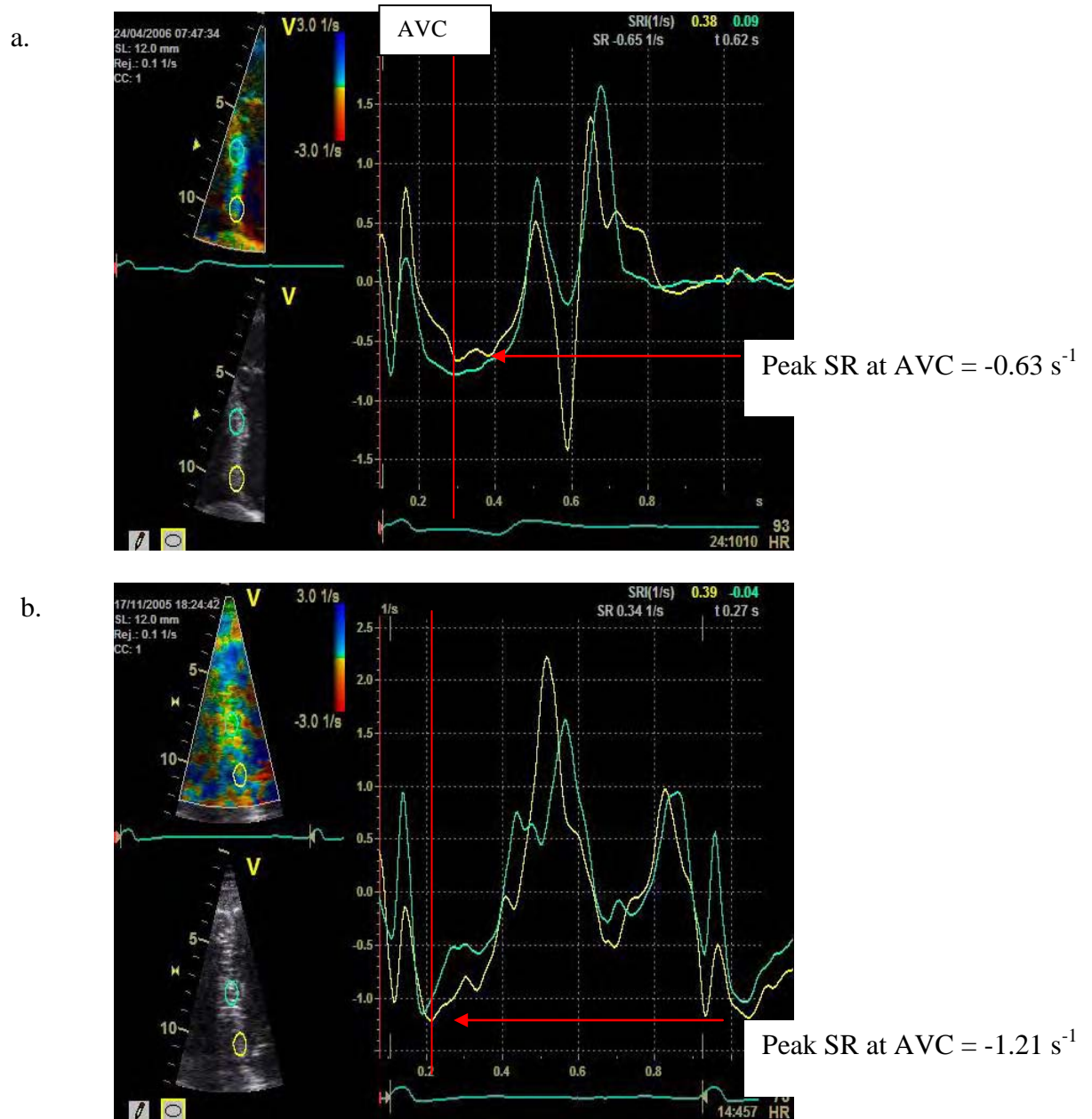
Peak S at AVC is significantly reduced in the basal segment in CKD compared to a control subject.

**Figure 4.2** Longitudinal systolic strain (S) curve over one heart cycle in the basal septal (yellow) and mid septal (blue) segments in a patient with chronic kidney disease demonstrating post-systolic shortening



Basal peak systolic S at AVC is reduced (14.1%). Abnormal basal post-systolic shortening (PSS 20.7%) is present after AVC signifying further shortening of the LV against a closed aortic valve

**Figure 4.3** Longitudinal strain rate (SR) curve over one heart cycle from the basal septal (yellow) and mid septal (blue) segments in **a)** patient with chronic kidney disease and **b)** healthy control



Peak SR is significantly reduced in the basal segment in a patient with CKD compared with a control subject

## **4.4 RESULTS**

### **4.4.1 Demographic data**

There were no significant differences in demographic data between subjects and controls (Table 4.1). The aetiology of CKD was; glomerulonephritis 40%, quiescent vasculitis / SLE 30%, adult polycystic kidney disease 8%, reflux 8%, other 14%). Fifty one percent of patients had a history of hypertension as a complication of their renal pathology but all were well controlled on an average of 2.1 antihypertensive agents per patient. Concomitant treatments in the CKD group included ACE inhibitors 75%, ARBs 18%, ACE inhibitor + ARB 5%, statins 29%, beta blockers 20% and calcium channel blockers 32%. There was no difference in systolic or diastolic blood pressure between patients and controls.

### **4.4.2 Standard echocardiography**

Data from 10 patients were excluded due to inadequate image quality. Standard echocardiographic parameters are summarised in Table 4.2. The septal wall was thicker in early CKD than in controls but the LV cavity size and posterior wall thickness did not differ. Mean LV mass index was increased with CKD compared to controls but there was no difference in eccentric/concentric remodelling as assessed by relative wall thickness.

**Table 4.1** Population characteristics

|                                   | Controls    | CKD         | Significance |
|-----------------------------------|-------------|-------------|--------------|
|                                   | n = 30      | n = 40      |              |
| Male (%)                          | 16 (53)     | 24 (60)     | NS           |
| Age (years)                       | 48 ± 9      | 48 ± 11     | NS           |
| BSA (g/m <sup>2</sup> )           | 1.83 ± 0.17 | 1.86 ± 0.18 | NS           |
| Office systolic BP (mmHg)         | 125 ± 13    | 130 ± 13    | NS           |
| Office diastolic BP (mmHg)        | 78 ± 11     | 79 ± 8      | NS           |
| Heart rate (bpm)                  | 65 ± 11     | 66 ± 10     | NS           |
| Serum creatinine (μmol/L)         | 97 ± 11     | 126 ± 34††  | p < 0.01     |
| eGFR (ml/min/1.73m <sup>2</sup> ) | 70 ± 5      | 56 ± 12 ††  | p < 0.01     |
| Haemoglobin (g/dL)                | 14.4 ± 1.5  | 13.5 ± 1.8  | NS           |
| Number of patients on treatment:  |             |             |              |
| ACE Inhibitors                    | 0           | 30          |              |
| ARB                               | 0           | 7           |              |
| ACE inhibitor + ARB               | 0           | 2           |              |
| Beta Blockers                     | 0           | 8           |              |
| Calcium Channel Blockers          | 0           | 13          |              |
| Diuretics                         | 0           | 10          |              |
| Statins                           | 0           | 10          |              |

Values are mean ± standard deviation

†† p < 0.01 CKD versus controls

**Table 4.2** Standard echocardiographic indices

|                                       | Controls (n=30) | CKD (n=40)    |
|---------------------------------------|-----------------|---------------|
| LV end-diastolic dimension (cm)       | 4.8 ± 0.4       | 5.0 ± 0.4     |
| LV septal wall thickness (cm)         | 0.9 ± 0.1       | 1.0 ± 0.2††   |
| LV posterior wall thickness (cm)      | 0.8 ± 0.1       | 0.8 ± 0.2     |
| Regional wall thickness               | 32 ± 0.08       | 34 ± 0.06     |
| LV mass index (g/m <sup>2</sup> )     | 74 ± 13         | 87 ± 23††     |
| LV hypertrophy (%)                    | 0               | 18%††         |
| LV Ejection fraction (%)              | 65 ± 5          | 64 ± 6        |
| Mean basal Sm (cm/s)                  | 6.2 ± 1.5       | 6.1 ± 1.4     |
| Mitral E velocity (m/s)               | 0.69 ± 1.1      | 0.72 ± 1.2    |
| Mitral A velocity (m/s)               | 0.54 ± 1.1      | 0.61 ± 1.8†   |
| Deceleration time (ms)                | 208 ± 23        | 212 ± 36      |
| Left atrial volume (cm <sup>3</sup> ) | 20.2 ± 4.8      | 28.0 ± 6.2††  |
| Lateral annular TDI Em                | 9.8 ± 3.2       | 8.4 ± 3.3††   |
| E/Em                                  | 7.6 ± 2.2       | 9.8 ± 4.2†    |
| RV Tei Index                          | 0.27 ± 0.09     | 0.36 ± 0.16†† |
| LV Tei Index                          | 0.37 ± 0.05     | 0.47 ± 0.08†† |

Values are mean ± standard deviation

† p < 0.05 †† p < 0.01 CKD vs. controls

A; late mitral inflow velocity, E; early mitral inflow velocity, Em early diastolic tissue Doppler velocity, E/Em; ratio of mitral inflow velocity (E) to early diastolic tissue Doppler velocity (Em), Sm; systolic tissue Doppler velocity

### 4.4.3 Doppler myocardial imaging

Regional and global longitudinal myocardial deformation for all LV segments are shown in Figures 4.4 and 4.5. In early CKD, peak S was reduced in the basal lateral, basal septal and mid septal segments with a trend towards reduction in the mid lateral LV. Mean global S over the LV was significantly reduced in CKD ( $-15 \pm 4\%$  vs.  $-17 \pm 3\%$ ,  $p < 0.01$ ) (Figure 4.4). Post-systolic shortening was increased in CKD compared to controls in the basal lateral ( $25 \pm 5.7\%$  vs.  $9 \pm 3.0\%$ ,  $p < 0.05$ ) and basal septal segments ( $11 \pm 2.2\%$  vs.  $5 \pm 1.7\%$ ,  $p < 0.05$ ). Peak SRs was reduced in the basal lateral, mid lateral, and mid septal segments with a trend towards reduction in the basal septal LV. Mean global SR was reduced in CKD ( $-0.88 \pm 0.16 \text{ s}^{-1}$  vs.  $-1.06 \pm 0.31 \text{ s}^{-1}$ ,  $p < 0.05$ ) (Figure 4.5).

In view of the higher LV mass index and septal thickness observed in early CKD, a subgroup analysis of regional deformation was performed in i) patients with (n=7) and without LV hypertrophy (n=33) and ii) patients with a history of treated hypertension (n=21) compared to patients without a history of hypertension (n=19). There was no significant difference in either analysis in any echocardiographic parameter of regional deformation or tissue velocity measured. There was no correlation between parameters of deformation and LV mass or systolic and diastolic blood pressure. These numbers are small however, and a type II error cannot be excluded.

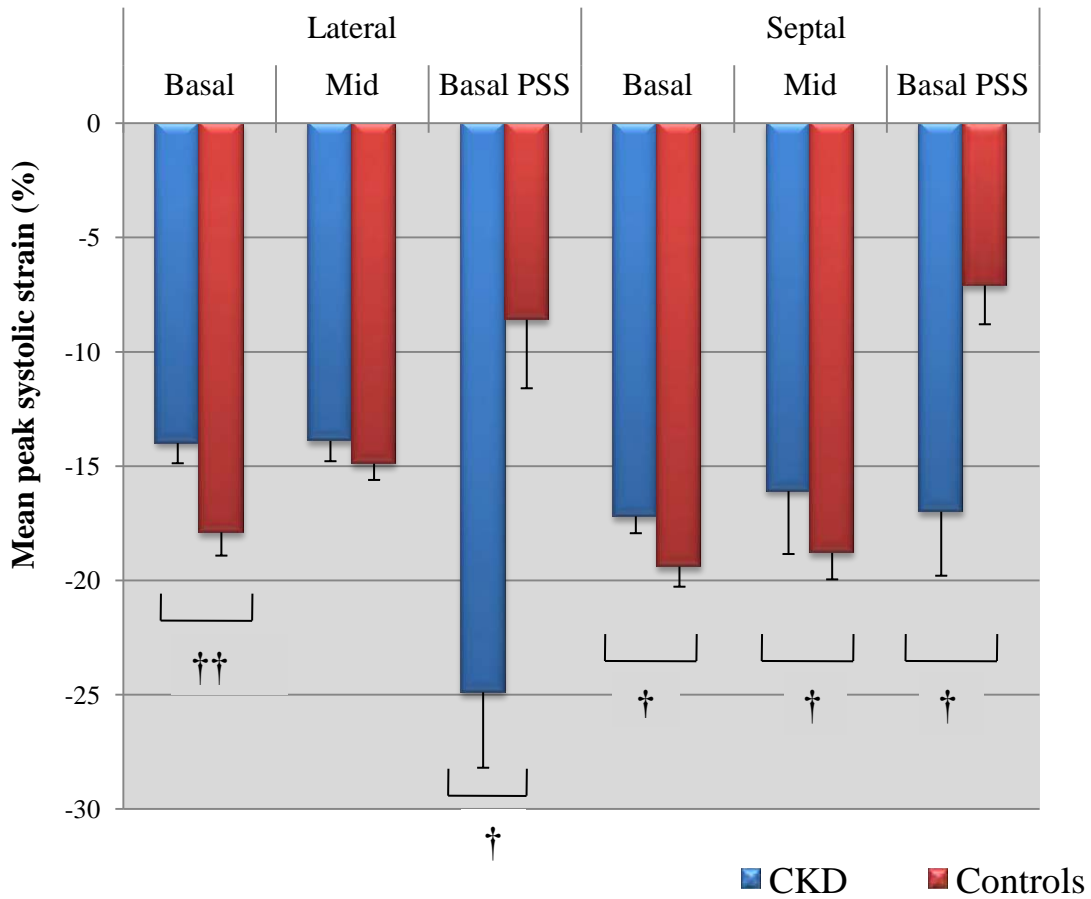
Haemoglobin, calcium, phosphate and parathyroid hormone were within normal range in all subjects. There was no detectable association between measures of myocardial function and any haematological or biochemical parameter by univariate analysis. There was no association between measures of myocardial function and any cardiac medication by univariate analysis.



#### **4.4.4 Reproducibility**

There was good intra and inter-observer agreement; peak systolic tissue velocities (Sm) ( $r = 0.96$ ;  $p < 0.01$ ) longitudinal peak SR ( $r = 0.95$ ,  $p < 0.01$ ) as well as longitudinal peak S ( $r = 0.98$ ;  $p < 0.01$ ). Bland-Altman limits of agreements revealed no systematic bias in differences between measurements with respect to their means for intra and inter-observers respectively: peak systolic tissue velocities 10.4 to -6.9% and 11.8 to -12.4%, longitudinal peak SR 13.3 to -12.7% and 19.9 to -21.6, longitudinal peak S 15.1 to -13.5% and 8.5 to -11.9%.

**Figure 4.4** Mean longitudinal peak systolic strain (S) and post-systolic shortening (PSS) in the left ventricular lateral and septal walls and global left ventricle in patients with chronic kidney disease and healthy controls

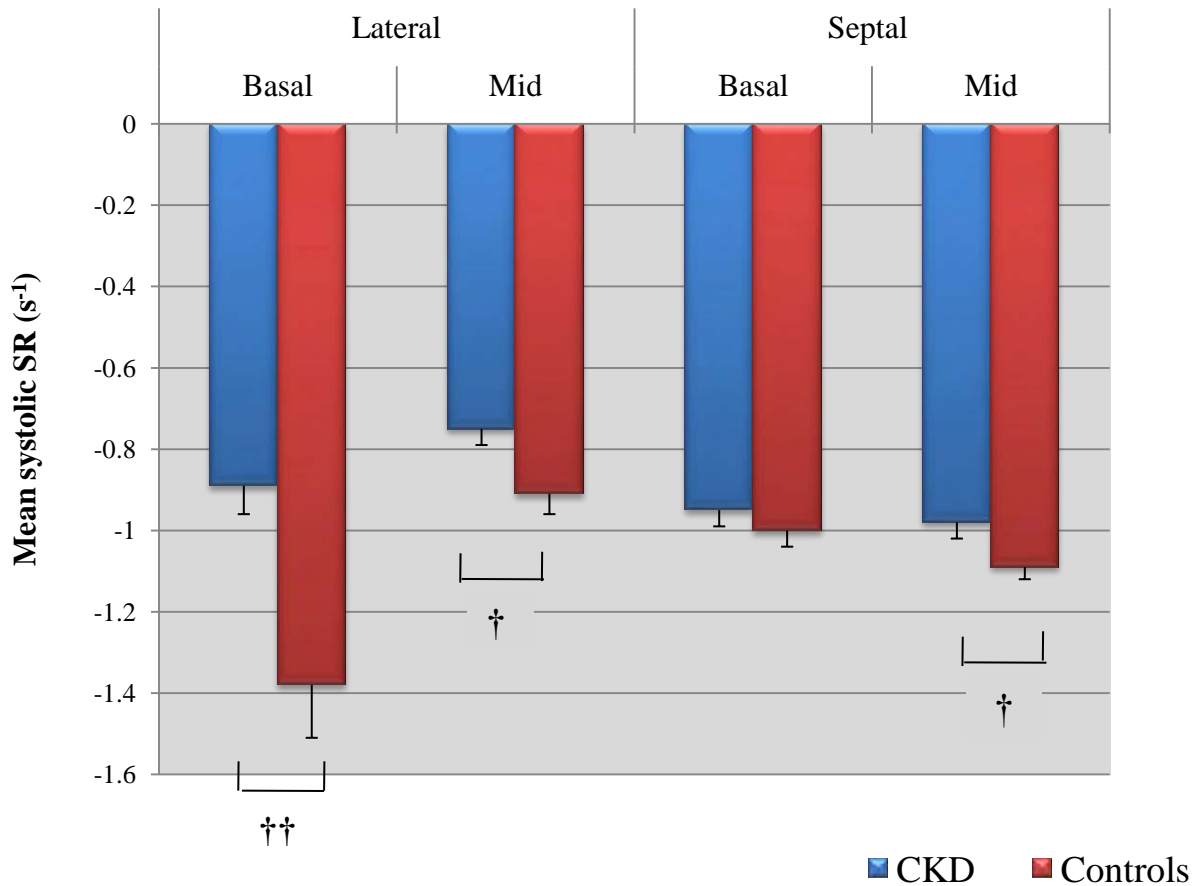


Values are mean ± standard error of the mean.

† p < 0.05 †† p < 0.01 CKD vs. controls.

S, strain; PSS, post-systolic shortening

**Figure 4.5** Peak systolic strain rate (SR) in the left ventricular lateral and septal walls and global left ventricle in patients with chronic kidney disease and healthy controls



Values are mean  $\pm$  standard error of the mean.

†  $p < 0.05$  ††  $p < 0.01$  CKD vs. controls.

SR, peak systolic strain rate

## **4.5. DISCUSSION**

This was the first study to demonstrate that patients with early CKD have abnormal LV systolic function detected by impaired longitudinal myocardial deformation imaging. These abnormalities occurred in the absence of abnormalities in conventional markers of LV dysfunction such as ejection fraction and tissue Doppler systolic velocities.

### **4.5.1 Echocardiographic changes in chronic kidney disease**

Left ventricular systolic function is abnormal at a stage when subjects have normal or near normal serum creatinine (mean serum creatinine 126  $\mu\text{mol/L}$ ) and are consistent with the epidemiological evidence that early stage CKD is associated with adverse cardiovascular outcomes with eGFRs as high as 60-90 ml/min/1.73m<sup>2</sup> (Van et al., 2007). Reductions in peak strain and peak strain rate have been reported before in late and end stage CKD (Rakhit et al., 2007). Rakhit et al. (2007) showed that these abnormalities were highly prevalent in late and end stage CKD and that a reduction in strain rate predicted adverse cardiac events despite otherwise near normal conventional echocardiographic findings.

Approximately 14% of the general population have the same degree of mild renal impairment as the subjects in our study (Coresh et al., 2007). These data therefore raise important questions regarding the prognostic significance of reduced strain rate in early stage CKD and the importance of identifying effective therapeutic treatment strategies.

#### **4.5.2 Changes in regional left ventricular function in early chronic kidney disease**

In this study, regional systolic function was abnormal but standard echocardiographic assessment of LV radial systolic function such as ejection fraction and regional myocardial systolic tissue velocities did not differ between early CKD and controls. This pattern is postulated to reflect an increase in contraction of the mid-wall circumferential fibres to compensate for the reduction in longitudinal contraction due to subendocardial dysfunction (Yip et al., 2002). From observational data however, it is not possible to determine if regional dysfunction is the precursor of major alterations in LV geometry and function characteristic of ESKD. In other cardiomyopathies abnormal longitudinal deformation has been demonstrated to precede clinical evidence of systolic dysfunction using conventional indices. Furthermore, abnormal longitudinal S / SR are useful in the pre-clinical diagnosis of a number of other cardiomyopathies, including diabetes, hypertrophic cardiomyopathy, Fabry's disease and amyloid (Kato et al., 2004; Koyama et al., 2003; Weidemann et al., 2005)

Abnormal longitudinal myocardial deformation in early CKD may reflect interstitial fibrosis with myocyte hypertrophy and disarray, which has been documented on endomyocardial biopsy (Aoki et al., 2005). Both human and animal work has shown uremia to be associated with myocardial fibrosis independent of traditional cardiovascular risk factors (Amann et al., 1998; Mall et al., 1990). Alongside the reduction in peak strain and strain rate, early CKD was associated with an increase in the frequency of post-systolic shortening / thickening. This may be due to microvascular ischaemia caused by the reduction in the density of myocardial capillaries which occurs in patients with CKD compared both to controls and to patients with essential hypertension (Amann et al., 1998).

There are few other echocardiographic data available in patients with early CKD for comparison with our results. In a small study of 29 patients with mild/moderate renal dysfunction (creatinine clearance  $\geq 29$  ml/min) and 19 patients with severe CKD (creatinine clearance  $\geq 29$  ml/min), early diastolic tissue velocities were found to be reduced in both groups compared to controls with no change in standard echocardiographic parameters of systolic or diastolic function (Hayashi et al., 2006). The reductions in early diastolic tissue velocities were present in patients with and without LVH, although were more pronounced in the sub-group with LVH, leading to the suggestion that factors other than hypertrophy could be responsible. This hypothesis is supported by a recent sub-group analysis of patients with early CKD from the Multi-Ethnic Study of Atherosclerosis (Nasir et al., 2007). The investigators used Harmonic Phase (HARP) tagged MRI studies (which has the advantage of high spatial resolution but at a cost of poor temporal resolution compared to echocardiography) to determine regional systolic and diastolic function. Significant reductions in systolic S / SR in patients with a creatinine clearance below 60 ml/min were observed, independent of age and coronary heart disease risk factors. These data however, only provide information on changes in regional circumferential strain and not regional longitudinal deformation as in our study but are consistent with the hypothesis of an early diffuse cardiomyopathy associated with early CKD.

A clinical outcome trial is required to investigate whether reductions in strain and strain rate are a marker of adverse prognosis in early stage CKD as in ESKD. They also raise the question as to whether patients with early CKD should be treated routinely with drugs such as ACE inhibitors, ARBs and mineralocorticoid receptor blocking agents such as spironolactone and eplerenone, which have been shown to be effective in improving the cardiac outcomes of other

groups of patients with symptomatic and asymptomatic LV systolic dysfunction (Mak et al., 2009; Mottram et al., 2004; Pitt et al., 1999; Zannad et al., 2011).

### **4.5.3 Limitations**

As described in chapter 3, the lack of a control group with treated hypertension and normal renal function means that it is not possible to exclude the impact of hypertension on the functional abnormalities documented in this study. A 5 mmHg difference in office systolic blood pressure was present between patients with CKD and controls, although this was not statistically significant. This was not a study however, of patients with hypertension and incidental renal dysfunction. All patients were recruited from a specialist renal clinic and had established renal disease, proven by biopsy in 70% of cases. None of the controls had CKD by definition. The surprisingly low mean eGFR in this group reflects the inherent inaccuracy of  $eGFR \geq 60$  ml/min/1.73m<sup>2</sup> using the 4-v MDRD formula (Poggio et al., 2005). We were not able to examine in detail the association between LV hypertrophy and the presence of subnormal myocardial deformation because of the small number of patients fulfilling the criteria for LVH; there were however, no significant differences in any parameter of global or regional deformation between the patients with LVH and those with a normal LV mass index, nor between patients with a history of hypertension and those with no history of hypertension. In addition, the regional reductions in deformation observed in this study differ from those found in hypertension, where a decrease in septal deformation appears to be accompanied by a concomitant increase in this parameter in the lateral wall, rather than the global reduction found in our study (Baltabaeva et al., 2008).

#### **4.5.4 Conclusion**

Abnormal longitudinal systolic deformation is present in asymptomatic individuals with early CKD without clinical evidence of heart disease. These changes represent the first detectable echocardiographic manifestations of systolic dysfunction in CKD and along with abnormalities of diastolic function, may be the precursors of the major alterations in LV geometry and function that are characteristic of ESKD.



## **CHAPTER 5**

### **EFFECT OF SPIRONOLACTONE ON LEFT VENTRICULAR MASS AND AORTIC STIFFNESS IN EARLY STAGE CHRONIC KIDNEY DISEASE: A RANDOMISED CONTROLLED TRIAL**

Contributory publications:

NC Edwards, RP Steeds, PM Stewart, CJ Ferro, JN Townend. Effect of Spironolactone on Left Ventricular Mass and Aortic Stiffness in Early Stage Chronic Kidney Disease: A Randomized Controlled Trial. *J Am Coll Cardiol*. 2009 Aug 4;54(6):505-12.

## 5.1 SUMMARY

Early stage chronic kidney disease (CKD) is associated with a high risk of cardiovascular disease and a high prevalence of left ventricular hypertrophy and arterial stiffness which confer an adverse prognosis. It is believed that these abnormalities are in part a result of activation of the renin-angiotensin-aldosterone system. It was hypothesised that the addition of spironolactone to ACE inhibitors and Angiotensin Receptor Blockers (ARBs) would improve left ventricular mass and arterial stiffness in CKD.

Following an active run in phase with spironolactone 25 mg once daily, 112 patients with stage 2 and 3 CKD with good blood pressure control (mean daytime ambulatory BP  $\leq$  130/85 mmHg) on established treatment with ACE inhibitors or ARBs were randomised to continue spironolactone or receive a matching placebo. Left ventricular mass (cardiac magnetic resonance) and arterial stiffness (pulse wave velocity / analysis, aortic distensibility) were measured before run in and after 40 weeks of treatment.

Compared to placebo, spironolactone resulted in significant improvements in LV mass ( $-14 \pm 13$  g vs.  $+3 \pm 11$  g,  $p < 0.01$ ), pulse wave velocity ( $-0.8$  m/s  $\pm 1.0$  vs.  $-0.1$  m/s  $\pm 0.9$ ,  $p < 0.01$ ), augmentation index ( $-5.2$  %  $\pm 6.1$  vs.  $-1.4$  %  $\pm 5.9$ ,  $p < 0.05$ ) and aortic distensibility ( $0.69 \times 10^{-3}$  mmHg  $\pm 0.86$  vs.  $0.04 \times 10^{-3}$  mmHg  $\pm 1.04$ ,  $p < 0.01$ ).

Spironolactone reduces left ventricular mass and improves arterial stiffness in early stage CKD. These effects suggest that aldosterone exerts adverse cardiovascular effects in CKD and that spironolactone is worthy of further study as a treatment that could reduce adverse cardiovascular events.

## 5.2 INTRODUCTION

In chapters 3 and 4, it was shown that early stage chronic kidney disease (CKD) is characterised by major abnormalities of cardiovascular structure and function. There are many possible causes which might promote these abnormalities but high on the list is activation of the renin-angiotensin-aldosterone system (RAAS).

The importance of the RAAS as a driver of progressive renal and cardiovascular disease in patients with CKD is increasingly apparent (Parfrey, 2008; Remuzzi et al., 2008). Both angiotensin II and aldosterone exert numerous adverse cardiovascular effects including the development of left ventricular hypertrophy (LVH) (Brilla & Weber, 1992) and increased arterial stiffness (Weber and Brilla, 1991a). Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are effective in reducing the progression of both renal and vascular damage in CKD but they do not result in complete suppression of aldosterone production (Bomback & Klemmer, 2007; Schrier, 2010), a powerful potential stimulus to arterial stiffness, ventricular hypertrophy and fibrosis (chapter 1).

It is therefore possible that in patients with early stage CKD, continuing aldosterone production might be an important cause of both LVH and increased arterial stiffness. These are powerful risk factors for cardiovascular disease in the general population (Dahlof et al., 2005; Levy et al., 1990) and are both highly prevalent and prognostically important in CKD (Blacher et al., 1999b; Foley & Parfrey, 1998). The Chronic Renal Impairment in Birmingham II (CRIB-II) trial therefore examined the effect of the addition of the aldosterone antagonist spironolactone to ACE inhibitors or ARBs on these prognostic markers in a group of patients with early (stage 2 and 3) CKD.

## **5.3 METHODS**

### **5.3.1 Study design**

The Chronic Renal Impairment in Birmingham (CRIB-II) study II was a single centre, prospective, double-blind, placebo controlled, randomised interventional trial over 40 weeks. It was registered on [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (Identifier: NCT00291720) and the European Clinical trials Database (EudraCT) (2007-003408-36). Medicines and Healthcare products Regulatory Agency (MHRA) approval was obtained for the use of spironolactone 25mg daily (MF8000/13148).

### **5.3.2 Study outline and treatment regimen**

Screening protocols and inclusion / exclusion criteria have been outlined in chapter 2. Reasons for ineligibility are described in chapter 3. Thus 287 patients were suitable for inclusion; 170 declined to participate and 117 patients provided written consent to participate. An outline of the study is presented in Figure 5.1

The study comprised 4-week open-label run-in phase of 25 mg of spironolactone once daily, after which patients were randomised to continue a further 36 weeks of treatment with 25 mg of spironolactone or to matching placebo.

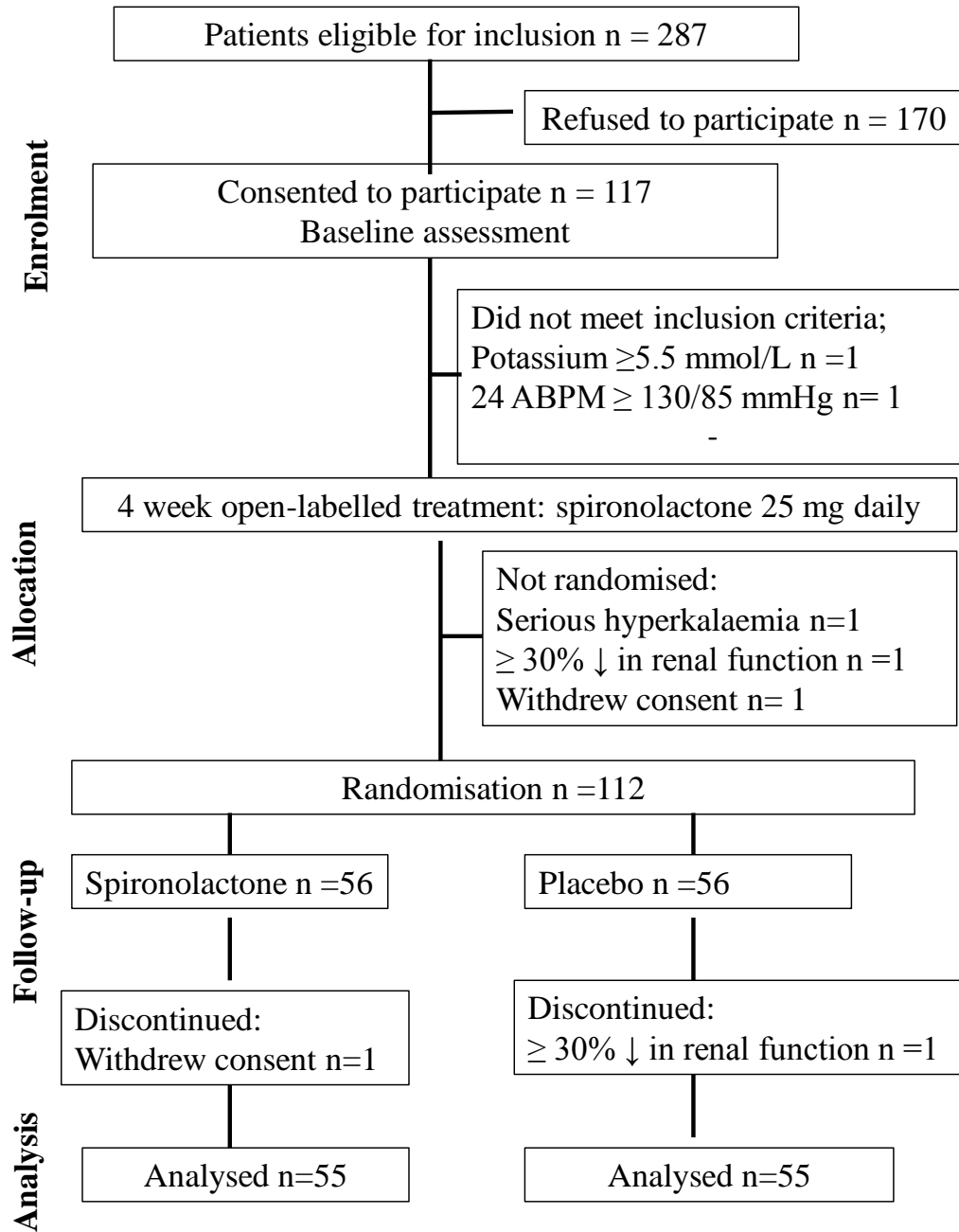
Patients were withdrawn at any stage for the following reasons;

1. Hyperkalaemia – protocol for management outlined in Table 5.1
2. Reduction in eGFR  $\geq$  30% compared to a previous sample
3. Intolerant of the study medication

#### 4. Patient withdrew consent

Patients unwell with vomiting or diarrheal illnesses had study medication temporarily suspended.

**Figure 5.1** Study Outline



### **5.3.2.1 Open labelled “run-in” phase (week 0 to week 4)**

After baseline clinical assessment, renal function, serum potassium and 24 hour ambulatory blood pressure results were reviewed to ensure the inclusion criteria were satisfied. All patients were dispensed 30 spironolactone (generic) tablets at a dose of 25mg daily from the pharmacy department in University Hospital Birmingham. Patients were requested to continue all regular medications at normal dosing times adding spironolactone to morning medications. Patients were advised to phone the Wellcome Research Clinical Facility (WRCF) study nurse or research fellow if they experienced any side effects or had any questions regarding treatment. Renal function and serum potassium levels were checked after weeks 1 and 2 of treatment at the WRCF or the patient’s local hospital phlebotomy unit. Patients GP’s were sent a letter detailing the study aims and protocol. They were requested not to change regular medications without prior discussion with the study research fellow and principle investigator.

### **5.3.2.2 Randomisation (week 4 to week 40)**

At week 4, patients re-attended the WRCF for further clinical assessment including; history, examination, 12 lead electrocardiogram, resting “office” and 24 hour ambulatory blood pressure monitoring, assessment of arterial stiffness (pulse wave analysis, carotid femoral pulse wave velocity), venous blood tests and urine analysis.

The unused spironolactone tablets were counted and returned to pharmacy to ensure compliance. Patients were randomised by the pharmacy department in University Hospital Birmingham to a capsule of spironolactone or placebo (manufactured by MHRA approved supplier; DHP Pharma, Powys, Wales). The shelf life of the spironolactone / placebo capsule was 6 months, hence up to a maximum of six containers were supplied to each patient at

randomisation. When a new supply was received, all patients were contacted and the appropriate remaining containers dispensed. Each container contained 32 tablets, enough for each calendar month after which they were advised to change to a new container. If commencing part way through a calendar month, patients were still advised to start a new container at the start of the next month. Renal function and potassium were checked at weeks 8, 16 and 32 at a nurse-led appointment at the WRCF or patients local hospital. At each review time point, patients were actively questioned as to possible side effects including disturbed menstrual cycle, breast tenderness, erectile dysfunction and gynecomastia. At week 40, all patients attended the WRCF for clinical review and investigations as per week 0. A tablet count was performed at each review to ensure compliance.

### **5.3.2.3 Biochemical monitoring protocol**

Serious hyperkalaemia was defined as a serum potassium  $\geq 6.5$  mmol/L or serum potassium  $\geq 6.0$  mmol/L on consecutive samples. Mild hyperkalaemia was defined as a serum potassium 5.5- 5.9 mmol/L. The protocol for management of hyperkalaemia is detailed in Table 5.1.



**Table 5.1** Management of hyperkalaemia

| Serum potassium (mmol/L)            | Action                                                   |
|-------------------------------------|----------------------------------------------------------|
| $\geq 6.5$ mmol/L                   | Appropriate treatment and withdrawal from study          |
| 6-6.4 mmol/L                        | Abstain for 1 week, recommence at 25mg on alternate days |
| $\geq 6.0$ mmol/L on repeat testing | Withdrawal from study                                    |
| $\geq 5.5$ -5.9 mmol/L              | Change to 25mg on alternate days                         |
| <5.4 mmol/L                         | No action                                                |

An independent data monitoring committee was established to review all biochemical data at 4 weekly intervals. The committee had the power to: i) un-blind the study if any significant trends were noted, ii) suspend the study if, for any reason, they feel patient safety is being compromised.

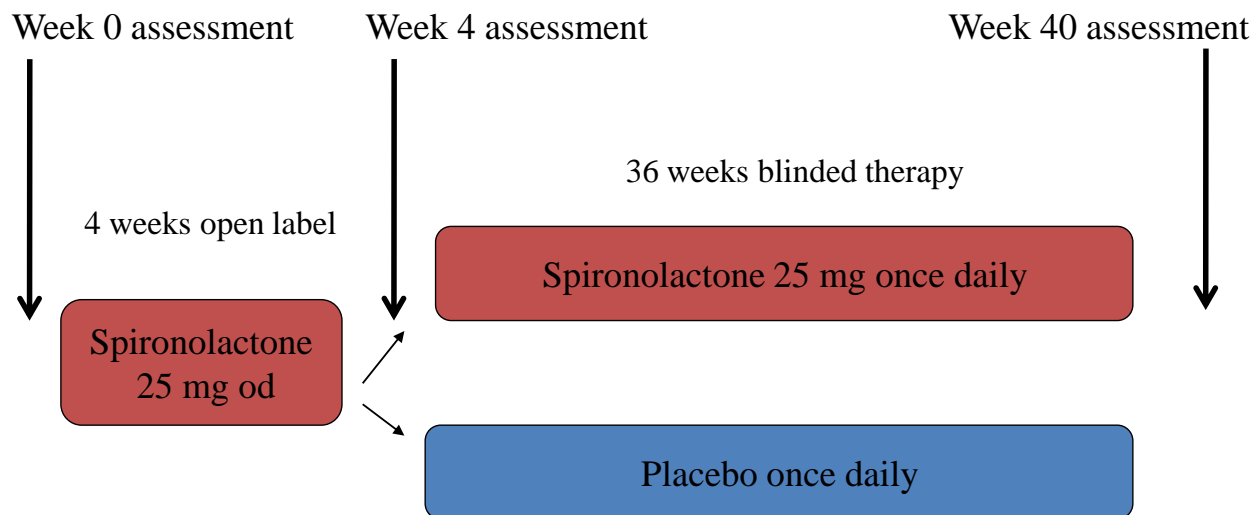
### **5.3.3 Clinical assessments**

Detailed methodology of the assessment techniques used are described in chapter 2. All patients underwent clinical assessments as described in chapter 3 at baseline / week 0 and at week 40 prior to stopping treatment. In this study the following additional assessments were performed:

1. Non-invasive assessment of arterial stiffness using applanation tonometry (pulse wave analysis (PWA), carotid-femoral pulse wave velocity (PWV)).

An outline of clinical assessments performed in this study is presented in Figure 5.2.

**Figure 5.2** An outline of clinical assessments performed in the Chronic Renal Impairment in Birmingham (CRIB)-II study



| Investigations                   |                                    |
|----------------------------------|------------------------------------|
| 1. Potassium and renal function: | weeks 0, 1, 2, 4, 8, 16, 28 and 40 |
| 2. Cardiac MRI:                  | weeks 0 & 40                       |
| 3. Arterial stiffness:           | weeks 0 & 40                       |
| 4. Echocardiography:             | weeks 0 & 40                       |
| 5. 24 hour ambulatory BP:        | weeks 0 & 40                       |
| 6. ACR:                          | weeks 0 & 40                       |

### **5.3.4 Outcomes and follow-up**

The study co- primary end-points were change in LV mass and arterial stiffness measured by PWV. Secondary end-points were aortic distensibility, augmentation index, blood pressure, and albuminuria.

### **5.3.5 Statistical analysis and power calculation**

Based on previous studies of LV mass determined by cardiac MRI and PWV by applanation tonometry, we assumed a standard deviation of change in these parameters of 12g and 1.0 m/s respectively. Thus we calculated a sample size of 90 patients assigned equally to the two treatment groups would provide 95% power to detect a change in LV mass of 10 g and 80% power to detect a change in PWV of 0.6m/s with an  $\alpha$ -value (type I error) of 5%. A 10% drop-out rate was assumed.

Statistical analyses were performed as described in Chapter 2. Adjustments for changes in mean arterial pressure from week 0 to week 40 for parameters of arterial stiffness were based on coefficients obtained from linear regressions using baseline data for both groups combined. These values are presented as PWV<sub>adj</sub>, aortic distensibility<sub>adj</sub>, augmentation<sub>adj</sub>, Aug Ix<sub>adj</sub>, Aix 75<sub>adj</sub>.

Independent predictors of changes in LV mass and arterial stiffness were determined using multivariate regression models as outlined in chapter 2.

## 5.4 RESULTS

### 5.4.1 Patient characteristics and follow-up

Baseline demographic and clinical characteristics are presented in Table 5.2. As expected, baseline levels of angiotensin II were significantly lower in patients on an ACE I than an ARB (data not shown,  $p < 0.01$ ) with no difference in but levels of aldosterone or renin.

Over 40 weeks of follow-up, no patients died, two patients did not complete the follow up period; 1 patient withdrew consent for further participation and 1 patient had a relapse of Wegener's granulomatosis causing acute renal failure. Two patients were hospitalised during their participation for unrelated medical conditions.

#### 5.4.2.1 Change in blood pressure

Spironolactone reduced office, central, daytime ambulatory and night-time ambulatory systolic blood pressure compared to placebo (Table 5.3). There were no changes in the equivalent diastolic pressures. Only 44% of patients at baseline were nocturnal dippers. There was no significant change at week 40 (47%) in either treatment group.

#### 5.4.2.2 Renal and endocrine effects

After 40 weeks of treatment there was no difference in eGFR; spironolactone  $-3 \pm 7$  ml/min/1.73m<sup>2</sup> vs. placebo  $-1 \pm 5$  ml/min/1.73m<sup>2</sup>, NS. Treatment with spironolactone reduced median ACR by  $-3.2$  (25) mg/mmol compared to  $-0.6 \pm (6.2)$  mg/mmol with placebo,  $p < 0.05$

(Table 5.4). Levels of plasma aldosterone, plasma renin, plasma angiotensin II were increased with spironolactone. There was no change in high sensitivity CRP concentrations are shown in Table 5.4.

#### **5.4.2.3 Changes in left ventricular mass, volumes and function**

Compared to placebo, treatment with spironolactone resulted in significant reductions in LV mass and LV mass index (Table 5.4, Figure 5.3). The prevalence of LVH declined by 50% with spironolactone but was unchanged with placebo (Table 5.4). Baseline LV mass was not a predictor of LV mass regression on multivariable analysis. The reduction in LV mass index on spironolactone for subjects with LVH at baseline was  $-8 \pm 8$ g compared to  $-6 \pm 5$ g for subjects with a normal baseline LV mass ( $p = \text{NS}$ ). Treatment with spironolactone did not affect LV volumes or ejection fraction.

**Table 5.2** Patient characteristics at baseline

|                                        | <b>Placebo</b><br><b>(n=56)</b> | <b>Spirolactone</b><br><b>(n=56)</b> |
|----------------------------------------|---------------------------------|--------------------------------------|
| Age (years)                            | 53 ± 12                         | 54 ± 12                              |
| Male (%)                               | 33 (59)                         | 32 (57)                              |
| Body surface area                      | 1.86 ± 0.2                      | 1.95 ± 0.2†                          |
| 24 hour ambulatory SBP (mmHg)          | 125 ± 10                        | 124 ± 11                             |
| 24 hour ambulatory DBP (mmHg)          | 77 ± 8                          | 76 ± 8                               |
| Serum creatinine (µmol/L)              | 124 ± 34                        | 133 ± 35                             |
| eGFR (ml/min/1.73m <sup>2</sup> )      | 53 ± 11                         | 49 ± 12                              |
| Heart rate (beats per minute)          | 65 ± 11                         | 66 ± 12                              |
| Haemoglobin (g/dL)                     | 13.5 ± 1.6                      | 13.5 ± 1.3                           |
| Cholesterol (mmol/L)                   | 4.7 ± 1.1                       | 4.9 ± 1.1                            |
| Serum potassium (mmol/L)               | 4.3 ± 0.3                       | 4.4 ± 0.8                            |
| <b>Number of patients on treatment</b> |                                 |                                      |
| ACE Inhibitors                         | 37                              | 37                                   |
| ARB                                    | 17                              | 18                                   |
| ACE Inhibitor + ARB                    | 2                               | 1                                    |
| Beta Blockers                          | 8                               | 15                                   |
| Calcium Channel Blockers               | 17                              | 13                                   |
| Diuretics                              | 13                              | 18                                   |
| Statins                                | 17                              | 27                                   |

Values are mean ± standard deviation. † p < 0.05.

ARB; angiotensin receptor blocker, DBP; diastolic blood pressure, SBP; systolic blood pressure

24 hour ambulatory; blood pressure every 30 minutes between 6am-10pm, 1 hourly 11pm-5am

**Table 5.3** Change in blood pressure after 40 weeks of treatment with spironolactone or placebo

|                          | Placebo  |          | Spironolactone |             |
|--------------------------|----------|----------|----------------|-------------|
|                          | Week 0   | Week 40  | Week 0         | Week 40     |
| Office SBP (mmHg)        | 130 ± 19 | 125 ± 17 | 130 ± 16       | 119 ± 13 †  |
| Office DBP (mmHg)        | 77 ± 10  | 73 ± 9   | 77 ± 10        | 71 ± 10     |
| Central SBP (mmHg)       | 120 ± 18 | 116 ± 16 | 121 ± 15       | 110 ± 13 †† |
| Central DBP (mmHg)       | 78 ± 10  | 74 ± 9   | 78 ± 10        | 72 ± 10     |
| 24 hr daytime SBP (mmHg) | 125 ± 11 | 124 ± 11 | 125 ± 11       | 119 ± 11 †† |
| 24 hr daytime DBP (mmHg) | 77 ± 9   | 76 ± 7   | 76 ± 8         | 73 ± 8      |
| 24 hr night SBP (mmHg)   | 113 ± 14 | 112 ± 11 | 115 ± 15       | 108 ± 14 †  |
| 24 hr night DBP (mmHg)   | 66 ± 10  | 66 ± 9   | 67 ± 9         | 63 ± 9      |

Values are mean ± standard deviation

To compare changes in the two groups we used repeated measures analysis of variance with the time point (week 0, week 40) as the ‘within subjects’ factor and the group (spironolactone and placebo) as the ‘between subjects’ factor and tested the significance of the interaction between the two. † p < 0.05, †† p < 0.01

DBP; diastolic blood pressure, SBP; systolic blood pressure, daytime; between 6am-10pm, night; 11pm-5am



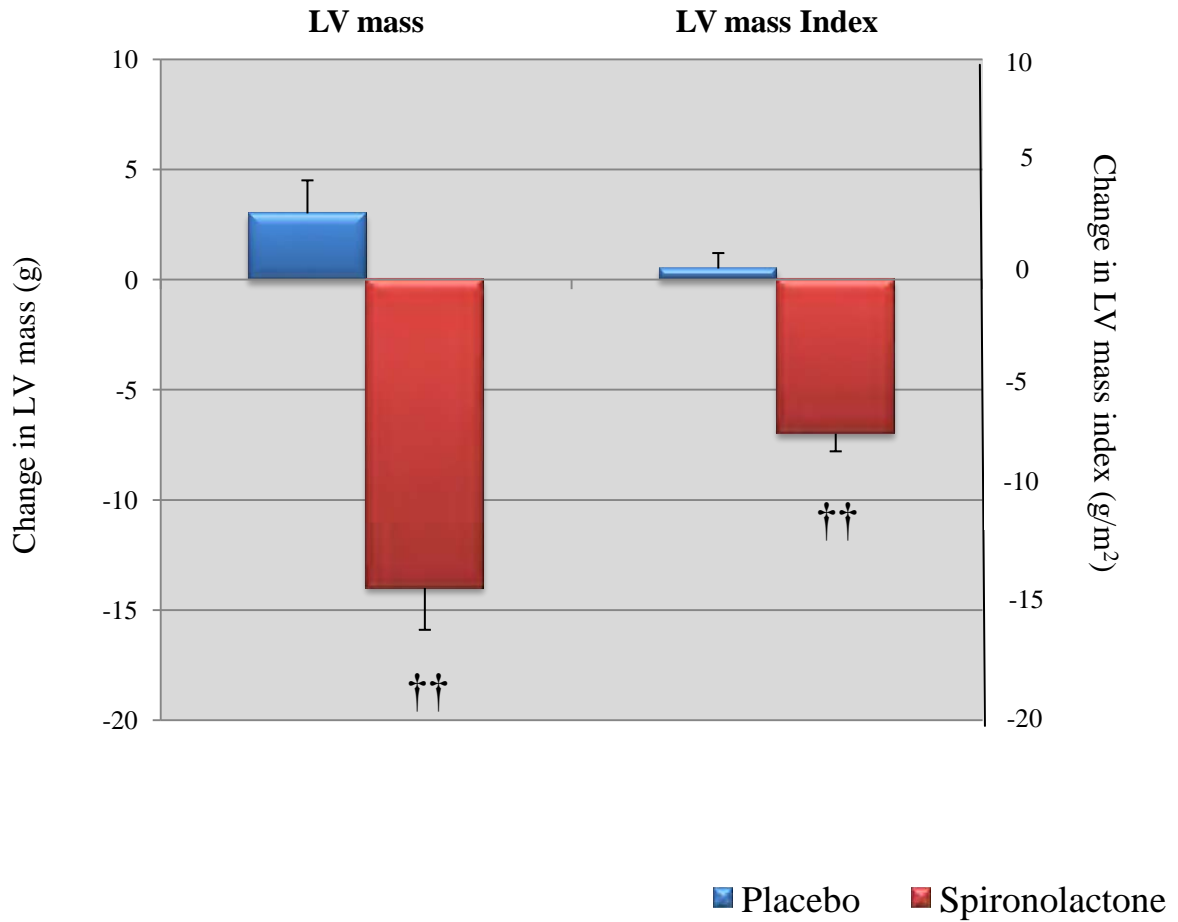
**Table 5.4** Changes in biochemical and cardiac MRI parameters

|                                   | Placebo     |             | Spironolactone |                |
|-----------------------------------|-------------|-------------|----------------|----------------|
|                                   | Week 0      | Week 40     | Week 0         | Week 40        |
| ACR (mg/mmol)#                    | 5.0 ± 46    | 5.0 ± 33    | 9.8 ± 41       | 3.3 ± 20†      |
| Renin (uU/ml)#                    | 1.94 ± 0.59 | 1.87 ± 0.60 | 1.85 ± 0.57    | 2.11 ± 0.56††  |
| Angiotensin II (pg/ml)#           | 0.90 ± 0.65 | 0.89 ± 0.59 | 0.90 ± 0.67    | 1.12 ± 0.89 †† |
| PAC (pg/ml)#                      | 1.84 ± 0.27 | 1.68 ± 0.44 | 1.78 ± 0.28    | 2.11 ± 0.38††  |
| hsCRP (mg/dL)#                    | 0.04 ± 1.00 | 0.14 ± 0.75 | 0.35 ± 0.83    | 0.37 ± 0.69    |
| LVEF (%)                          | 72 ± 8      | 72 ± 7      | 69 ± 8         | 72 ± 9         |
| LV mass (g)                       | 110 ± 26    | 113 ± 28    | 119 ± 34       | 105 ± 30††     |
| LV mass Index (g/m <sup>2</sup> ) | 59.2 ± 11.3 | 58.9 ± 12.0 | 60.7 ± 13.5    | 53.8 ± 12.5††  |
| LV hypertrophy                    | 8           | 8           | 11             | 5              |
| LVEDV/BSA (ml/m <sup>2</sup> )    | 54 ± 11     | 55 ± 12     | 56 ± 13        | 53 ± 11        |
| LVSV/BSA (ml/m <sup>2</sup> )     | 39 ± 8      | 39 ± 8      | 38 ± 7         | 38 ± 8         |
| RVEDV/BSA (ml/m <sup>2</sup> )    | 64 ± 12     | 66 ± 14     | 66 ± 12        | 64 ± 12        |
| RV EF (%)                         | 61 ± 6      | 59 ± 6      | 59 ± 7         | 59 ± 6         |

Normally distributed values are presented as values are mean ± standard deviation; the remainder (#) were log transformed before comparison and are presented as median and inter quartile range. To compare changes in the two groups we used repeated measures analysis of variance with the time point (week 0, week 40) as the ‘within subjects’ factor and the group (spironolactone and placebo) as the ‘between subjects’ factor and tested the significance of the interaction between the two. † p < 0.05, †† p < 0.01.

ACR; urinary albumin-creatinine ratio, BSA; body surface area, EF; ejection fraction, PAC; plasma aldosterone concentration

**Figure 5.3** Change in left ventricular mass (g) and left ventricular mass index ( $\text{g}/\text{m}^2$ ) in patients treated with spironolactone and placebo



Values are mean  $\pm$  standard error of the mean

††  $p < 0.01$  treatment by time interaction, repeated measures ANOVA

LV mass index; LV mass / body surface area

#### **5.4.2.4 Changes in pulse wave velocity, aortic distensibility and augmentation**

Compared to placebo, spironolactone resulted in a significant fall in PWV (Table 5.5, Figure 5.4a), central aortic pressure augmentation, Aug Ix and AIX 75 (Table 5.5, Figure 5.4b).

Consistent with these changes, aortic distensibility increased with spironolactone compared to placebo (Table 5.5, Figure 5.4c). All of the changes in arterial stiffness remained significant after adjustment for the reduction in mean blood pressure that occurred with treatment (Table 5.5).

#### **5.4.2.5 Effect of changes in blood pressure on left ventricular mass and pulse wave velocity**

The possible effects of the reduction in blood pressure caused by spironolactone on the changes in LV mass and PWV were examined by determining the association of these changes with the changes in systolic pressure using multivariate regression models (Table 5.6). Independent variables known to influence LV mass and arterial stiffness were entered into the models. Only the change in central aortic systolic BP was a significant independent predictor of change in LV mass. The difference in the strength of the association between the reduction in central aortic and ambulatory 24 hour systolic pressures and change in LV mass is illustrated in Figure 5.5. When we added treatment with spironolactone to the model, the changes in systolic BP (including central systolic BP) were rendered insignificant.

In a model with PWV as the dependent variable, the change in central systolic BP ( $r^2 = 0.28$ ,  $p < 0.01$ ) and office systolic BP ( $r^2 = 0.28$ ,  $p < 0.01$ ) were independent predictors and remained significant after the addition of treatment with spironolactone to the models ( $r^2 = 0.33$ ,  $p < 0.01$ ,  $r^2 = 0.36$ ,  $p < 0.01$ ).

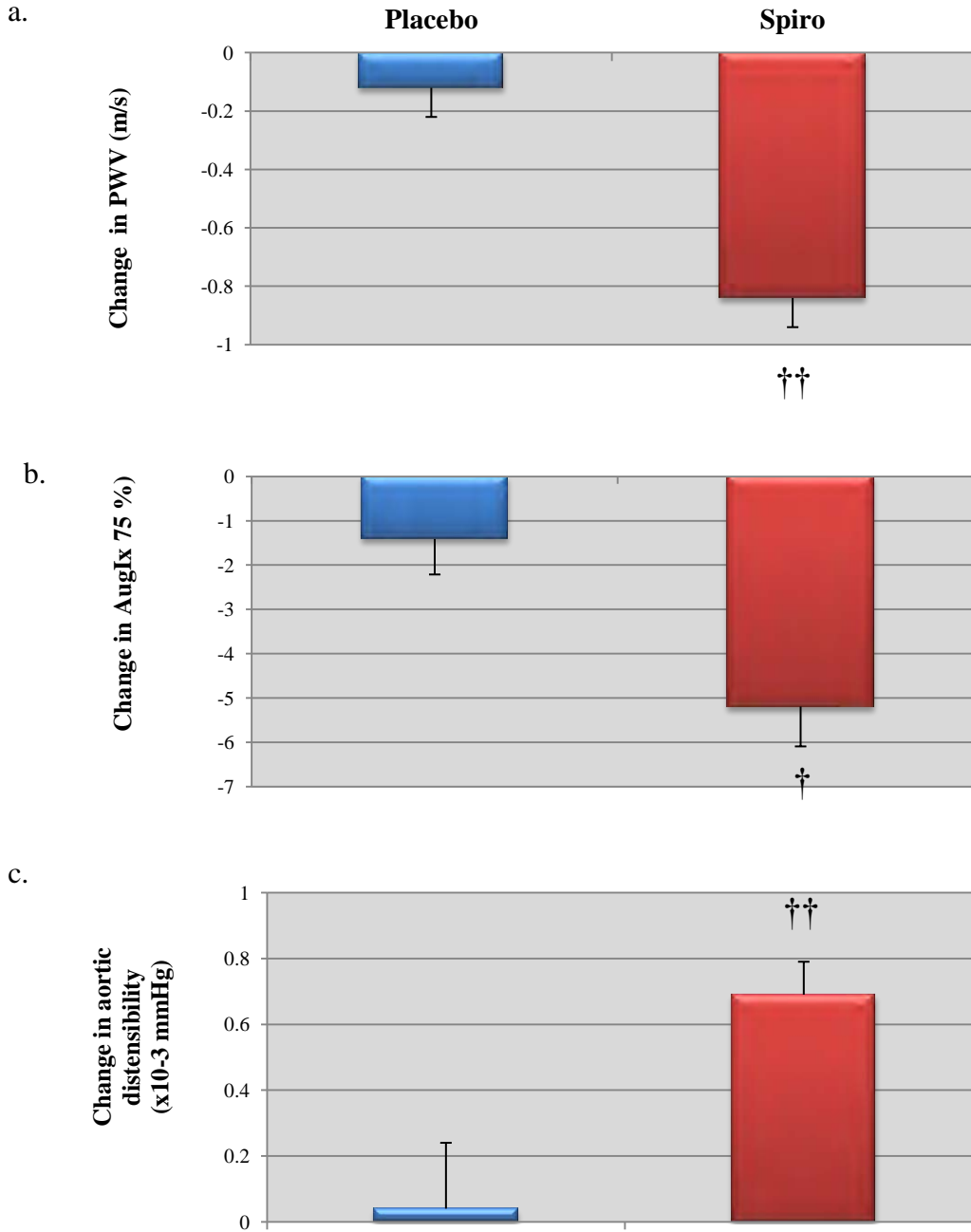
**Table 5.5.** Arterial stiffness values (absolute and adjusted for changes in mean arterial pressure over 40 weeks of treatment)

|                                                | Placebo     |             | Spironolactone |               |
|------------------------------------------------|-------------|-------------|----------------|---------------|
|                                                | Week 0      | Week 40     | Week 0         | Week 40       |
| PWV (m/s)                                      | 8.3 ± 1.7   | 8.1 ± 1.9   | 8.3 ± 1.6      | 7.5 ± 1.4††   |
| PWV <sub>adj</sub>                             |             | 8.3 ± 1.9   |                | 7.9 ± 1.5††   |
| Aortic Distensibility (x10 <sup>-3</sup> mmHg) | 2.3 ± 1.6   | 2.4 ± 1.6   | 2.6 ± 1.6      | 3.4 ± 1.9††   |
| Aortic Distensibility <sub>adj</sub>           |             | 2.4 ± 1.6   |                | 3.4 ± 1.8††   |
| Augmentation (mmHg)                            | 12.9 ± 8.8  | 12.4 ± 8.4  | 13.5 ± 5.9     | 10.2 ± 5.2 †† |
| Augmentation <sub>adj</sub>                    |             | 13.2 ± 8.1  |                | 11.7 ± 5.3 †† |
| Augmentation Index (%)                         | 28.3 ± 10.8 | 27.9 ± 10.1 | 30.8 ± 10.8    | 26.2 ± 9.5†   |
| Augmentation Index <sub>adj</sub>              |             | 28.2 ± 10.2 |                | 26.7 ± 9.7 †  |
| Augmentation Index 75 (%)                      | 25.1 ± 10.9 | 23.4 ± 10.1 | 25.6 ± 9.6     | 20.6 ± 9.2†   |
| Augmentation Index 75 <sub>adj</sub>           |             | 22.4 ± 10.5 |                | 19.3 ± 9.3††  |

Values are mean ± standard deviation. † p <0.05; †† p <0.01 treatment by time interaction, repeated measures ANOVA. Adjusted results (<sub>adj</sub>) are corrected for change in mean arterial pressure from week 0 to week 40. Adjustments were based on coefficients obtained from linear regressions using baseline data for both groups combined.

AIx 75; augmentation index standardised for a heart rate of 75 beats per minute

**Figure 5.4** Changes in (a) pulse wave velocity, (b) augmentation Index at 75 beats per minute (c) aortic distensibility on cardiac MRI in patients treated with spironolactone and placebo



Values are mean ± standard error of the mean. † p < 0.05; †† p < 0.01 treatment by time interaction, repeated measures ANOVA.

**Table 5.6** Multivariate regression models for the prediction of change in left ventricular mass

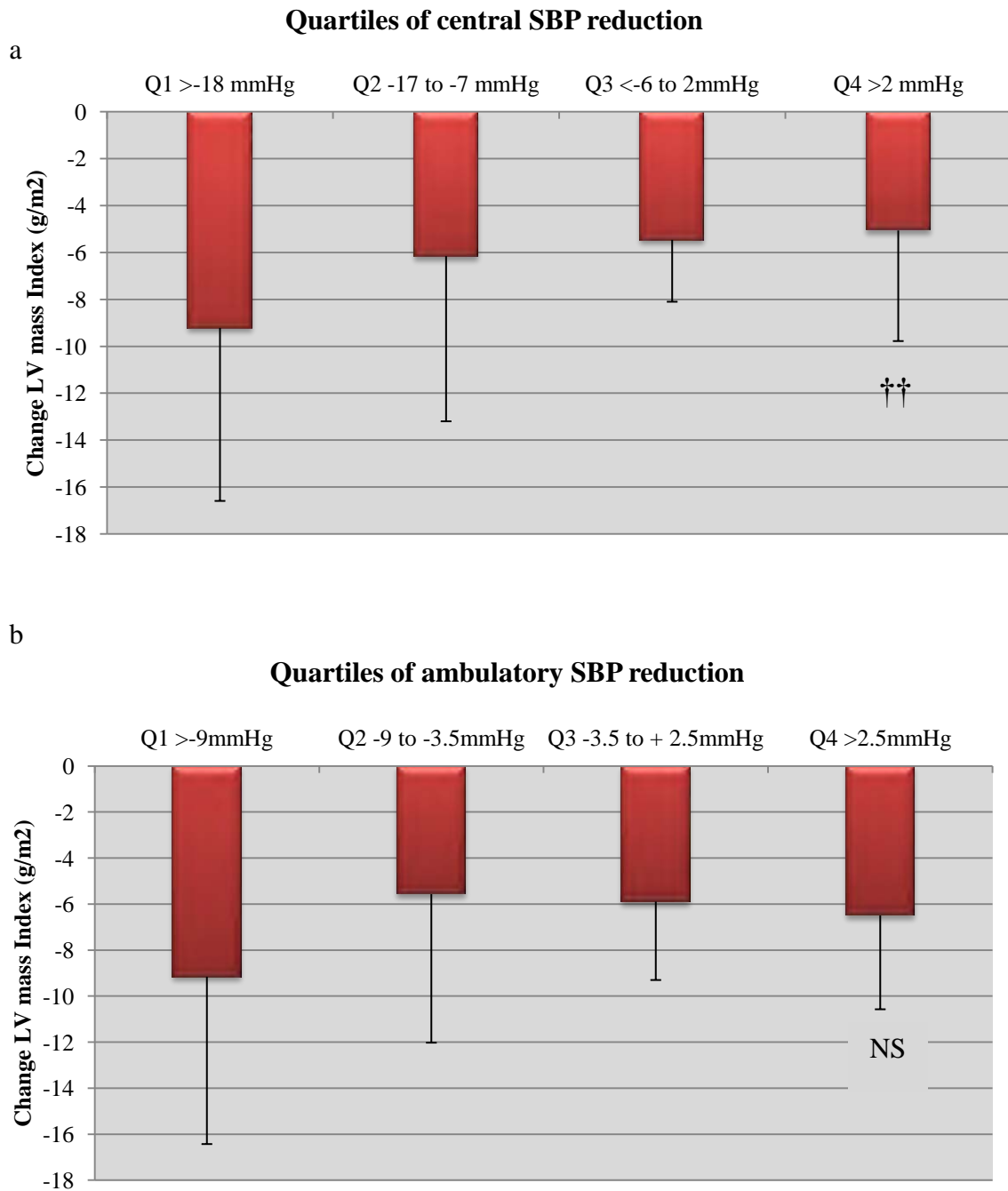
|                       |                  | Treatment not<br>included in model | Significance | Treatment effect<br>included in model | Significance |
|-----------------------|------------------|------------------------------------|--------------|---------------------------------------|--------------|
| <b>Model 1</b>        | $r^2$            | 0.16                               |              | 0.38                                  |              |
| <b>Change in BP</b>   | <i>Beta + SE</i> | 0.109 + 0.05                       | <0.05        | 0.065 + 0.044                         | 0.15         |
| <b>Spironolactone</b> | <i>Beta + SE</i> |                                    |              | -6.717 + 1.203                        | <0.01        |
| <b>Model 2</b>        | $r^2$            | 0.15                               |              | 0.38                                  |              |
| <b>Change in BP</b>   | <i>Beta + SE</i> | 0.1 + 0.051                        | 0.08         | 0.059 + 0.045                         | 0.19         |
| <b>Spironolactone</b> | <i>Beta + SE</i> |                                    |              | -6.778 + 1.202                        | <0.01        |
| <b>Model 3</b>        | $r^2$            | 0.13                               |              | 0.37                                  |              |
| <b>Change in BP</b>   | <i>Beta + SE</i> | 0.109 + 0.086                      | 0.21         | 0.027 + 0.076                         | 0.72         |
| <b>Spironolactone</b> | <i>Beta + SE</i> |                                    |              | -6.973 + 1.212                        | <0.01        |

**Model 1;** age, sex, GFR, ACE Inhibitor, Angiotensin Receptor Blockers, Calcium Channel Blocker Beta Blocker, Statins, change in central aortic SBP ± treatment group

**Model 2;** age, sex, GFR, ACE Inhibitor, Angiotensin Receptor Blockers, Calcium Channel Blocker Beta Blocker, Statins, change in office peripheral SBP ± treatment group

**Model 3;** age, sex, GFR, ACE Inhibitor, Angiotensin Receptor Blockers, Calcium Channel Blocker Beta Blocker, Statins, change in 24 hour ambulatory SBP ± treatment group

**Figure 5.5** Changes in left ventricular mass index associated with a) quartiles of central systolic blood pressure reduction, b) quartiles of ambulatory brachial systolic blood pressure reduction in patients randomised to spironolactone



Values are mean  $\pm$  standard deviation. ††  $p < 0.01$  trend between quartiles.

#### **5.4.2.6 Adverse effects**

During the 4 week open-label run in phase, one patient was withdrawn with serious hyperkalaemia (potassium 6.5 mmol/L), one patient was withdrawn with hypotension and acute deterioration in renal function (eGFR declined from 31 to 24 ml/min/1.73m<sup>2</sup>) and one patient withdrew consent. Six patients (5%) had potassium levels between 5.5 and 5.9 mmol/L and were changed to spironolactone on alternate days as per protocol.

On blinded treatment between weeks 4 and 40, four patients had potassium levels between 5.5 and 5.9 mmol/L requiring a dose reduction to alternate day treatment. Two of these four patients were found to have been on placebo after un-blinding. Following randomisation, no patients were withdrawn because of serious hyperkalaemia and there were no reported side effects including gynaecomastia. At week 40, serum potassium was slightly higher in the spironolactone group than in the placebo group ( $4.6 \pm 0.6$  mmol/L vs.  $4.4 \pm 0.4$  mmol/L,  $p < 0.05$ ).

#### **5.4.2.7 Reproducibility of measurements**

There was good intra-observer agreement for the primary end points: LV mass:  $r = 0.97$  ( $112 \pm 22$ g vs.  $115 \pm 22$ g,  $p < 0.01$ ), PWV:  $r = 0.92$  ( $7.2 \pm 1.6$  m/s vs.  $7.2 \pm 1.3$  m/s,  $p < 0.01$ ).



## **5.5 DISCUSSION**

This randomised trial has demonstrated that important structural and functional cardiovascular abnormalities present in early stage CKD (described in chapter 3) can be improved by mineralocorticoid receptor blockade. The addition of spironolactone to established treatment with ACE inhibitors or ARBs resulted in reduction in LV mass and improved arterial stiffness along with reduced blood pressure and albuminuria. These effects occurred despite excellent blood pressure control and a low prevalence of LVH at baseline. These findings provide support to published experimental data using remnant models of kidney disease (described in chapter 1) regarding the importance of aldosterone as a major cause of the development of ventricular hypertrophy and vascular and ventricular stiffness in patients with early stage CKD. They also suggest that aldosterone antagonism should be further evaluated in larger trials as a possible powerful therapeutic option in the treatment of this high risk group of patients. Such trials would of course have to examine carefully the safety of such treatment, most importantly the frequency of hyperkalaemia. This study was not large enough or of sufficient duration to provide reliable data on this adverse outcome but the incidence was surprisingly low. This may have been due to the active run in phase and the exclusion of patients with diabetic and renovascular CKD.

### **5.5.1 Cardiovascular disease in chronic kidney disease**

Although CKD is a major risk factor for atheromatous coronary artery disease, it is now evident from epidemiological studies that heart failure and arrhythmias, arising due to left ventricular hypertrophy and fibrosis, are the most common causes of cardiovascular morbidity and mortality (Herzog et al., 2008; Tonelli et al., 2006). Increased arterial stiffness is a major

factor in the development of left ventricular hypertrophy, fibrosis and ventricular dysfunction in CKD, diabetes and systolic hypertension (Kimoto et al., 2006;London et al., 2004;Mottram et al., 2005). Thus, the finding that spironolactone can improve these fundamental pathophysiological abnormalities is of importance and suggests that treatment commenced early in CKD may reduce the later burden of adverse cardiovascular events. Furthermore, there is increasing evidence that aldosterone causes progressive renal injury in CKD, thus the use of aldosterone antagonists might also retard the progression of renal disease (Bomback et al., 2008a;Remuzzi et al., 2008). The prevention of further decline in renal function may have secondary benefits in the development of cardiovascular disease in CKD as the magnitude of risk is related to GFR.

To date, beneficial effects of aldosterone antagonists on cardiovascular events and mortality have been observed in patient with heart failure, hypertension and hyperaldosteronism but not in patients with CKD (Catena et al., 2007;Mottram et al., 2005;Pitt et al., 1999;Pitt et al., 2003b). The use of aldosterone antagonists in CKD has been restricted due to concerns about adverse effects on serum potassium and renal function. Reported studies have been small without cardiovascular end points but have consistently shown reductions in proteinuria and slowing of progression to ESKD (Bomback et al., 2008a).

Reductions in LVH are associated with prognostic benefit in hypertension and CKD (Devereux et al., 2004;London et al., 2001). In the LIFE study which examined hypertensive patients with LV hypertrophy, a reduction in LV mass index of 11% over 12 months was associated with a 15% reduction in relative risk of cardiovascular events (Devereux et al., 2004). In patients with end stage CKD, a 10% reduction in left ventricular mass achieved by

multiple interventions was associated with a hazard ratio of 0.72 for cardiovascular death (London et al., 2001). Patients in this study had an approximate 10% reduction in left ventricular mass using spironolactone in combination with an ACE inhibitor or an ARB despite low rates of LVH (17%) and well controlled blood pressure on entry. These data are consistent with published reports demonstrating additive reductions in LV mass and blood pressure using combination treatment with MRBs and ACE inhibitor in subjects with LVH due to hypertension (Pitt et al., 2003a).

In the CRIB-II study, patients had systolic blood pressure within the normal range and a low prevalence of LVH, yet spironolactone produced additional reductions in LV mass. These findings are consistent with data in patients with treated hypertension considered within the normal range and LVH, where an additional -9 mmHg reduction in systolic pressure produced a  $5\text{g/m}^2$  reduction in LV mass index on cardiac MRI (Simpson et al., 2010). These data suggest that potentially important additional reductions in LV mass can be achieved through extra blood pressure lowering in individuals with end-organ damage. The mechanism of LV mass regression is uncertain but might be contributed to by mechanisms other than blood pressure as the relationship between the two variables becomes flatter at non-hypertensive blood pressures (Fraser, 2003). One potential contributory factor is arterial stiffness, a strong prognostic marker in end-stage CKD, hypertension and the general population (Blacher et al., 1999a; Blacher et al., 1999b; Willum-Hansen et al., 2006). In our study indices of arterial stiffness including PWV, aortic distensibility and augmentation index were all improved with spironolactone. These changes appear independent of the change in blood pressure and might have contributed to the reduction in LV mass.

### 5.5.2 The effect of blood pressure on cardiovascular structure and function

An important question raised by this study is the degree to which the reductions in LV mass and arterial stiffness (which remained significant after mathematical correction for the mean arterial pressure at which they were measured) were due to the effect of lowering systolic blood pressure relative to the direct effects of mineralocorticoid receptor blockade. The use of an inactive placebo rather than a control anti-hypertensive agent means that it is not possible to provide a definitive answer to this question. The significant relationships between change in LV mass and PWV and the reduction in central aortic systolic blood pressure suggest that this might be at least part of the mechanism of action of spironolactone. The blood pressure effects were much weaker than the treatment effect so it is plausible that blockade of cardiac and vascular mineralocorticoid receptors reduced adverse effects of aldosterone such as inflammation, fibrosis and hypertrophy (Rocha & Stier, Jr., 2001). It is also possible that ACE-2 activity may have increased under the influence of spironolactone leading to an increase in Ang (1-7) which has vasodilatory, anti-fibrotic and hypertrophic effects (Mercure et al., 2008). Indeed, it is possible that the reduction in systolic pressure that occurred with spironolactone was a result rather than a cause of reduced arterial stiffness. In support of this theory is the finding that eplerenone treatment in hypertension resulted in a reduction in the collagen/elastin ratio and *in vitro* arterial stiffness of resistance arteries in hypertensive patients (Savoia et al., 2008). The lack of effect of spironolactone on CRP provides no support for an anti-inflammatory effect but does not exclude local effects on vascular inflammation. The significant associations between central aortic but not ambulatory blood pressure changes and the improvements in LV mass and PWV are consistent with recent work showing that central but not peripheral pressures are determinants of clinical outcome (Williams et al., 2006).

### **5.5.3 Limitations**

This study does not address whether maximizing doses of ACE inhibitors or ARBs would be as effective as adding 25mg daily of spironolactone to standard therapy but all the patients were on doses of ACE Inhibitors and ARBs that achieved blood pressure control for at least 6 months prior to recruitment. The dose of 25mg of spironolactone was chosen because this low dose is efficacious and safe in heart failure (when GFR is often reduced) (Pitt et al., 1999) and because tolerability was demonstrated in several small studies in CKD (Bomback et al., 2008a; Bomback et al., 2008b). Peripheral blood pressure was used for the calculation of aortic distensibility. It might have been more appropriate to use central aortic blood pressure (measured by applanation tonometry) as this is the pressure ‘seen’ by the left ventricle and aorta but due to the constraints of magnetic resonance technology it was not possible to acquire this data at the time of imaging.

### **5.5.4 Conclusion**

In patients with early stage CKD spironolactone resulted in improvements in important prognostic markers of cardiovascular disease; most of these effects were statistically independent of the change in blood pressure. These data provide strong support for the further evaluation of spironolactone in patients with CKD in trials with clinical outcomes.

## **CHAPTER 6**

### **THE EFFECT OF SPIRONOLACTONE ON LEFT VENTRICULAR SYSTOLIC AND DIASTOLIC FUNCTION IN PATIENTS WITH EARLY STAGE CHRONIC KIDNEY DISEASE**

Published by NC Edwards, CJ Ferro, H Kirkwood, CD Chue, AA Young, PM Stewart, RP Steeds, JN Townend. Effect of Spironolactone on Left Ventricular Systolic and Diastolic Function in Patients with Early Stage Chronic Kidney Disease. *Am J Cardiol.* 2010, Nov 15;106 (10):1505-11.

## 6.1 SUMMARY

Aldosterone levels are elevated in chronic kidney disease (CKD) and might impair ventricular function through adverse myocardial and vascular pro-inflammatory and fibrotic effects. In the Chronic Renal Impairment in Birmingham II study (CRIB-II) it was hypothesised that mineralocorticoid receptor blockade with spironolactone in addition to ACE Inhibitors or Angiotensin Receptor Blockers, would improve left ventricular (LV) function and markers of inflammation, ventricular stretch and collagen turnover in early CKD.

A total of 112 patients with early CKD were randomised to spironolactone 25mg once a day or placebo for 40 weeks. Left ventricular function was assessed by echocardiography and cardiac MRI tagging. High sensitivity C reactive protein, N-terminal pro-B-type natriuretic peptide (NT-proBNP) and aminoterminal propeptide of type III procollagen (PIIINP) were measured.

Spironolactone improved LV long axis systolic function (Sm  $8.2 \pm 1.4$  cm/s vs.  $7.7 \pm 1.3$  cm/s,  $p < 0.05$ ), torsion ( $7.77 \pm 1.61$  vs.  $6.77 \pm 1.48$ ,  $p < 0.05$ ) and myocardial deformation (strain rate  $-1.14 \pm 0.24$  s<sup>-1</sup> vs.  $-1.09 \pm 0.20$  s<sup>-1</sup>,  $p < 0.05$ ) compared with placebo, without a change in ejection fraction. Markers of LV filling ( $E/e'$   $7.2 \pm 2.3$  vs.  $8.5 \pm 2.3$ ,  $p < 0.05$ ) and suction (M-mode propagation velocities  $56 \pm 12$  cm/s vs.  $50 \pm 12$  cm/s,  $p < 0.05$ ) were also improved. Spironolactone reduced NT-proBNP (24.8 (0.4-122.4 (pmol/L)) vs. 39.4 (10.8-102.4 (pmol/L)),  $p < 0.01$ ) and attenuated an increase in PIIINP observed with placebo.

In conclusion, spironolactone improved markers of regional LV systolic and diastolic function in early CKD.

## 6.2 INTRODUCTION

In chapter 5 it was demonstrated that the addition of spironolactone to established treatment with ACE inhibitor or Angiotensin Receptor Blockers (ARBs) in patients with early stage chronic kidney disease (CKD) resulted in a reduction in left ventricular (LV) mass and improved arterial stiffness and aortic distensibility. In a further analysis of prospectively defined secondary end-points from the Chronic Renal Impairment in Birmingham II (CRIB-II) study, the effect of spironolactone on LV function and on serological markers of inflammation, ventricular stress and collagen turnover were examined.



## **6.3 METHODS**

### **6.3.1 Study design, participants and treatment regimen**

The study design, clinical characteristics of patients and treatment regimen used in CRIB-II have been described in chapter 5.

### **6.3.2 Clinical assessments**

All patients underwent clinical assessments as described in chapter 3 at baseline / week 0 and at week 40 prior to stopping treatment. Detailed methodology is described in chapter 2.

In this pre-defined sub study of secondary end-points, the following specific parameters are reported;

1. Transthoracic echocardiography for assessment of:
  - i) LV volumes
  - ii) LV mass determined by 2D area length formula and indexed to body surface area (LVMI  $\text{g}/\text{m}^2$ )
  - iii) LV systolic function
  - iv) LV diastolic function
  - v) Myocardial deformation using 2D speckle tracking
  - vi) Arterial-ventricular interaction
2. Cardiac MRI:
  - i) Myocardial tagging
3. Plasma / serum biomarkers:
  - i) N-terminal pro-B-type natriuretic peptide (NT-proBNP)

- ii) High-sensitivity C-reactive protein (hsCRP)
- iii) Intact aminoterminal propeptide of type III procollagen (PIIINP)
- iv) Carboxyterminal telopeptide of type I collagen (CTx)

### **6.3.3 Statistical analysis**

Statistical analyses were performed as described in Chapter 2. Multivariate regression was used to identify the independent predictors of the change in LV function. The model included; treatment group, change in systolic blood pressure, age, medication usage and eGFR. Intra-observer variability for the major parameters of ventricular function was assessed using interclass correlation coefficient (ICC) with a 95% confidence interval as described in section 2.3.4.

## 6.4 RESULTS

### 6.4.1 Patient demographics

Baseline demographic and clinical characteristics are presented in Table 5.2.

### 6.4.2 Echo data

Twenty one patients (9 in the spironolactone group) were excluded due to poor image quality. Treatment groups were matched for LV geometry (regional wall thickness (RWT)  $0.35 \pm 0.06$  vs.  $0.32 \pm 0.06$ ,  $p = 0.07$ ), volumes (LVED volume index  $46 \pm 12$  ml/m<sup>2</sup> vs.  $46 \pm 9$  ml/m<sup>2</sup>,  $p = 0.23$ ) and mass index ( $88 \pm 20$  g/m<sup>2</sup> vs.  $91 \pm 22$  g/m<sup>2</sup>,  $p = 0.15$ ). After treatment with spironolactone, there was a fall in LV mass index ( $-8.9$  g/m<sup>2</sup>  $\pm 1.6$  vs.  $2.2$  g/m<sup>2</sup>  $\pm 1.7$ ,  $p < 0.01$ ) but no change in LVEDV or RWT. These data are consistent with the results using cardiac MRI (data presented in chapter 5).

Measures of LV filling pressure were within normal limits ( $E/V_p < 1.9$ ,  $Ar-A < 30$  ms,  $LA$  volume index  $< 34$  ml/m<sup>2</sup>,  $E/Em < 8$ ) in both groups at baseline (Table 6.1). Following treatment with spironolactone, markers of LV filling pressure were improved; reduced pulmonary atrial velocity duration – transmitral atrial duration ( $Ar-A$ ), reduced left atrial volume index ( $LAVI$ ) and reduced ratio of mitral inflow velocity to early diastolic myocardial velocities ( $E/Em$ ). Markers of LV relaxation were also improved; attenuation of isovolumic relaxation time ( $IVRT$ ), increased early myocardial velocities ( $Em$ ) and increased m-mode propagation velocity (an approximation of the intraventricular pressure gradient and hence LV suction) (Table 6.1 and Figure 6.1).

Treatment with spironolactone increased systolic ( $S_m$ ) tissue velocities but did not change ejection fraction. Longitudinal systolic strain ( $S$ ) in each wall was significantly

increased resulting in a global improvement in LV deformation. Global longitudinal systolic strain rate (SR) was also improved with spironolactone (Table 6.1).

Arterial elastance, end-systolic and diastolic elastance were all reduced with spironolactone. The arterial–ventricular coupling ratio was maintained in both groups (Table 6.1).

### **6.4.3 Cardiac MRI tagging data**

Patients randomised to CMR tagging were younger ( $48 \pm 10$  yrs vs.  $58 \pm 11$  yrs,  $p < 0.01$ ) but did not differ in any other characteristic from the other patients recruited to the study.

Treatment groups in the tagging study were well matched for age ( $48\text{yrs} \pm 12$  vs.  $46\text{yrs} \pm 8$ ,  $p = 0.34$ ), males (30% vs. 33%), eGFR ( $54 \pm 13$  ml/min/1.73m<sup>2</sup> vs.  $53 \pm 14$  ml/min/1.73m<sup>2</sup>,  $p = 0.86$ ) and blood pressure ( $123 \pm 13$  mmHg vs.  $125 \pm 9$  mmHg,  $p = 0.68$ ). Two data sets were excluded due to poor image quality. Systolic S and SR were significantly improved with spironolactone, consistent with the changes documented on 2D echocardiography (Table 6.3; Figure 6.2). In addition, diastolic SR was improved consistent with a reduction in diastolic ventricular stiffness. Left ventricular peak apical rotation and left ventricular peak torsion were both increased after treatment with spironolactone while basal rotation remained unchanged (Table 6.3).

**Table 6.1** Measurements of left ventricular function

|                                                                        | <b>Placebo (n=55)</b> |              | <b>Spironolactone (n=55)</b> |               |
|------------------------------------------------------------------------|-----------------------|--------------|------------------------------|---------------|
|                                                                        | Week 0                | Week 40      | Week 0                       | Week 40       |
| <b>Systolic Function</b>                                               |                       |              |                              |               |
| Left ventricular ejection fraction (%)                                 | 63 ± 7                | 66 ± 7       | 63 ± 6                       | 66 ± 7        |
| Tissue Doppler systolic velocity (cm/s)                                | 7.6 ± 1.4             | 7.7 ± 1.3    | 7.5 ± 1.5                    | 8.2 ± 1.4†    |
| Global peak strain (%)                                                 | -17.8 ± 3.0           | -17.9 ± 2.4  | -17.3 ± 3.2                  | -19.7 ± 2.7†† |
| Global peak strain rate (s <sup>-1</sup> )                             | -1.01 ± 0.18          | -1.09 ± 0.20 | -1.02 ± 0.23                 | -1.14 ± 0.24† |
| <b>Diastolic Function; LV Filling Pressures</b>                        |                       |              |                              |               |
| Transmitral early velocity / m-mode flow propagation velocity          | 1.54 ± 0.6            | 1.49 ± 0.5   | 1.40 ± 0.4                   | 1.27 ± 0.4    |
| Pulmonary atrial velocity duration – trans mitral atrial duration (ms) | -21 ± 23              | -25 ± 25     | -24 ± 18                     | -17 ± 23†     |
| Left atrial volume index (ml/m <sup>2</sup> )                          | 27 ± 6                | 26 ± 6       | 28 ± 5                       | 24 ± 6††      |
| Transmitral E / average annular early myocardial TDI ratio             | 8.9 ± 2.6             | 8.5 ± 2.3    | 8.7 ± 2.0                    | 7.2 ± 2.3††   |
| <b>Diastolic Function ;LV Relaxation</b>                               |                       |              |                              |               |
| Ratio of early to late mitral inflow velocities (E/A)                  | 1.17 ± 0.29           | 1.07 ± 0.32  | 1.08 ± 0.3                   | 1.09 ± 0.29   |
| Deceleration time (ms)                                                 | 223 ± 50              | 235 ± 42     | 216 ± 50                     | 227 ± 38      |
| Isovolumic relaxation time (ms)                                        | 93 ± 14               | 102 ± 15     | 97 ± 14                      | 95 ± 15†      |

|                                                                     |             |             |             |               |
|---------------------------------------------------------------------|-------------|-------------|-------------|---------------|
| Mean septal / lateral annular early myocardial TDI velocities(cm/s) | 8.4 ± 2.4   | 8.8 ± 2.5   | 8.1 ± 2.0   | 9.7 ± 2.3††   |
| M-mode flow propagation velocity (cm/s)                             | 50 ± 13     | 50 ± 12     | 50 ± 15     | 56 ± 12†      |
| <b>Ventricular -Vascular Interaction</b>                            |             |             |             |               |
| Arterial elastance (mm Hg/ml)                                       | 1.76 ± 0.45 | 1.69 ± 0.38 | 1.79 ± 0.56 | 1.43 ± 0.37†† |
| End systolic elastance (mm Hg/ml)                                   | 2.42 ± 0.79 | 2.45 ± 0.71 | 2.37 ± 0.76 | 1.98 ± 0.59†† |
| End diastolic elastance                                             | 0.12 ± 0.04 | 0.12 ± 0.46 | 0.11 ± 0.04 | 0.09 ± 0.39†  |
| Ratio of Arterial elastance / End systolic elastance                | 0.76 ± 0.20 | 0.73 ± 0.21 | 0.79 ± 0.22 | 0.74 ± 0.17   |

Values are mean ± standard deviation

† p < 0.05, †† p < 0.01 (treatment group\* time interaction in the repeated measures analysis of variance after adjustment for baseline differences in age, sex, eGFR, blood pressure, heart rate, medications, NT-proBNP)

Abbreviations;

Transmitral early velocity (E)

Transmitral early velocity / m-mode flow propagation velocity (E/Vp)

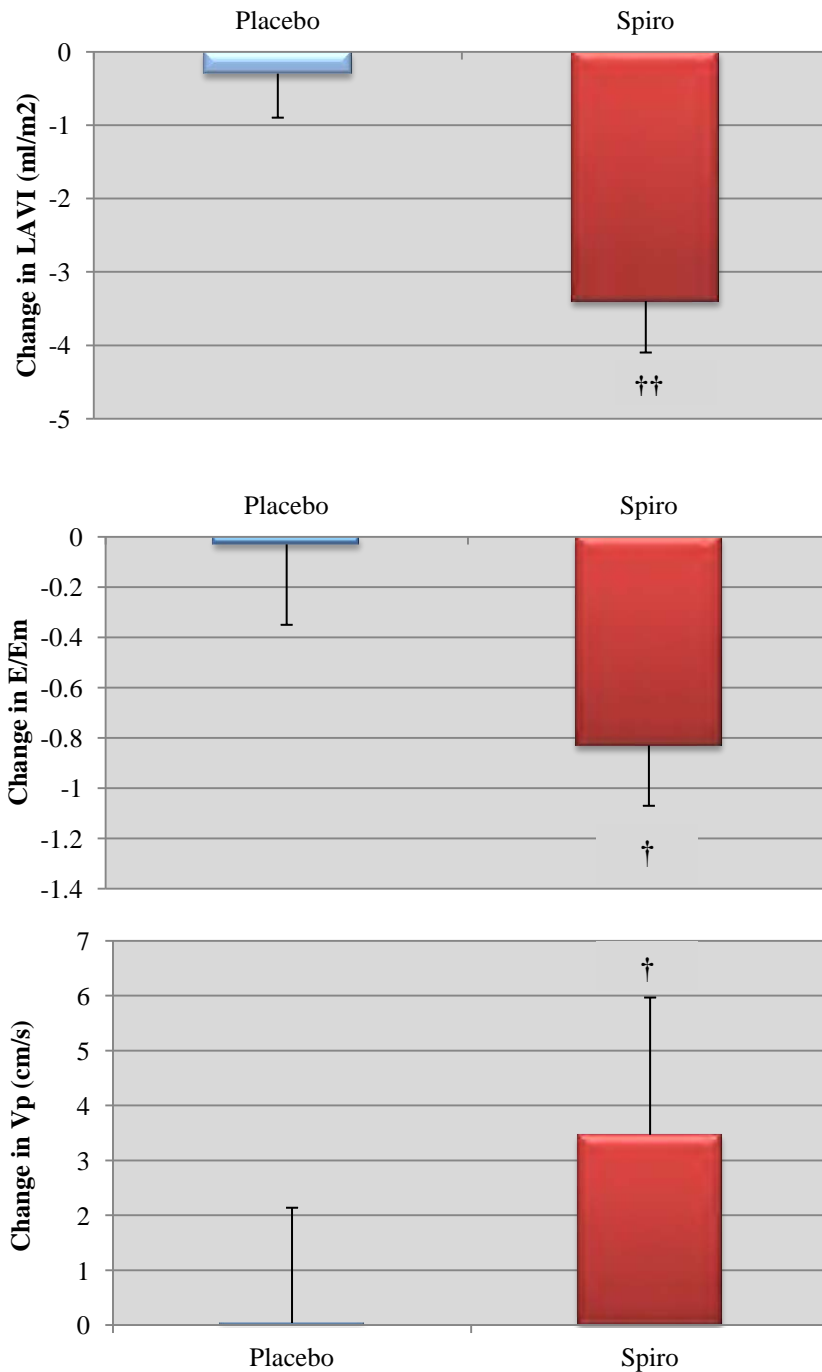
Pulmonary atrial velocity duration – transmitral atrial duration (Ar-A)

Left atrial volume index (LAVI)

Mean septal and lateral annular early myocardial TDI velocities (E/Em)

Transmitral E / average annular myocardial TDI ratio (E/Em)

**Figure 6.1** Changes in markers of left ventricular function: a) left atrial volume, b) ratio of transmitral early filling velocity and early myocardial diastolic velocity, c) m-mode flow propagation velocity



Values are mean  $\pm$  standard error of the mean. †  $p < 0.05$ , ††  $p < 0.01$

LAVI; left atrial; volume index, E/Em; ratio of transmitral early filling velocity and early myocardial diastolic velocity (lateral annulus), Vp; m-mode flow propagation velocity.

**Table 6.2** Cardiac MRI tagging parameters

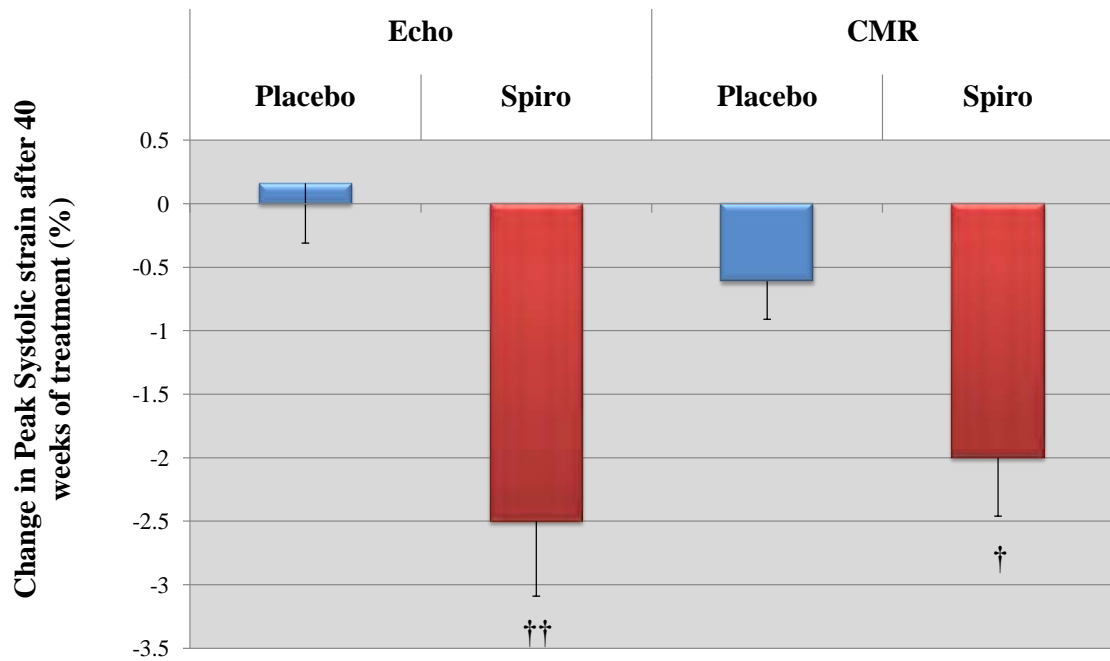
| Variable                                | Placebo (n=18) |              | Spironolactone (n=20) |               |
|-----------------------------------------|----------------|--------------|-----------------------|---------------|
|                                         | Week 0         | Week 40      | Week 0                | Week 40       |
| Peak Strain (%)                         | -14.8 ± 2.2    | -15.4 ± 2.0  | -13.7 ± 1.8           | -15.7 ± 1.9†  |
| Systolic Strain Rate (s <sup>-1</sup> ) | -85 ± 18       | -82 ± 11     | -78 ± 13              | -88 ± 11      |
| Diastolic SR % s <sup>-1</sup>          | 60 ± 24        | 54 ± 30      | 57 ± 17               | 66 ± 25†      |
| Apical Rotation (°)                     | 10.87 ± 5.75   | 9.98 ± 5.02  | 11.13 ± 5.12          | 14.21 ± 5.95† |
| Basal Rotation (°)                      | -5.05 ± 2.99   | -5.06 ± 1.92 | -5.38 ± 2.72          | -4.91 ± 2.44  |
| Peak Torsion (°)                        | 7.28 ± 1.40    | 6.77 ± 1.48  | 7.34 ± 1.85           | 7.77 ± 1.61†  |

Values are mean ± standard deviation

† p < 0.05, †† p < 0.01 (treatment group\* time interaction in the repeated measures analysis of variance after adjustment for baseline differences)



**Figure 6.2** Change in peak systolic strain measured by echo and cardiac MRI over 40 weeks of treatment



Values are mean  $\pm$  standard error of the mean

†  $p < 0.05$ ; ††  $p < 0.01$  treatment by time interaction, repeated measures ANOVA

#### **6.4.4 Biomarker analysis**

There were no differences in plasma renin activity (PRA), circulating angiotensin II (AngII) or plasma aldosterone concentration (PAC) (Table 6.3) or biomarkers at baseline (Table 6.3). Estimated GFR was inversely correlated with PAC ( $r = -0.33$ ,  $p < 0.01$ ), PIIINP ( $r = -0.52$   $p < 0.01$ ), hsCRP ( $r = -0.191$   $p = 0.04$ ) and NT-proBNP ( $r = -0.220$   $p = 0.03$ ) but not with CTx. At week 40, NT-pro-BNP was significantly reduced after treatment with spironolactone ( $-24.8 \pm 13.5$  vs.  $+12.5 \pm 13.4$ ,  $p < 0.01$ ) (Figure 6.3). PIIINP increased significantly in the placebo group but was attenuated with spironolactone ( $+0.2 \pm 0.1$  vs.  $-0.01 \pm 0.01$ ,  $p = 0.01$ ). There was no difference in post treatment levels of CTx or hsCRP. As expected, PRA, AngII and PAC all increased with spironolactone (Table 6.3).

#### **6.4.5 Haemodynamic effects**

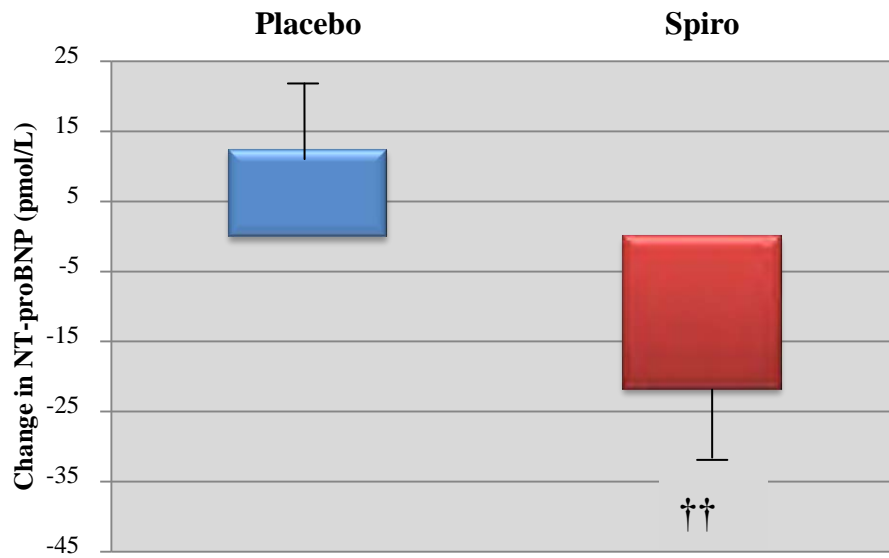
Twenty four hour daytime systolic blood pressure ( $-6 \pm 1$  mmHg vs.  $-1 \pm 1$  mmHg,  $p = 0.01$ ) was decreased with spironolactone but there was no difference in diastolic pressures. In multivariate regression examining the independent predictors of changes in markers of LV systolic and diastolic function, only treatment with spironolactone was predictive of all changes except m-mode propagation velocity, after adjusting for other variables in the model (Table 6.4). The change in systolic blood pressure was not predictive of any changes in LV function (Table 6.4).

#### **6.4.6 Intra-operator variability**

The repeated analysis of major parameters of ventricular function showed good intra-observer reproducibility (Intra-class correlation coefficient (ICC) with a 95% confidence interval; global LV strain ICC = 0.96 (95% CI 0.86-0.99), global LV strain rate ICC = 0.93 (0.75-

0.98), long-axis Sm at the lateral annulus ICC = 0.99 (0.97-0.99), LA volume index ICC 0.94 (0.79-0.99) and m-mode propagation velocity ICC 0.96 (0.87-0.99).

**Figure 6.3** Change in N-terminal pro-B-type natriuretic peptide over 40 weeks of treatment



Values are mean  $\pm$  standard error of the mean

††  $p < 0.01$

NT-proBNP; N-terminal pro-B-type natriuretic peptide

**Table 6.3** Changes in biomarkers of collagen, inflammation and left ventricular stress over 40 weeks of treatment with spironolactone or placebo

|                                                                   | Placebo          |                  | Spironolactone    |                               |
|-------------------------------------------------------------------|------------------|------------------|-------------------|-------------------------------|
|                                                                   | Week 0           | Week 40          | Week 0            | Week 40                       |
| Aminoterminal propeptide type III procollagen ( $\mu\text{g/L}$ ) | 3.73 $\pm$ 0.98  | 4.16 $\pm$ 1.19  | 4.01 $\pm$ 0.77   | 4.00 $\pm$ 0.79 <sup>††</sup> |
| Carboxyterminal telopeptide type I collagen (ng/mL)               | 0.20 (0.13-0.34) | 0.22 (0.13-0.34) | 0.22 (0.15-0.36)  | 0.26 (0.13-0.48)              |
| High sensitivity C-reactive protein (mg/L)                        | 1.1 (0.4-4.3)    | 1.4 (0.5-2.5)    | 2.2 (0.6-4.1)     | 2.3 (0.9-4.6)                 |
| N-terminal pro-B-type natriuretic peptide (pmol/L)                | 25.7 (2.7-72.9)  | 39 (2.8-72.0)    | 62.1 (10.8-130.8) | 25 (0.6-132) <sup>††</sup>    |

Values are mean  $\pm$  standard deviation. Log transformed data are median (IQR)

<sup>†</sup> p < 0.05, <sup>††</sup> p < 0.01.

**Table 6.4** Predictive effects of treatment with spironolactone and changes in systolic blood pressure on markers of left ventricular function in multivariate regression

|                                                | Spironolactone |                           | Change 24 hour systolic blood pressure |                           |
|------------------------------------------------|----------------|---------------------------|----------------------------------------|---------------------------|
|                                                | p value        | $\beta$ -Coefficient (SE) | p value                                | $\beta$ -Coefficient (SE) |
| Global strain                                  | 0.01           | -2.7 (0.77)               | 0.62                                   | 0.02 (0.05)               |
| Global strain rate                             | 0.04           | -1.44 (0.070)             | 0.19                                   | 0.006 (0.004)             |
| Change in E/Em ratio                           | 0.04           | -0.87 (0.42)              | 0.33                                   | 0.02 (0.02)               |
| Change left atrial volume index                | 0.001          | -3.60 (1.02)              | 0.85                                   | -0.12 (0.06)              |
| Change M-mode propagation velocity             | 0.65           | 1.61 (3.56)               | 0.90                                   | -0.26 (0.21)              |
| Change in average TDI Sm myocardial velocities | 0.02           | -2.02 (0.84)              | 0.56                                   | 0.03(0.05)                |

Included in the model; treatment group, change systolic blood pressure, age, baseline eGFR, use of medications; ACE inhibitor, ARB, beta blocker, calcium channel blocker, diuretic, statin.

Spironolactone was the only parameter in the model which was an independent predictor of the echo markers.

$\beta$ -coefficient; unstandardised  $\beta$ -coefficient, E/Em; ratio of early transmitral inflow velocity to early diastolic myocardial velocity, TDI Sm; lateral annular systolic tissue Doppler velocity, SE; Standard Error.

## **6.5 DISCUSSION**

Using the complimentary imaging techniques of echo and cardiac MRI tagging, this study has shown that abnormalities of myocardial systolic and diastolic LV function present in patients with early stage CKD were improved by the addition of spironolactone to standard therapy including ACE inhibitors and ARBs. These data were prospectively defined secondary endpoints from the CRIB-II double blind randomised placebo controlled trial (described in chapter 5) which showed benefits of spironolactone in reducing LV mass and improving markers of arterial stiffness. The improvements in LV function were accompanied by a fall in NT-proBNP, a peptide hormone released from ventricular myocytes in response to stretch, further confirming the beneficial effects of spironolactone on cardiac performance in CKD.

### **6.5.1 Changes in cardiac function in chronic kidney disease**

Left ventricular dilatation, hypertrophy and impaired systolic function, a combination frequently termed ureamic cardiomyopathy, are strong predictors of an adverse cardiovascular prognosis (Foley et al., 1995b). In chapter 3 and 4 it was shown that in patients with early stage CKD diastolic relaxation was impaired, arterial and ventricular elastance were increased and systolic deformation decreased compared to healthy controls. These changes might reflect an increase in myocardial fibrosis and collagen content and were present before detectable change in LV geometry, mass and systolic function using conventional measurements. This hypothesis is supported by data from Salvetti et al (2007) using acoustic densitometry (integrated backscatter), an ultrasound derived technique which detects increasing backscatter reflectivity as myocardial collagen content and fibrosis increase (Salvetti et al., 2007). Compared to patients with hypertension with normal renal function, backscatter was increased

even in patients with mild CKD and increased progressively with advancing stages, being greatest in the dialysis patients. Furthermore cyclic variation of backscatter, a marker of LV contractile performance was reduced at all stages of CKD and was inversely correlated with fibrosis.

Many of the parameters of LV filling, LV relaxation and myocardial deformation which were improved after treatment with spironolactone have been shown to be of prognostic value. For example, LA volume index is independently predictive of mortality in patients with CKD (Chan et al., 2008) and predictive of the onset of cardiac failure in patients with apparently normal systolic function (Takemoto et al., 2005). Similarly, early myocardial diastolic velocities provide significant incremental value for predicting cardiovascular mortality and heart failure onset, compared to both ejection fraction and systolic velocities (Wang et al., 2005a). Abnormalities in strain rate are detectable early in heart disease due to both hypertension (Mottram et al., 2004) and CKD and this sensitive index of systolic function is of prognostic value in dialysis dependent patients (Rakhit et al., 2007).

### **6.5.2 Changes in cardiac biomarkers with spironolactone**

Brain-type natriuretic peptide (BNP) is a hormone secreted in the ventricular myocardium due to increased ventricular stretch and wall-tension. After secretion it is split into the biologically active peptide and the more stable amino terminal prohormone fragment (N-BNP or NT-proBNP) and plays an important role in the regulation of blood pressure, blood volume, and sodium balance. The observed reduction in NT-proBNP with spironolactone in this study provides powerful evidence of a significant improvement in central hemodynamics and is also suggestive of a prognostically important effect. The use of BNP as a marker of outcome in heart failure due to left ventricular systolic dysfunction is well described (Berger et al., 2002)



but it has also been shown to predict mortality independent of LV mass in end stage CKD (Zoccali et al., 2001) patients on peritoneal dialysis (Wang et al., 2007) and in pre-dialysis CKD (Vickery et al., 2008).

### **6.5.3 A role for mineralocorticoid receptor blockers in chronic kidney disease?**

There are no other published data on the effect of MRB therapy on cardiovascular function in patients with early CKD. Mineralocorticoid receptor blockade with eplerenone has however recently been shown to exert beneficial effects on markers of diastolic function in patients with heart failure with preserved ejection fraction (HFpEF) (Mak et al., 2009). These data are highly relevant to patients with CKD who have abnormalities of myocardial and arterial stiffness which closely resemble those of HFpEF (Lam et al., 2007). Furthermore, in patients with hypertension, treatment with spironolactone improves both diastolic function and systolic function assessed by strain and strain rate, possibly independently of blood pressure (Mottram et al., 2004). Thus, there is consistent evidence that MR blockade may be an effective method of improving LV function in patients with increased ventricular and arterial stiffness.

There is a wide body of experimental evidence showing that the pro-inflammatory and pro-fibrotic effects of aldosterone on the heart, vasculature and kidneys can be prevented by MR blockade (Rocha & Stier, Jr., 2001). In this study, the increase in the collagen synthesis marker PIIINP observed in the placebo group after 40 weeks of treatment did not occur in the spironolactone treatment group. This finding was in keeping with reports showing that eplerenone reduced markers of collagen turnover after myocardial infarction (Hayashi et al., 2003) and prevented a progressive increase in PIIINP over a 12 month treatment period in

patients with HFpEF (Mak et al., 2009). Indeed, effects on collagen metabolism may be used to identify patients who respond to treatment with aldosterone blockade (Zannad et al., 2000).

#### **6.5 4 Limitations**

Changes in afterload caused by spironolactone were evident from the reductions in blood pressure and arterial elastance but did not predict the major echo changes in regression analysis. However, the relative contribution of load-related effects and sustained myocardial and vascular effects from the tissue action of MRBs cannot be determined from the results of this study. Furthermore, it is recognised that sustained preload reduction due to increased sodium and water excretion as a result of MR blockade may also have exerted significant effects on myocardial performance. Studies performed early after discontinuation of spironolactone might have helped to elucidate these effects.

Measurement of other markers of collagen turnover (such as aminoterminal propeptide of type I procollagen (PINP)) or inflammation (such as TNF- $\alpha$ ) might have provided further information on the effect of spironolactone on cardiac and vascular inflammation. The higher use of statins and beta blockers in patients randomised to spironolactone could have had a positive effect on cardiac performance. These differences were adjusted for using multivariate regression analyses. No changes in medications were allowed during follow up. Finally, multiple analyses will have increased the chance of type 1 errors. However, the findings were consistent on two different imaging techniques and biochemical analyses of circulating markers, supporting the concept that the majority of the significant results represented real effects.

### **6.5.5 Conclusion**

Mineralocorticoid receptor blockade with spironolactone improved ventricular systolic and diastolic function in early stage CKD. Early treatment of patients with asymptomatic reductions in left ventricular function might be an important step in reducing the mortality and morbidity related to myocardial disease seen in later stages of CKD. Given the likely role of aldosterone and the promise such a strategy has demonstrated, further exploration appears warranted in longer-term clinical outcome trials.

## **CHAPTER 7**

### **THE SAFETY AND TOLERABILITY OF SPIRONOLACTONE IN PATIENTS WITH MILD-MODERATE CHRONIC KIDNEY DISEASE**

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## 7.1 SUMMARY

Mineralocorticoid receptor blockers (MRBs) in combination with ACE inhibitors and angiotensin receptor blockers (ARBs) improve prognostic markers of cardiovascular and renal disease in early stage chronic kidney disease (CKD). Concerns relating to the safety and tolerability of MRBs in CKD might limit their use in a non clinical trial setting.

In the Chronic Renal Impairment in Birmingham II study (CRIB-II), 115 patients with non-diabetic early stage CKD (eGFR 30-89 ml/min/1.73m<sup>2</sup>) received 25 mg daily of spironolactone for 4 weeks before randomisation to continuing treatment or placebo for a further 36 weeks. All patients were on ACE inhibitor and / or ARB therapy. Potassium and renal function were checked at weeks 1, 2, 4, 8, 16, 28 and 40. The incidence of hyperkalaemia, significant renal dysfunction (defined as a reduction eGFR  $\geq$  25%) and patient-related side effects were assessed.

After 40 weeks of treatment serious hyperkalaemia (serum potassium  $\geq$  6.0 mmol/L) occurred in 1 patient, an incidence of < 1%. Mild hyperkalaemia (serum potassium 5.5-5.9 mmol/L) occurred on one or more occasions over follow-up in 11 patients (9 on spironolactone) and was predicted by baseline potassium  $\geq$  5.0 mmol/L and eGFR  $\leq$  45 ml/min/1.73m<sup>2</sup>. Over follow-up, 3 patients on spironolactone experienced significant renal dysfunction but no patients withdrew due to intolerance or side effects. Changes in potassium, eGFR and systolic blood pressure were most apparent in the first 4 weeks but remained stable after randomisation.

Spironolactone is well tolerated in selected non-diabetic patients with early stage CKD. Strict monitoring over the first month of treatment followed by standard surveillance as for ACE inhibitors and ARBs is suggested.

## 7.2 INTRODUCTION

In the Chronic Renal Impairment in Birmingham II (CRIB-II) study described in chapters 5 and 6, treatment with spironolactone over 40 weeks produced beneficial effects on prognostic markers of arterial stiffness and cardiac function in patients with early stage chronic kidney disease (CKD). In addition, mineralocorticoids receptor blockers (MRBs) have been shown to slow the decline in renal function in patients with proteinuric CKD (Bomback et al., 2008a). These data provide support for the use of MRBs in patients with CKD. However, concerns regarding the risk of hyperkalaemia and deterioration of renal function with these drugs, particularly when used in combination with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) necessitate the urgent need for prospective data on the safety and tolerability of MRBs in patients with CKD.

This sub-analysis of the CRIB-II study reports in detail the effects on renal function, potassium, blood pressure and tolerability that occurred over 40 weeks of treatment with spironolactone.

## **7.3 METHODS**

### **7.3.1 Study design, participants and treatment regimen**

The study design, clinical characteristics of patients and treatment regimen used in CRIB II have been described in chapter 5.

In this sub analysis, the effect of treatment with spironolactone for 40 weeks on the following parameters are reported and compared to placebo:

1. Serum potassium
2. Estimated GFR by the 4-v MDRD formula (eGFR)
3. Twenty four hour ambulatory blood pressure (24 hour ABPM)
4. Proteinuria assessed by albumin-creatinine ratio (ACR)
5. Patient side-effects

### **7.3.2 Statistical analysis**

Statistical analyses were performed as described in Chapter 2. Odds ratios (OR) and 95% CI for a reduction in eGFR ( $\geq 10\%$ ), serum potassium  $\geq 5.5$  mmol/L and systolic blood pressure ( $\geq 10\%$ ) were derived using logistic regression. Multiple linear regression models were used to assess the predictors of change in eGFR, potassium and blood pressure. Models included baseline demographic and clinical features known to increase the risk of occurrence (age, male gender, concurrent ACE inhibitor, ARB or spironolactone use, eGFR  $\leq 45$  ml/min/1.73m<sup>2</sup>, and baseline potassium  $\geq 5$  mmol/L) (de Denus S. et al., 2006). Analysis was adjusted for baseline differences.

## 7.4 RESULTS

### 7.4.1 Patient demographics

Baseline demographic and clinical characteristics have been presented in Tables 5.2.

### 7.4.2 Open-label treatment

#### Open-label treatment

Three patients were withdrawn during the 4 weeks of open-label spironolactone treatment; two patients for safety reasons: one with serious hyperkalaemia (potassium 6.8 mmol/L) at week 3, and one with symptomatic hypotension and significant deterioration of eGFR ( $\geq 30\%$ ) at week 3. One patient withdrew consent.

After 4 weeks of spironolactone the mean eGFR was reduced by 3%, an absolute change of  $-1.6 \text{ ml/min/1.73m}^2$  (95% CI,  $-2.5 - -0.8$ ),  $p < 0.01$ . Serum creatinine increased by  $+7 \text{ } \mu\text{mol/L}$  (95% CI  $5 - 9$ )  $p < 0.01$ . The change in mean eGFR was not different between quartiles of baseline GFR ( $p = 0.80$ ) (Figure 7.1), quartiles of age ( $p = 0.07$ ) or gender ( $p = 0.9$ ) or predicted by any variable in a multivariate regression model (Table 7.1).

Mean serum potassium was increased by  $0.22 \text{ mmol/L}$  (95% CI,  $0.14 - 0.30$ )  $p < 0.01$  over the first 4 weeks of treatment with spironolactone. In accordance with our protocol, 5 patients with mild hyperkalaemia ( $5.5 - 5.9 \text{ mmol/L}$ ) were switched to alternate day spironolactone at week one and one further patient was switched to alternate day treatment at week two. Patients in the lowest quartile of baseline potassium had the greatest absolute change over the first 4 weeks (Figure 7.2 & Table 7.1). Predictors of the change in potassium in multivariate regression were; baseline potassium (each  $0.1 \text{ mmol/L}$  increase predicted a -

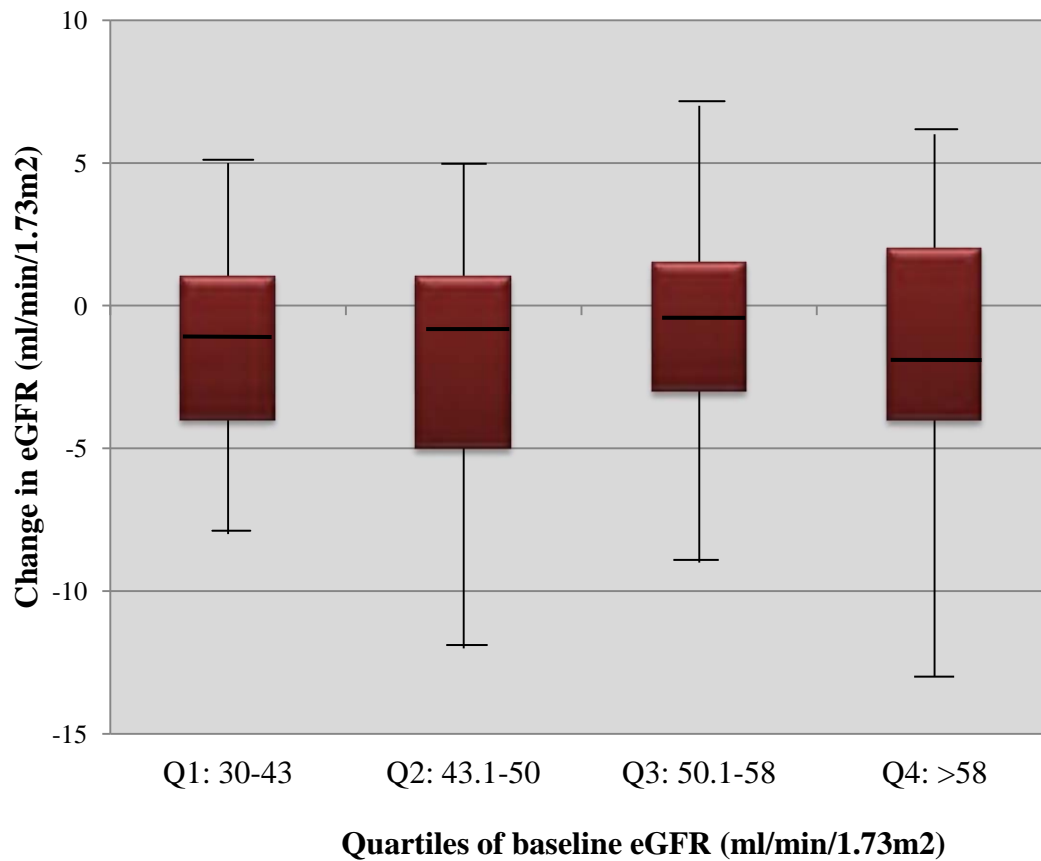


0.47 mmol/L decrease at week 4), baseline eGFR (each 10 ml/min/1.73m<sup>2</sup> increase predicted a -0.11 mmol/L reduction) and age (each increase of 10 years predicted an increase 0.10 mmol/L). However, the only predictor for the development of hyperkalaemia (potassium  $\geq$  5.5 mmol/L) during weeks 0-4 in a logistic regression model was baseline potassium  $\geq$  5.0 mmol/L (OR 5.0; 95% CI, 1.0 - 25, p = 0.04).

Ambulatory systolic and diastolic blood pressures were reduced at week 4 compared to baseline: systolic -7mmHg (95% CI, -10 -5) p < 0.01, diastolic blood pressure -5mmHg (95% CI,-7 -4) p < 0.01. In multivariate regression, the change in ambulatory systolic blood pressure was predicted by baseline systolic blood pressure (each 10 mmHg increase in baseline systolic predicted -2.5mmHg reduction at week 4) and gender (Table 7.1).

Urinary ACR was also significantly reduced after 4 weeks of spironolactone therapy from median 7.4 mg/mmol (95% CI, 0 - 603) to 4.1 mg/mmol (95% CI, 0 - 518), p < 0.01.

**Figure 7.1** Change in eGFR over the first four weeks of open-labelled treatment with spironolactone by quartiles of baseline eGFR



Data are median (horizontal bold line), upper and lower quartiles (box) and range (error bars) using one way analysis of variance with post-hoc Tukey test.

No significant differences between quartiles.

**Table 7.1** Linear regression models for change in eGFR, potassium and systolic blood pressure after 4 weeks of spironolactone treatment

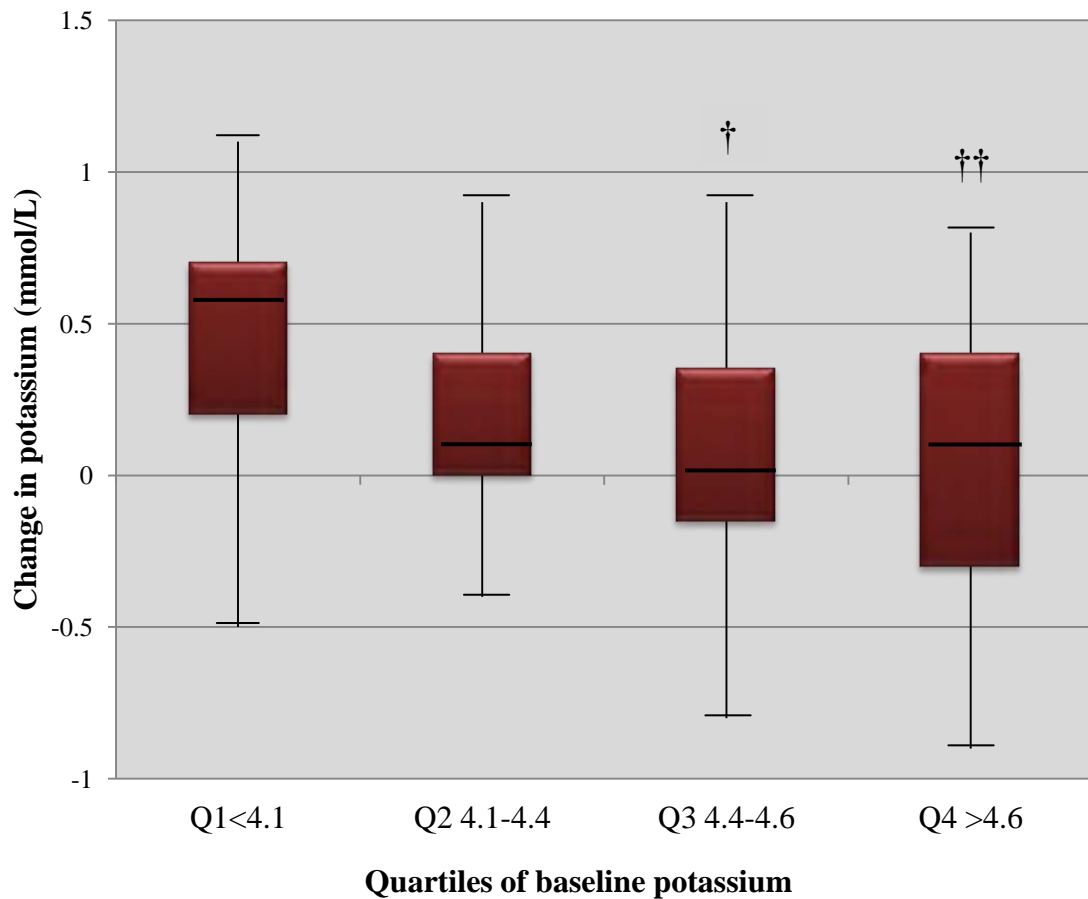
| Variables                    | Change in eGFR             | Change in potassium        | Change in systolic BP      |
|------------------------------|----------------------------|----------------------------|----------------------------|
|                              | $\beta$ coefficient + (SE) | $\beta$ coefficient + (SE) | $\beta$ coefficient + (SE) |
| Age                          | -0.003 (0.005) p = 0.96    | 0.01 (0.003) p < 0.01      | -0.02 (0.10) p = 0.82      |
| Male gender                  | 0.61 (1.27) p = 0.63       | -0.07 (0.07) p = 0.35      | -5.78 (2.61) p = 0.03      |
| Baseline eGFR                | -0.02 (0.05) p = 0.64      | -0.01 (0.003) p < 0.01     | 0.08 (0.08) p = 0.34       |
| Baseline 24 hour systolic BP | 0.04 (0.06) p = 0.45       | 0.002 (0.003) p = 0.43     | -0.25 (0.11) p = 0.03      |
| Baseline potassium           | N/A                        | -0.50 (0.08) p < 0.01      | N/A                        |
| ACE inhibitor‡               | -1.56 (1.34) p = 0.25      | -0.02 (0.07) p = 0.75      | 2.05 (2.26) p = 0.37       |

Three models are shown: the first the 4 weeks of open-labelled treatment with spironolactone. Estimates for all models are adjusted for the variables listed and also for use of beta blockers, statins, diuretics, calcium channel blockers.

‡ ACE inhibitor and ARB were used in separate models in view of co-linearity. Substituting ARBs did not significantly alter the data (not shown).

$\beta$  coefficient; Unstandardised  $\beta$ -coefficient, SE; standard error, BP; blood pressure, eGFR; estimated glomerular filtration rate, N/A; variable not known to influence dependent variable

**Figure 7.2** Change in serum potassium levels over the first four weeks of open-labelled treatment with spironolactone by quartiles of baseline potassium



Data are median (horizontal bold line), upper and lower quartiles (box) and range (error bars) using one way analysis of variance with post-hoc Tukey test.

Reference group Q1: †  $p < 0.05$ , ††  $p < 0.01$

### 7.4.3 Randomised treatment with spironolactone or placebo

Two patients were withdrawn during the double blind randomised phase of the study; one patient randomised to spironolactone withdrew consent in week 6 and 1 patient randomised to placebo had a symptomatic relapse of Wegener's granulomatosis in week 15. No patients were withdrawn for iatrogenic complications or side effects.

Compared to baseline, eGFR was reduced in patients on spironolactone at week 40 (49 ml/min/1.73m<sup>2</sup> vs. 46 ml/min/1.73m<sup>2</sup>,  $p < 0.01$ ) (Figure 7.3). On placebo, baseline eGFR did not change significantly over follow-up (53 ml/min/1.73m<sup>2</sup> vs. 52 ml/min/1.73m<sup>2</sup>,  $p = 0.48$ ).

During the first 24 weeks of treatment eGFR was lower on spironolactone compared to placebo but there was no significant difference by week 40 (46.1 ml/min/1.73m<sup>2</sup> vs. 52.3 ml/min/1.73m<sup>2</sup>,  $p = 0.09$ ). Four patients (3 on spironolactone) experienced a clinically significant reduction in eGFR (25-29%) but with such small numbers it was not possible to identify co-variables which were predictive in a logistic regression model. Minor reductions in eGFR (10-20%) were more common with spironolactone; 17 patients verses 9 patients on placebo. In a logistic regression model, a reduction in eGFR of  $\geq 10\%$  was predicted by baseline eGFR  $\leq 45$  ml/min/1.73m<sup>2</sup> and treatment assignment to spironolactone (Table 7.2).

Compared to patients with  $<10\%$  reduction in eGFR, these patients had a greater reduction in office systolic blood pressure (-7 mmHg  $\pm$  8 vs. 2 mmHg  $\pm$  8)  $p = 0.01$  and lower baseline eGFR (47 ml/min/1.73m<sup>2</sup>  $\pm$  13 vs. 53 ml/min/1.73m<sup>2</sup>  $\pm$  12)  $p < 0.01$ .

After randomisation mean serum potassium levels were persistently higher ( $p < 0.05$ ) with spironolactone than with placebo but remained stable over the 36 weeks of follow-up (Figure 7.4). No patients experienced serum potassium  $\geq 6$  mmol/L and only 4 patients had a

potassium  $\geq 5.5$  mmol/L; 2 with spironolactone and 2 with placebo. Nine patients on spironolactone and 2 on placebo had a potassium  $\geq 5.5$  mmol/L on at least one occasion over 40 weeks of treatment. The risk of developing a serum potassium  $\geq 5.5$  mmol/L during 40 weeks of treatment was increased if baseline potassium was  $\geq 5.0$  mmol/L and baseline eGFR  $\leq 45$  ml/min/1.73m<sup>2</sup> (Table 7.2).

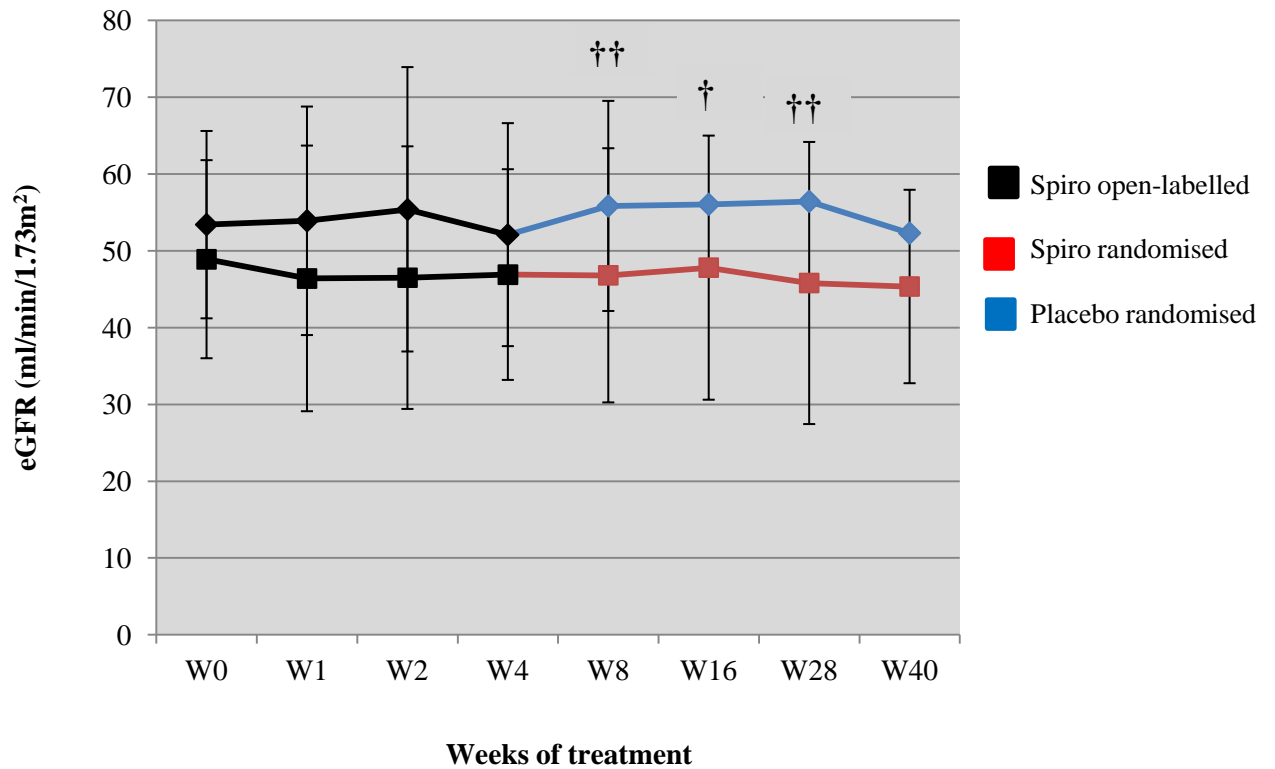
Spironolactone significantly reduced ambulatory systolic blood pressure at week 40 compared to placebo: systolic -6 mmHg (95% CI, -8 -3) vs. -1 mmHg (95% CI -3 1)  $p < 0.01$ . Diastolic blood pressure was reduced but did not reach statistical significance: -3 mmHg (95% CI, -5 -1) vs. -1 (95% CI -3 0)  $p = 0.20$ . In multivariate regression, the change in ambulatory systolic blood pressure was predicted by baseline ambulatory systolic blood pressure (Table 7.2).

Urinary ACR was significantly reduced with spironolactone (-3.2mg/mmol (-38-+11) vs. -0.6 (-14-+5) compared to placebo ( $p < 0.05$ ).

#### **7.4.4 Tolerability**

There were no reports of disturbed menstrual cycle, breast tenderness, erectile dysfunction or gynaecomastia.

**Figure 7.3** Change in eGFR over 40 weeks in patients treated with spironolactone or placebo



Data are mean  $\pm$  standard deviation.

†p < 0.05, †† p < 0.01, repeated measures analysis of variance with the time point (week 0, week 40) as the within-subjects factor and the group (spironolactone and placebo) as the between-subjects factor.

**Table 7.2** Logistic regression models for change in eGFR, potassium and systolic blood pressure over 40 weeks of treatment

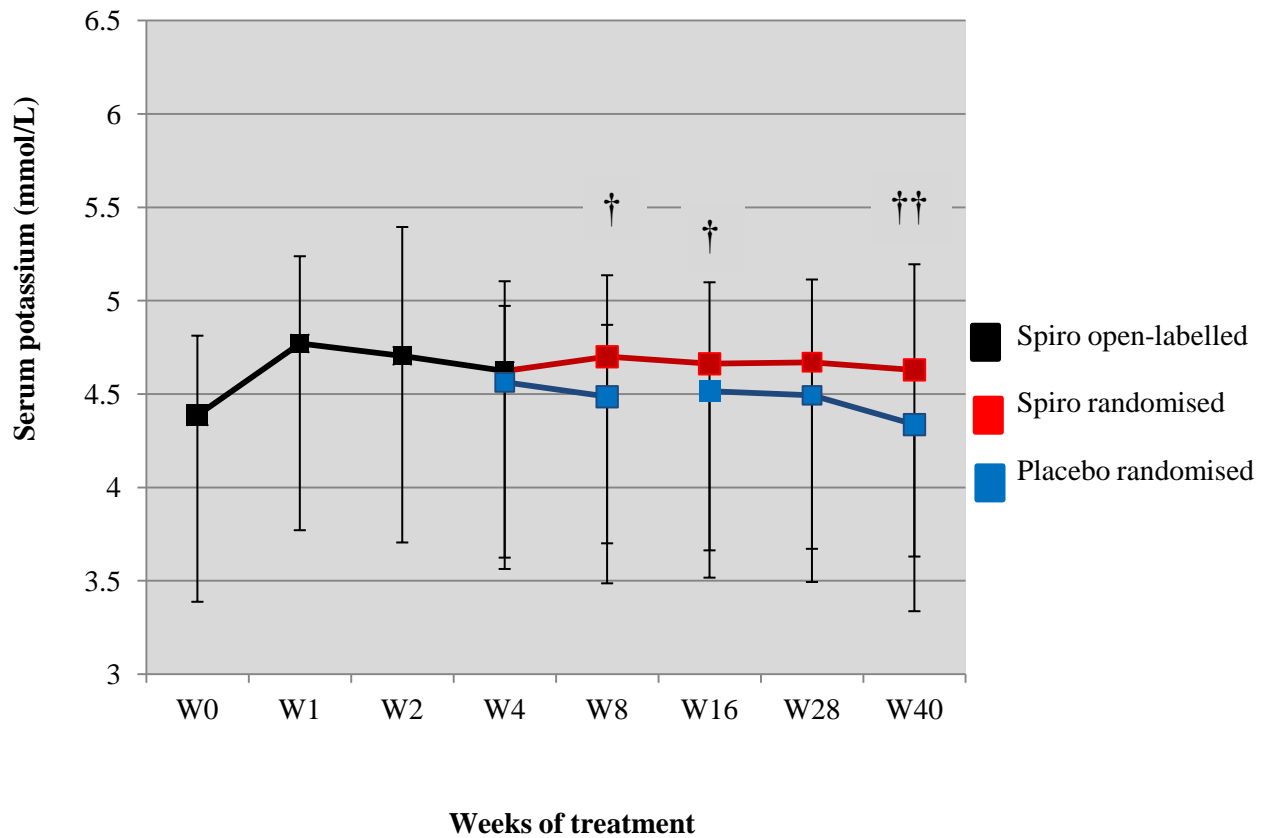
| <b>Variables</b>      | <b>eGFR<br/>reduction &gt;10%<br/>OR (95% CI)</b> | <b>Potassium<br/>&gt; 5.5 mmol/L anytime<br/>OR (95% CI)</b> | <b>Systolic BP<br/>reduction &gt;10%<br/>OR (95% CI)</b> |
|-----------------------|---------------------------------------------------|--------------------------------------------------------------|----------------------------------------------------------|
| Age                   | 1.0 (0.1-1.1) p = 0.70                            | 1.0 (0.9-1.0) p = 0.10                                       | 0.96 (0.9-1.0) p = 0.19                                  |
| Male gender           | 0.7 (0.3-1.7) p = 0.30                            | 0.4 (0.1-2.4) p = 0.33                                       | 3.3 (0.8-13) p = 0.10                                    |
| eGFR ≤45 ml/min/1.73  | 3.4 (0.12-0.84) p = 0.02                          | 5.8 (1.1-29.0) p = 0.04                                      | 1.0 (0.2-4.6) p = 0.96                                   |
| Baseline 24 hr SBP    | 1.0 (1.0-1.1) p = 0.56                            | 1.0 (0.9-1.0) p = 0.40                                       | 1.1 (1.0-1.3) p < 0.01                                   |
| Baseline K ≥ 5 mmol/L | NA                                                | 16.5 (4.4-61.0) p < 0.01                                     | NA                                                       |
| ACE inhibitor‡        | 2.2 (0.7-6.6) p = 0.18                            | 0.7 (0.1-4.1) p = 0.69                                       | 0.8 (0.2-3.3) p = 0.80                                   |
| Spirolactone          | 3.5 (1.3-9.4) p = 0.01                            | 0.7 (0.1-3.5) p = 0.63                                       | 1.2 (0.2-6.3) p = 0.3                                    |

Three models are shown: 40 weeks of treatment with spironolactone and placebo. Estimates for all models are adjusted for the variables listed and also for use of beta blockers, statins, diuretics, calcium channel blockers. Baseline potassium was not thought to contribute to reduction in eGFR or BP and is not examined in the model.

‡ ACE inhibitor and ARB were used in separate models in view of co-linearity. Substituting ARBs did not significantly alter the data (not shown). OR; Odds ratio (95% CI)



**Figure 7.4** Change in serum potassium over 40 weeks in patients treated with spironolactone or placebo.



Data are mean  $\pm$  standard deviation.

†  $p < 0.05$ . ††  $p < 0.01$ , repeated measures analysis of variance with the time point (week 0, week 40) as the within-subjects factor and the group (spironolactone and placebo) as the between-subjects factor.

## 7.5 DISCUSSION

These results provide support for the safety and tolerability of low dose spironolactone with concurrent ACE inhibitor or ARB treatment in patients with non-diabetic early stage CKD. In a cohort of patients with well characterised renal disease treated with spironolactone, the incidence of serious hyperkalaemia ( $\geq 6$  mmol/L) was  $< 1\%$  and that of a clinically significant ( $\geq 25\%$ ) reduction in eGFR was  $\leq 5\%$  over the 40 weeks of treatment. Changes in potassium, eGFR and systolic blood pressure were most apparent in the first month of treatment but only 2% of patients required treatment discontinuation within 4 weeks. Of the 56 patients randomised to continuing therapy with spironolactone, all remained stable thereafter with no serious adverse events occurring during 36 weeks of follow-up. A pre-treatment serum potassium  $\geq 5$  mmol/L was predictive for the development of hyperkalaemia in our study and thus we would agree with the original RALES criterion and not recommend spironolactone in such patients.

### 7.5.1 The safety of mineralocorticoid receptor blockers in heart failure

Following publication of the RALES study, several studies raised safety concerns about MRBs in combination with ACE inhibitors and ARBs in patients with heart failure (Cruz et al., 2003; Juurlink et al., 2004; Masoudi et al., 2005). A large increase in the number of prescriptions for spironolactone in patients with heart failure over 65 years of age was mirrored by an increase in the number of hospital admissions with hyperkalaemia and an increase in associated mortality. It was estimated that for every 1000 additional prescriptions of spironolactone there were 50 additional admissions for hyperkalaemia (Juurlink et al., 2004). These analyses identified several factors which increased the risk of developing hyperkalaemia on spironolactone: i) inadequate monitoring of potassium

levels; ii) neglect of predisposing factors to hyperkalaemia including diabetes, newly developing conditions while on treatment such as renal dysfunction; iii) inappropriately high doses with concomitant medications such as beta blockers which increase the risk of hyperkalaemia; iv) inappropriate increases in dietary potassium due to concomitant diuretic therapy and prescriptions to patients not satisfying the RALES entry criteria.

It is therefore reassuring that a recent publication reporting the use of spironolactone in Scotland between 1994-2007, reported a marked increase in prescriptions without an increase in hospital admissions for hyperkalaemia and low rates of serious hyperkalaemia (Wei et al., 2010). These data reflect appropriate patient selection and an increase in the rates of monitoring of serum potassium and creatinine. Indeed rates of mild hyperkalaemia (5.0-6.0 mmol/L) were increased but without an increase in the incidence of serious hyperkalaemia ( $\geq 6.0$  mmol/L) (Wei et al., 2010).

### **7.5.2 The safety of mineralocorticoid receptor blockers in chronic kidney disease**

To date, safety data on the use of MRBs in patients with CKD has been limited to small studies investigating their role in reducing proteinuria and have been reassuring (Bianchi et al., 2006; Chrysostomou & Becker, 2001; Sato et al., 2003). Bomback et al. published a systematic review of the effect of adding a MRB to ACE inhibitor and ARBs for proteinuria (Bomback et al., 2008a). In the 15 studies and 436 patients reviewed, a serum potassium  $\geq 5.5$  mmol/L was identified in 5.5% of patients. Post treatment mean eGFR was reduced in three studies with study durations up to 12 months, although these reductions were considered clinically insignificant (74 to 67, 87 to 74, 57 to 54 ml/min/1.73m<sup>2</sup>).

A recent randomised control trial in patients with idiopathic glomerular disease and an eGFR of  $\geq 30$  ml/min/1.73m<sup>2</sup>, provided information on long term (3 years) treatment

with a MRB (Bianchi et al., 2010). Spironolactone was combined with an ACE inhibitor and an ARB plus a statin in the ‘intensive’ treatment group. While mean serum potassium increased only slightly in comparison to the control group, the rate of hyperkalaemia (potassium  $\geq 5.5$  mmol/L after dose frequency reduction) was 9/64 in the intensive regimen compared to 3/64 in the conventional group. This high rate might have been due to the concomitant use of both ARBs and ACE inhibitors, a treatment strategy not commonly employed. In the spironolactone group, eGFR fell approximately 2% over the first three months before subsequently improving and was not significantly different to baseline value at the end of follow-up (Bianchi et al., 2010). This change is consistent with data in the CRIB-II study where a mean 3% reduction in eGFR over the first month of open-label treatment was observed before improving over further follow-up. This change is similar to that observed following initiation of ACE inhibitors (Bakris and Weir, 2000).

### **7.5.3 An optimum level for serum potassium?**

Mild hyperkalaemia (5.5-5.9 mmol/L) during MRB therapy is common but is probably of no adverse clinical significance (Wei et al., 2010). In the CRIB-II study, 8% of patients on spironolactone and 2% on placebo had a potassium  $\geq 5.5$  mmol/L on one or more occasion over 40 weeks of treatment. In a recent prospective observational study of 820 patients with CKD stages 3-5, a potassium level between 5.5-5.9 mmol/L was not associated with an increased mortality rate or progression to end-stage CKD over an average follow-up of 2.6 years (Korgaonkar et al., 2010). Indeed, there was an increased risk of end-stage CKD and death with serum potassium levels  $\leq 4.0$  mmol/L. Thus there may be a U-shaped relationship between potassium and mortality in CKD, with an optimum “eukalemic” range for serum potassium level of between 4.1-5.5 mmol/L. In CRIB-II, 16 patients

randomised to placebo had a serum potassium  $\leq 4$  mmol/L during follow-up compared to 8 patients with spironolactone.

#### **7.5.4 The optimum dose and monitoring regimen in chronic kidney disease?**

An early reduction in eGFR can be anticipated with spironolactone just as it can following treatment with ACE inhibitors and ARBs, necessitating monitoring of renal function. The frequency of monitoring in CRIB-II was greater in the first month of treatment than is commonly employed following initiation of ACE inhibitors and ARBs but does not differ significantly to protocols used for anticoagulants such as warfarin where the importance of frequent monitoring is accepted. This decision reflected concurrent treatment with an ACE inhibitor and / or ARB and the significant range of renal function; 34% of patients in CRIB-II had an eGFR  $\leq 45$  ml/min/1.73m<sup>2</sup>. After randomisation, no increase in the risk of hyperkalaemia or reduction in eGFR was observed compared to placebo. Thus monitoring for adverse effects of spironolactone after 4 weeks of therapy is probably required no more frequently than is recommended for monitoring of patients on ACE inhibitor/ARB therapy. Attention to the risk factors for the development of hyperkalaemia (eGFR  $\leq 45$  ml/min/1.73m<sup>2</sup> and baseline potassium  $\geq 5.0$  mmol/L) and clear advice to temporarily suspend spironolactone with acute illness such as diarrhoea and vomiting should help reduce such risks along with careful patient selection.

The optimum dose of spironolactone and monitoring strategy of spironolactone in CKD are still to be determined and data from large scale prospective studies will be required. Eminent figures in this area have suggested a role for very low dose spironolactone 12.5mg daily (Bomback et al., 2009b) or using a titration schedule of 25mg on alternate days for one month with an increase to daily treatment if potassium remains  $\leq$

5 mmol/L (Pitt, 2009). This study provides preliminary data to support the dose of 25 mg once daily provided adequate monitoring is employed.

### **7.5.5 Limitations**

It is important to note that patients recruited in this study were selected to minimise the chances of serious iatrogenic hyperkalaemia. Patients with a history of hyperkalaemia, diabetes and CKD stage >3, considered to be at high risk of hyperkalaemia were excluded. Thus these data provide no support for use of MRBs in such population, in particular diabetics. All patients were recruited from specialist nephrology clinics limiting the applicability of these data to patients treated in primary care who represent the majority of patients with CKD. Doses of ACE inhibitors and ARBs were titrated to those maximally tolerated but were not the same in all patients. Finally, only 40 weeks of follow-up data is reported. No conclusions can be drawn as to the frequency of later adverse event rates. Such adverse events can be precipitated by acute illness or surgery occurring during treatment and these events are unlikely during short treatment periods.

### **7.5.6 Conclusion**

Low-dose spironolactone is well tolerated in early stage CKD. The risk of serious hyperkalaemia and significant renal deterioration appear rare particularly after the first month of treatment. Careful patient selection and biochemical monitoring should ensure these adverse effects are minimised and may be an early and “acceptable price” in reducing cardiovascular events and preventing the progression of renal disease in these high risk patients. These data serve to heighten the need for a large scale clinical outcome trial from which much needed further safety data would be available.

## **CHAPTER 8**

### **ASSESSMENT OF REPRODUCIBILITY**

## 8.1 SUMMARY

The ability to perform repeated clinical measurements accurately and reproducibly is of pivotal importance for clinical practice and research. Identifying “real” differences in cardiovascular structure and function between patients with chronic kidney disease (CKD) and controls as well as monitoring patients response to treatment with spironolactone or placebo were the principal aims of this thesis. In this chapter, I sought to determine intra-operator and inter-operator variability for measurements of left ventricular mass, systolic function and arterial stiffness performed using well validated techniques of cardiac MRI and echocardiography.

Ten patients with early stage CKD recruited for the Chronic Renal Impairment in Birmingham II study (CRIB-II) (presented in chapter 5) and 10 healthy volunteers recruited for the cross-sectional observational study (presented in chapter 3) were randomly chosen for intra-operator and inter-operator variability studies.

There was good agreement of measurements. The overall (patients and volunteers) absolute differences (median (range)) for the single operator performing repeated measurements were: cardiac MRI LV mass index -1.4 (9.2 g/m<sup>2</sup>), aortic distensibility 0.21 (0.27 mmHg<sup>-3</sup>), cardiac MRI LV ejection fraction (EF) 1.0 (6%), echo LV EF -3 (14%). Inter-operator variability between two clinicians analysing the same data sets were: CMR LV mass index -2.9 (9.2 g/m<sup>2</sup>), aortic distensibility 0.23 (0.51 mmHg<sup>-1</sup>), CMR LV ejection fraction (EF) -0.4 (11%), echo LV EF 2.0 (12%).

The reproducibility for measurements performed in this thesis were good. These data are comparable to previous published reports, suggesting that operators assessing data in this thesis were reliable for serial measurements.



## 8.2 INTRODUCTION

The accuracy and reliability of repeated clinical measurements has important implications for clinical practice and research. Observed differences in measurements over time can reflect “real” changes in clinical status such as disease progression or an altered response to treatment. Alternatively they can reflect variation in quantitative analysis between individuals or limitations of the chosen imaging modality. Thus the ability to identify “real” changes over time necessitates analysis of variability factors which will affect the interpretation of data in serial studies and the calculation of study samples sizes in the research setting.

Intra- and inter-observer variability factors are introduced during the quantitative analysis phase, when measurements are performed twice by one observer or by two or more different observers, respectively. To allow complete interpretation of data, investigators should quantify the expected variation relating to the operator (s) performing repeated measurements on individuals over time.

The accuracy of quantitative analysis on repeated measurements can depend on a number of factors including; the quality of imaging, the observer’s experience at analysing specific measurements / data sets, and presence or absence of pathology. Operator variability is higher with abnormal cardiac geometry such as asymmetric remodelling with ischaemic cardiomyopathies (Grothues et al., 2002).

In this chapter, I have reported the intra-operator and inter-operator variability for prospectively defined end-points in studies described in studies included in this thesis. These include: left ventricular (LV) mass, LV function and aortic distensibility in patients and healthy volunteers. The measurements selected are all important intermediate markers of cardiovascular prognosis.

The hypothesis of this study was that experienced operators performing repeated measurements using gold standard imaging techniques would be reliable and reproducible, allowing complete interpretation of data presented in this thesis.

## **8.3 METHODS**

### **8.3.1 Participants**

Ten patients with early stage chronic kidney disease recruited for the Chronic Renal Impairment in Birmingham (CRIB-II) study (presented in chapter 5) and 10 healthy volunteers recruited for the cross-sectional observational study (presented in chapter 3) were randomly chosen for intra-operator and inter-operator variability studies.

### **8.3.2 Measurements assessed**

The following measurements were assessed using gold standard techniques (described in chapter 2):

1. LV mass: on cardiac MRI
2. Aortic distensibility on cardiac MRI
3. LV systolic function: ejection fraction (EF) on cardiac MRI and echo
4. Longitudinal LV systolic function: tissue Doppler (TDI) myocardial systolic ( $S_m$ ) velocities and myocardial deformation by 1D peak Strain
5. LV diastolic function: early diastolic myocardial velocities ( $E_m$ ) at the lateral annulus

### **8.3.3 Intra-observer variability study**

Intra-operator variability is a measure of the extent to which the same observer agrees when analysing the same set of data at different time points. In this study, a single experienced operator (NE) blinded to all treatment and clinical data, measured the variables off-line on all 20 patients / controls using commercially available cardiac MRI

and echo software. Blind re-analysis was repeated by the same operator on a second occasion at least six weeks later.

#### **8.3.4 Inter-observer variability study**

Inter-operator variability is a measure of the extent to which two or more observers agree when analysing the same set of data. In this study, two experienced operators (NE and RPS for cardiac MRI, NE and AH for echo) blinded to all clinical data and results from the other operator, performed measurements off-line on the same 20 individuals.

#### **8.3.5 Statistical analysis**

Data are presented as intra-class correlation coefficients (ICC) + 95% confidence intervals (95% CI). A 2-way mixed model with absolute agreement was used. This model assumes that the operators performing measurements were not selected from a larger population of operators, so their effect is included in the model. It also takes into account the systematic differences between the two measurements. An assessment of the agreement within and between two measurements made by operators was evaluated by Bland-Altman analysis (Bland & Altman, 1986). Spearman's correlation coefficients were also performed to discern if differences in measurements change with the magnitude of the measurements.

## 8.4 RESULTS

### 8.4.1 Study population

There were no differences in demographic or clinical data for patients and volunteers randomly selected for the variability studies (Table 8.1).

### 8.4.2 Reproducibility

The intra-observer and inter- observer reproducibility for measures of LV mass, function and aortic distensibility are presented in Tables 8.2 and 8.3. All of the variables gave highly significant results, suggesting strong agreement in their measurements. As expected, reproducibility with cardiac MRI was superior to echo, reproducibility for controls was superior to patients and intra-operator reproducibility was superior to inter-operator variability.

Bland-Altman analysis confirmed high reproducibility with acceptable bias (Figure 8.1 and Table 8.4). There were no significant correlation for the difference between measurements and average measurement: LV mass; Spearman correlation  $r = 0.25$ ,  $p = 0.28$ , aortic distensibility  $r = 0.11$ ,  $p = 0.64$ , cardiac MRI EF;  $r = 0.12$ ,  $p = 0.60$ , echo EF;  $r = -0.08$ ,  $p = 0.72$ . This suggests that differences in measurements change with the magnitude of the measurements.

Taken over both groups (controls and patients); absolute differences between measurements were small. For the single operator; LV mass; 1.4 (9.2 g) [median difference (range)], aortic distensibility; 0.21 (0.27 mmHg<sup>-1</sup>), LV ejection fraction (EF) on cardiac MRI; 1.0 (6.0%), LVEF on echo; 3 % (6.0), LVEDVi on cardiac MRI; 4.0 (19 ml/m<sup>2</sup>), TDI Sm; 0.3 (1.2 cm/s), TDI Em; 0.2 (1.7 cm/s), LV global strain; 0.3 (3.4%). Between operators; cardiac MRI LV mass index; 2.9 (9.2 g/m<sup>2</sup>), aortic distensibility; 0.23

(0.51 mmHg<sup>-3</sup>), LVEF on cardiac MRI; 0.4 (11%), LVEDVi on cardiac MRI; 2.6 (19 ml/m<sup>2</sup>), echo EF; 2.0 (12%), TDI Sm; 2.0 (12 cm/s), TDI Em; 0.01 (0.84 cm/s), LV global strain; 0.6 (8%).

**Table 8.1** Patient characteristics

|                       | <b>Controls (n=10)</b> | <b>CKD (n=10)</b> | <b>Significance</b> |
|-----------------------|------------------------|-------------------|---------------------|
| Age (years)           | 45 ± 11                | 44 ± 8            | 0.9                 |
| Male (%)              | 50%                    | 50%               | 1.0                 |
| BSA (m <sup>2</sup> ) | 1.8 ± 0.2              | 1.89 ± 0.2        | 0.6                 |
| Heart rate (bpm)      | 71 ± 8                 | 66 ± 10           | 0.15                |
| Systolic BP (mmHg)    | 123 ± 12               | 126 ± 12          | 0.6                 |
| Diastolic BP (mmHg)   | 78 ± 8                 | 77 ± 7            | 0.8                 |

BSA; body surface area, BP; blood pressure, CKD; chronic kidney disease

**Table 8.2** Intra-operator reproducibility of measurements

|                                                | Controls (n = 10) |            |         | Patients (n = 10) |           |         |
|------------------------------------------------|-------------------|------------|---------|-------------------|-----------|---------|
|                                                | ICC               | 95% CI     | P-value | ICC               | 95% CI    | P-value |
| LVMi on cardiac MRI (g)                        | 0.96              | 0.85-0.99  | <0.01   | 0.93              | 0.77-0.98 | <0.01   |
| Aortic distensibility ( $\times 10^{-3}$ mmHg) | 0.99              | 0.96-0.99  | <0.01   | 0.99              | 0.96-0.99 | <0.01   |
| LV EF on cardiac MRI (%)                       | 0.83              | -0.03-0.97 | <0.01   | 0.77              | 0.36-0.94 | <0.01   |
| LV EDVI cardiac MRI (ml/m <sup>2</sup> )       | 0.97              | 0.88-0.99  | <0.01   | 0.84              | 0.50-0.95 | <0.01   |
| LV EF on echo (%)                              | 0.76              | 0.07-0.94  | <0.01   | 0.75              | 0.30-0.92 | <0.01   |
| TDI Sm velocity (cm/s)                         | 0.98              | 0.89-0.99  | <0.01   | 0.96              | 0.87-0.99 | <0.01   |
| TDI Em velocity (cm/s)                         | 0.99              | 0.31-0.99  | <0.01   | 0.80              | 0.43-0.94 | <0.01   |
| LV global Strain (%)                           | 0.96              | 0.79-0.99  | <0.01   | 0.95              | 0.83-0.99 | <0.01   |

ICC; intra class correlation coefficient, 95% CI; 95% confidence intervals

LV EF; left ventricular ejection fraction, LVMi; left ventricular mass index, LVEDVI; left ventricular end-diastolic volume index, PWV; pulse wave velocity, TDI Sm; tissue Doppler imaging myocardial systolic velocity, TDI Em; tissue Doppler imaging myocardial early diastolic velocity

All of the variables gave highly significant results, suggesting strong agreement in their measurements



**Table 8.3** Inter-operator study reproducibility of measurements

|                                                | Controls (n = 10) |           |         | Patients (n = 10) |           |         |
|------------------------------------------------|-------------------|-----------|---------|-------------------|-----------|---------|
|                                                | ICC               | 95% CI    | P-value | ICC               | 95% CI    | P-value |
| LVMI on cardiac MRI (g)                        | 0.92              | 0.52-0.98 | <0.01   | 0.91              | 0.72-0.98 | <0.01   |
| Aortic distensibility ( $\times 10^{-3}$ mmHg) | 0.97              | 0.90-0.99 | <0.01   | 0.97              | 0.87-0.99 | <0.01   |
| LV EF on cardiac MRI (%)                       | 0.80              | 0.42-0.95 | <0.01   | 0.78              | 0.34-0.94 | <0.01   |
| LV EDVI cardiac MRI (ml/m <sup>2</sup> )       | 0.86              | 0.60-0.97 | <0.01   | 0.84              | 0.5-0.95  | <0.01   |
| LV EF on echo (%)                              | 0.75              | 0.28-0.93 | <0.01   | 0.73              | 0.21-0.93 | <0.01   |
| TDI Sm velocity (cm/s)                         | 0.97              | 0.89-0.99 | <0.01   | 0.81              | 0.40-0.95 | <0.01   |
| TDI Em velocity (cm/s)                         | 0.78              | 0.40-0.94 | <0.01   | 0.92              | 0.66-0.98 | <0.01   |
| LV global Strain (%)                           | 0.84              | 0.50-0.95 | <0.01   | 0.81              | 0.39-0.95 | <0.01   |

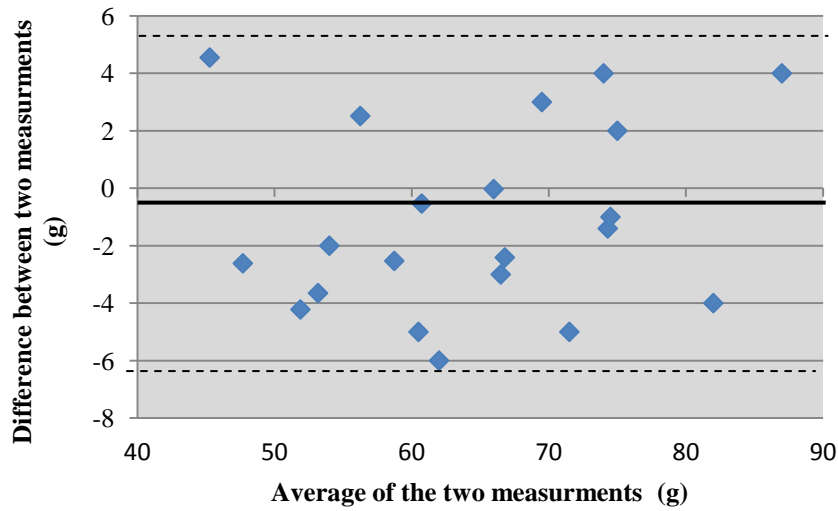
ICC; intra class correlation coefficient, 95% CI; 95% confidence intervals

LV EF; left ventricular ejection fraction, LVMI; left ventricular mass index, LVEDVI; left ventricular end-diastolic volume index, TDI Sm; tissue Doppler imaging myocardial systolic velocity, TDI Em; tissue Doppler imaging myocardial early diastolic velocity

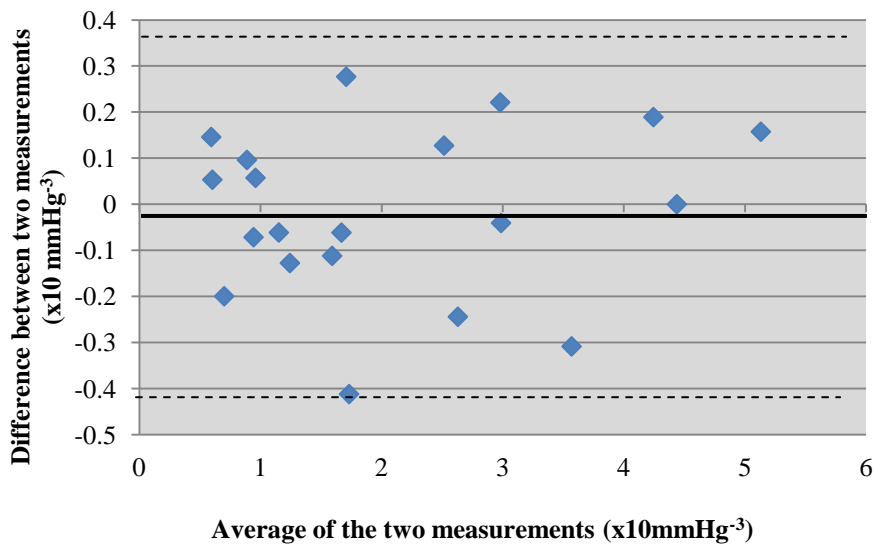
All of the variables gave highly significant results, suggesting strong agreement in their measurements

**Figure 8.1** Intra-operator reproducibility for measurements of left ventricular mass and function in the overall (patients and controls) cohort using Bland-Altman Plots

**8.1a)** Left ventricular mass on cardiac MRI

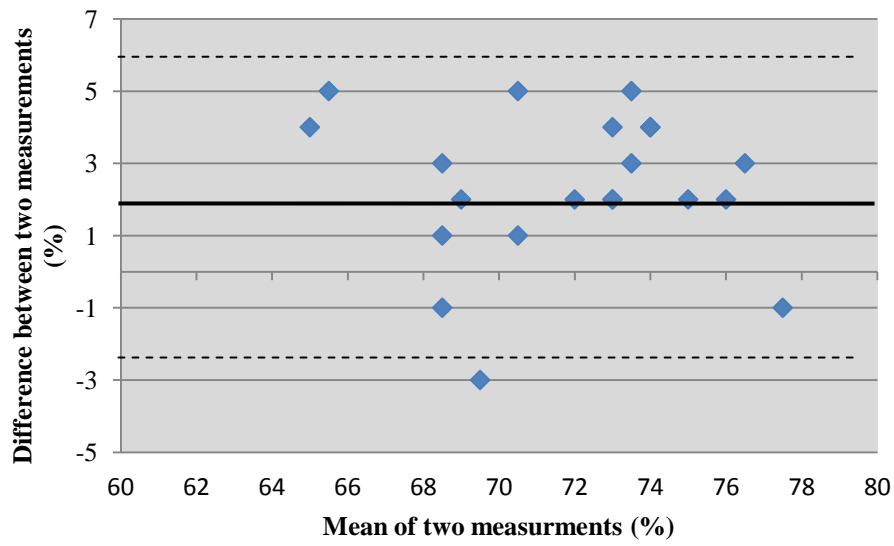


**8.1b)** Aortic distensibility on cardiac MRI

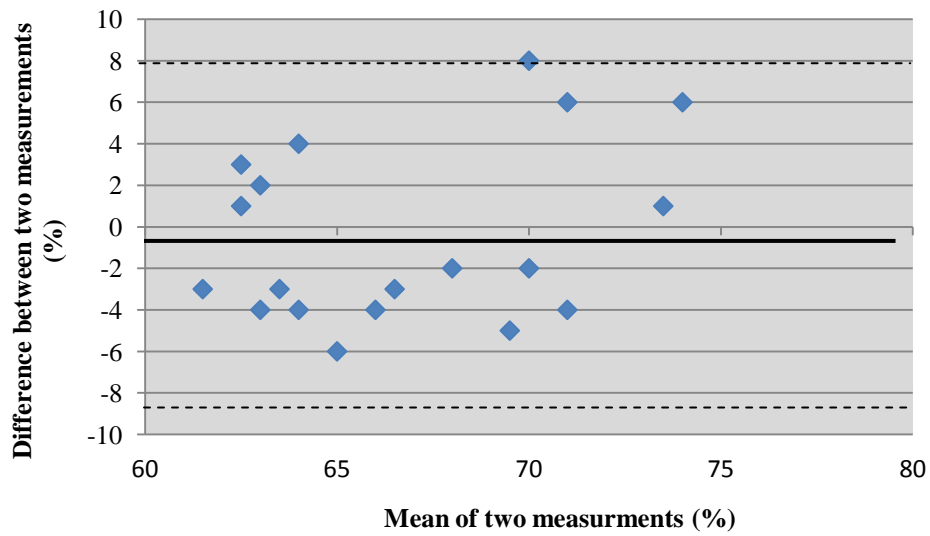


Solid line represents the mean (bias) and dotted lines represent the limits of agreement (defined as the mean difference plus and minus 1.96 times the standard deviation of the differences)

**8.1c)** Ejection fraction on cardiac MRI



**8.1d)** Ejection fraction on echo



Solid line represents the mean (bias) and dotted lines represent the limits of agreement (defined as the mean difference plus and minus 1.96 times the standard deviation of the differences)

**Table 8.4** Intra-observer and inter-operator reproducibility of measurements for total cohort (patients and controls)

|                                                | <b>Intra-observer (n = 20)</b>            | <b>Inter-observer (n = 20)</b>            |
|------------------------------------------------|-------------------------------------------|-------------------------------------------|
|                                                | <b>Bias<br/>(95% limits of agreement)</b> | <b>Bias<br/>(95% limits of agreement)</b> |
| LVMI on cardiac MRI (g)                        | -1.1 (-7.5 - +5.3)                        | -2.2 (-7.0 - +2.5)                        |
| Aortic distensibility (x10mmHg <sup>-3</sup> ) | -0.02 (-0.38 - +0.34)                     | -0.15 (-0.81- +0.50)                      |
| LV EF on cardiac MRI (%)                       | 2.3 (-1.9 - +6.4)                         | -1.4 (-5.8 - +3.2)                        |
| LV EDVI cardiac MRI (ml/m <sup>2</sup> )       | -2.2 (-10.0 - +5.8)                       | 0.58 (-2.0 - +3.2)                        |
| LV EF on echo (%)                              | -0.5 (-9.0 - +7.8)                        | 1.9 (-4.9 - +8.7)                         |
| TDI Sm velocity (cm/s)                         | 0.2 (-1.2 - +0.6)                         | -0.04 (-1.1 - +1.0)                       |
| TDI Em velocity (cm/s)                         | -0.2 (-1.2 - +0.7)                        | -0.2 (-0.7 - +0.3)                        |
| LV global Strain (%)                           | -0.3 (-2.3 - +1.7)                        | -0.19 (-4.5 - +4.5)                       |

Analysis by the Bland-Altman method; absolute difference and limits of agreement.

EDVI; end-diastolic volume index, EF; ejection fraction, Em; early myocardial velocity, LV; left ventricle, Sm systolic myocardial velocity, TDI; tissue Doppler imaging

## **8.5 DISCUSSION**

### **8.5.1 Reproducibility of measurements compared to previous works**

The results of this study have demonstrated high reproducibility for the measurements of LV mass, LV function and aortic stiffness selected for intra-operator and inter-operator variability studies. These data support the hypothesis that measured differences are due to “real” changes in the cardiovascular system. The variability between experienced operators in this study is consistent with previous reports using similar imaging protocols in patients with cardiovascular disease and healthy controls (Grothues et al., 2002;Hudsmith et al., 2005).

Assessment of intra-observer variability was important to ensure consistency of analysis over time. As a single operator was responsible for analysing all imaging data in this thesis, it was important to demonstrate that changes observed reflected the effects of disease or treatment and not inconsistencies in analysis. These data were further supported by high reproducibility in the inter-operator variability studies which confirmed consistent analysis techniques between observers and helped exclude potential bias introduced using a single operator.

Reproducibility was higher for a single operator than between two different operators reflecting the improved consistency of a single individual. Reproducibility was generally higher for healthy controls than patients with structurally abnormal hearts and is consistent with previous reports (Grothues et al., 2002). This is postulated to reflect better cardiac function (better endocardial / epicardial definition, smaller LV volumes) and image quality (breath-holding, slower heart rates, less arrhythmias). Also in keeping with previous reports, we demonstrated higher reproducibility with cardiac MRI than with echocardiography for assessment of LV mass dimensions and function (Bellenger et al., 2000;Myerson et al., 2002). Cardiac MRI has become established as the gold standard method for these prognostic

markers as it provides a 3D assessment of the ventricle and is not limited by geometric assumptions or acoustic windows as in 2D echo (Myerson et al., 2002;Pennell et al., 2004). However, this was not a study examining the accuracy and precision of individual imaging techniques. It was designed to identify the disparity and error introduced by quantitative analysis. The combination of reliable operators and gold standard imaging modalities, allows the smallest level of change which can be reliably detected to be defined. This is important for repeated follow-up examinations, monitoring the response to the therapeutic intervention (such as in the CRIB-II study ) in the individual patient as well as helping to determine the sample sizes required for future clinical trials.

### **8.5.1 Limitations**

In view of time and financial constraints we were unable to assess inter-study (test-retest) variation in which the same individual would undergo a second identical examination separated by a specified time interval. This determines the variability associated with physiological variation and operator related changes in patient positioning and scan re-planning. These potential sources of error were minimised this using set protocols and with the same individual acquiring all echo (NE) and cardiac MRI (RPS) images. The study sample size was small but was based on feasibility and is similar to that used in previous studies. Finally, I acknowledge that reproducibility is limited not only by measurement error but by extrinsic variables such as altered haemodynamics, medications and disease status.

### **8.5.2 Conclusion**

Intra and inter-operator reproducibility in this study was high in keeping with previous reports using the same imaging modalities. It provides greater reliability of observed changes in the parameters under measure throughout this thesis.

## **CHAPTER 9**

### **CONCLUSIONS AND FUTURE DIRECTIONS**



## 9.1 Summary of thesis findings

In the work described in this thesis, I sought firstly to determine whether large artery stiffness and ventricular function are abnormal in patients with early stage chronic kidney disease (CKD) and secondly to determine the effect of treatment with the mineralocorticoid receptor blocker (MRB) spironolactone on these prognostically important parameters of cardiovascular function.

Work presented in chapter 3 demonstrated that compared to healthy controls, patients with early stage CKD had reduced aortic distensibility and that this displayed a graded positive relationship with eGFR. In addition, left ventricular (LV) systolic and diastolic stiffness were increased and LV diastolic function was impaired. In chapter 4, I demonstrated that regional LV systolic function was also abnormal in early stage CKD compared to healthy controls. In both chapters, abnormalities in regional LV systolic, diastolic and aortic stiffness were present in the absence of alterations in conventional markers of LV systolic function. These data provided evidence in support of the hypothesis that early stage CKD is characterised by abnormalities of arterial and LV function.

Chapters 5 and 6 examined the role of spironolactone in improving the abnormalities of arterial and LV function described in chapters 3 and 4. Patients with early stage CKD randomised to treatment with spironolactone for 40 weeks had marked reductions in LV mass and aortic stiffness. In addition, LV regional systolic and diastolic function were improved. There was also a significant fall in peripheral and central blood pressures. Spironolactone reduced serological markers of collagen turnover and cardiac peptides which are known to be increased with cardiac dysfunction. These were the first published data demonstrating

improvements in intermediate prognostic markers of cardiovascular function with spironolactone in early stage CKD.

Chapter 7 described detailed biochemical, safety and tolerability data for the use of spironolactone in early stage CKD from the CRIB-II study described in chapter 5. Rates of serious hyperkalaemia were < 1 % and there was no significant change in eGFR compared to placebo. Patients at higher risk of biochemical complications were identified by a baseline potassium  $\geq 5.0$  mmol/L and an eGFR  $\leq 45$  ml/min/1.73m<sup>2</sup>. These data add to the small but increasing number of publications reporting the safe use of spironolactone in early CKD, provided patients are carefully selected and receive close biochemical monitoring.

Taken together, this work suggests that: i) arterial stiffness is increased at the very earliest stages of CKD and has a graded relationship with eGFR; ii) ventricular function is impaired in early stage CKD, iii) aldosterone might be the important stimulus at the mineralocorticoid receptor which mediates arterial and cardiac injury in early stage CKD and iv) mineralocorticoid receptor blockade is an effective method of preventing or reversing these adverse markers of cardiovascular function. These data complement animal work which has demonstrated that aldosterone can induce arterial injury and cardiac fibrosis (Rocha & Stier, Jr., 2001). It is therefore possible that increased arterial stiffness in early CKD subsequently promotes the development of adverse changes in cardiac function which are almost universally present at more advanced stages of CKD.

## **9.2 Future directions**

Data described in this thesis have proposed that aldosterone is a key factor mediating cardiovascular disease in early CKD. There is a potential need for a large multi-centre clinical outcome randomised controlled trial of MRB therapy in CKD. However, several clinical and experimental issues must first be addressed.

### **9.2.1 Were the improvements in cardiovascular structure and function observed with spironolactone in chronic kidney disease due to sustained reductions in blood pressure?**

In the CRIB-II study described in this thesis, spironolactone used at a modest dose of 25mg daily was highly effective at lowering blood pressure in patients with blood pressure considered within the normal range. Mean 24 hour systolic blood pressure was reduced by -6 mmHg with spironolactone compared to -1 mmHg with placebo. The effects of this sustained fall in blood pressure over 40 weeks of treatment with spironolactone may well have contributed to the observed changes in outcome measures. I was able to examine the possible influence of blood pressure changes on values such as LV mass and PWV using statistical models such as linear regression analyses. However, the use of an inactive placebo means it is not possible to completely exclude the possibility that the effects observed with spironolactone were mediated by sustained reductions in blood pressure.

To address this question, it would be necessary to re-design and then repeat the CRIB-II study with the same primary end-points but substituting in an active placebo for comparison with spironolactone. The question then arises as to which drug would be the most suitable

comparator. In theory, the most important property of the active control drug is that it should produce an equal fall in blood pressure to spironolactone. In the CRIB-II study, spironolactone produced an average reduction in office blood pressure of -11 / -6 mmHg over 40 weeks. These reductions occurred despite baseline blood pressure within the normal range, a low incidence of LV hypertrophy and established use of an average of 2.1 other anti-hypertensive agents at enrolment. These data are consistent with previous reports using spironolactone. In a sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA), patients with resistant hypertension (mean baseline office blood pressure 157 / 86 mmHg) treated with spironolactone in addition to  $\geq 3$  established alternative antihypertensive agents, had an average reduction in office blood pressure of -22 / -10 mmHg over a median of 1.3 years treatment (Chapman et al., 2007). The efficacy of spironolactone suggests that identifying a suitable active control agent which is as effective might in practice be a difficult requirement.

It is clear from these data that the response of any individual to specific blood pressure lowering drugs is difficult to predict and will vary according to the state of the numerous factors that control arterial blood pressure. This point is illustrated by the ASCOT study which recruited nearly 20,000 patients with hypertension to two different blood pressure lowering regimens. An amlodipine based regimen was compared to atenolol based regimen in a stepped design with perindopril and doxazosin or bendroflumethiazide and doxazosin added respectively to achieve the specified blood pressure goal (Dahlof et al., 2005). Despite the size of the study and 5.5 year follow-up period, patients randomised to the amlodipine based regimen had lower blood pressure values throughout the study with an average difference of 2.7 / 1.9 mmHg which might have mediated the observed reductions in cardiovascular mortality.

It is clear that producing an equal reduction in systolic blood pressure with spironolactone and the chosen active placebo agent would be difficult to achieve. An alternative approach to prevent differential blood pressures between treatment groups would be to withdraw patients who do not achieve a pre-specified reduction in systolic blood pressure from baseline within a set time frame after enrolment. An additional “add-on” antihypertensive regimen, applicable to both treatment groups could also be used to try and achieve a pre-specified blood pressure reduction.

Active control agents which could be considered for such a study include:

i) Calcium channel blockers (CCBs) - in a retrospective analysis from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), 1,516 high risk hypertensive patients with  $eGFR \leq 60 \text{ ml/min/1.73m}^2$  were randomised to amlodipine (Rahman et al., 2005). This agent was safely tolerated over 6 years of follow-up. Rates of ESKD did not differ compared to the ACE inhibitor lisinopril and the thiazide diuretic chlorthalidone. The antihypertensive effects were very similar to spironolactone in the CRIB-II study, with a mean reduction in office systolic blood pressure of -12 mmHg over 6 years (Rahman et al., 2005). However, potential limitations if used as a comparator include the high number of patients already taking CCBs which might make recruitment difficult and the absence of an equivalent diuretic effect.

ii) Alpha adrenergic blockers – a small pilot study of 15 patients showed the selective alpha 1-adrenoceptor antagonist doxazosin to significantly reduced blood pressure in ESKD (Mori et al., 2001). An average reduction of -30 mmHg in systolic pressure was reported with an associated reduction in proteinuria and improvement in eGFR. This agent was further assessed in the prospective ALLHAT study which compared doxazosin, lisinopril and amlodipine to chlorthalidone in high risk hypertensive patients (Davis et al., 1996). Over

9,000 patients were randomised to doxazosin with a mean reduction in systolic blood pressure of 8 mmHg from 145 mmHg to 137 mmHg after 4 years of treatment. However, systolic blood pressure was consistently greater than was observed with chlorthalidone by an average of 3 mmHg over follow-up. This arm of ALLHAT was prematurely terminated after an interim analysis demonstrated an increased risk of heart failure with doxazosin independent of higher systolic blood pressure (ALLHAT Collaborative, 2000). This data and other associated side effects such as urinary frequency in women make this agent an unsuitable comparator to spironolactone.

iii) Thiazides - in the retrospective analysis of ALLHAT, patients with  $eGFR \leq 60$  ml/min/1.73m<sup>2</sup> randomised to the thiazide diuretic chlorthalidone had an equivalent safety profile, rates of progression to ESKD and blood pressure reduction as lisinopril and amlodipine. Over 6 years, office systolic blood pressure was reduced by a mean of 12 mmHg from 146 mmHg to 134 mmHg (Rahman et al., 2005). Other benefits when considered as a comparator agent include its ready availability and a similar diuretic / natriuretic action to that of spironolactone. Potential problems include reduced efficacy in reducing extracellular volume at  $eGFR \leq 45$  ml/min/1.73m<sup>2</sup> (Wilcox, 2002) and hypokalaemia. The latter action reflects increased delivery of sodium to the distal tubule and increased potassium excretion, thereby making blinding of therapy difficult compared to spironolactone.

### **9.2.2 Could improvements in cardiovascular structure and function observed with spironolactone be replicated with more selective mineralocorticoid receptor antagonists with potentially better side effect profiles?**

In the CRIB-II study, we elected to study the effect of spironolactone, a non-selective blocker of mineralocorticoid, glucocorticoid, progesterone and androgen receptors. At the time this study was originally devised this low cost, generic preparation was readily available and had been shown to be highly efficacious in experimental and clinical studies of hypertension (Chapman et al., 2007), heart failure (Pitt et al., 1999) and proteinuria (Chrysostomou & Becker, 2001). In addition, a dosing study in patients with heart failure had established that 12.5mg and 25mg daily were pharmacologically effective at blocking MRs, decreasing atrial natriuretic peptide levels without the risks of serious hyperkalaemia associated with doses of 50mg daily or greater (The RALES Investigators, 1996).

Despite the clinical efficacy of spironolactone, its use remains hindered by side-effects related to its lack of selectivity toward androgen, progesterone and glucocorticoid steroid receptors. These limitations warrant further study of alternative more selective MRBs and alternative new modes of MR blockade.

The effects of improved MR selectivity could be assessed by repeating the CRIB-II study presented in chapter 5 with eplerenone (and an active placebo agent as discussed in section 9.3.1). This highly selective MRB offers some potential advantages over spironolactone (Ravis et al., 2005): i) it has reduced potential for androgen and progesterone receptor side effects such as gynaecomastia and breast pain observed with spironolactone (gynaecomastia 9% spironolactone vs. 0.5% eplerenone; breast pain 2% spironolactone vs. 0.8% eplerenone); ii) It has a beneficial pharmacokinetic profile for CKD with a short elimination half life and extensive metabolism into an inactive form with < 5% of active

metabolite being renally excreted; iii) Despite having a lower binding affinity for the MR than spironolactone, it is efficacious in reducing end-organ damage in both the experimental (Blasi et al., 2003; Rocha et al., 2002a) and clinical research settings (Pitt et al., 2003a). However, rates of hyperkalaemia are similar to spironolactone (serious hyperkalaemia in RALES 2%, EPHESUS 5.5%, EMPHASIS 2.5%) when combined with an ACE inhibitor or ARB and would therefore necessitate close biochemical monitoring throughout a study.

An alternative approach which holds promise for an improved safety profile would be to study new modes of MR blockade using non-steroidal MRBs. Biochemical studies have identified BR-4628, a dihydropyridine-based prototype MR antagonist with high in-vitro and in-vivo MR potency as well as selectivity with respect to the other steroid hormone receptors (Fagart et al., 2010). In DOCA-salt hypertensive rat models, BR-4628 significantly reduced pro-inflammatory genes and proteins (MCP-1), proteinuria and renal hypertrophy independent of an anti-hypertensive effect (Schupp et al., 2011). SM-368229 (*N*-4,4-dimethyl-2-thioxo-1,4-dihydro-2*H*-3,1-benzoxazin-6-yl-thiophene-2-sulfonamide) is another potent oral non-steroidal MR antagonist under development. In-vivo it was four and six times more selective for the MR than spironolactone or eplerenone respectively. In-vitro it was 18 times more selective for the MR than androgen, progesterone or glucocorticoid receptors, producing no anti-androgenic or progesterone related side effects (Nariai et al., 2011). Development of non-steroidal MRBs remains at a very early stage but in the future might allow a direct “head to head” comparison with spironolactone and eplerenone as part of a “proof of concept” study.



### **9.2.3 Additional data is required to support the widespread use of mineralocorticoid receptor antagonists in early stage chronic kidney disease?**

Data published after RALES confirmed that use of spironolactone in a non-clinical trial setting where patient selection and biochemical monitoring are less rigorous, the incidence of hyperkalaemia and related complications are increased (Juurlink et al., 2004). Therefore before the use of MRBs can be contemplated in routine clinical practice for early CKD, it will be necessary to perform a long-term multi-centre trial with prospectively defined end-points for safety data and local monitoring of renal function and serum potassium.

Any future study should be designed with advice from a Clinical Trials Unit to ensure it is adequately powered with long-term follow-up in order to address the drug safety and tolerability concerns which have limited MRB use to date. Additional information required would include:

i) Optimum dosing strategy in CKD: Work contributing to this thesis used spironolactone at a dose of 25mg daily which was reduced to alternate day therapy if potassium levels were  $\geq 5.5$  mmol/L. This protocol was based on a dosing study from patients with heart failure in which 12.5mg and 25mg daily of spironolactone were shown to be effective at blocking aldosterone with acceptable levels of hyperkalaemia (5% and 13% respectively). Most studies examining the use of spironolactone in reducing proteinuria have also used 25mg daily (Chrysostomou & Becker, 2001; Sato et al., 2003). Eminent figures in this field have suggested regimens including low-dose spironolactone 12.5mg daily (Bombardier et al., 2009b) or using a titration schedule of 25 mg on alternate days and increasing after 1 month if potassium remains  $\leq 5.0$  mmol/L (Pitt, 2009). However, objective evidence of an optimum dose in CKD would necessitate a randomised crossover trial in which

patients would be allocated in random order to different doses of spironolactone with monitoring of serum and urinary potassium as well as components of the RAAS and other steroid metabolites.

ii) Optimum monitoring schedule in CKD: Studies using MRBs in CKD have not reported consistent monitoring regimens. Work in this thesis performed biochemical monitoring more frequently than in RALES in the first month of treatment, reflecting initial concerns about hyperkalaemia and worsening renal dysfunction in CKD. Data in chapter 7 demonstrated an increase in potassium over the first four weeks of treatment which remained stable thereafter with no episodes of serious hyperkalaemia. This suggests a role for frequent monitoring in the immediate weeks after MRB treatment is initiated. In the longer term, a regimen in keeping with that used for ACE inhibitors and ARBs such as 3 monthly for the first year and 6 monthly thereafter might be appropriate. A dosing study should also help define this schedule.

Work in this thesis and by other groups would suggest that pre-treatment factors (outlined in section 1.9.3.3 and in chapter 7) can be defined which predict an increased risk of developing hyperkalaemia with MRBs (Khosla et al., 2009). Future work could also test the role for new oral potassium binders. These agents might provide a new therapeutic strategy for the prevention of hyperkalaemia in high risk individuals including patients with diabetes and CKD. A recent study of patients with heart failure on an ACE inhibitor and /or ARB, randomised patients to treatment with spironolactone 25mg daily plus placebo or the oral polymeric potassium binder RLY5016 for 4 weeks (Pitt et al., 2011). This study is of particular relevance to data presented in this thesis as it included two groups at increased risk of hyperkalaemia; 50% of patients had an eGFR  $\leq 60$  ml/min/1.73m<sup>2</sup> and a third of patients had diabetes. Despite concomitant initiation of spironolactone, RLY5016 reduced mean

serum potassium within two days of treatment and levels remained consistently lower over follow-up with an overall mean reduction of 0.45 mmol/L compared to placebo. The incidence of hyperkalaemia (defined as  $\geq 5.5$  mmol/L) was also significantly reduced. In patients with CKD the mean reduction in serum potassium with RLY5016 was 0.52 mmol/L and the incidence of hyperkalaemia was 7% compared to 39% with placebo. Longer term studies and dosing regimens need to be examined for RLY5016. Furthermore the risks of associated hypokalaemia (potassium  $\leq 4.0$  mmol/L) need to be addressed. Nearly 50% of patients with CKD developed hypokalaemia in the potassium binder study which is highly significant in light of recent data demonstrating increased rates of mortality and progression to ESKD in patients with mild-moderate CKD who had a potassium  $\leq 4.0$  mmol/L (Korgaonkar et al., 2010).

iii) Active post-marketing surveillance will be critical in any future studies examining use of MRBs in CKD. Participating centres would be required to maintain a system of adverse event reporting and risk assessment reporting to identify the incidence of known and new adverse events. These data would need to be assessed regularly by an independent monitoring committee and relayed to the Medicines and Healthcare products Regulatory Agency.

#### **9.2.4 What other mechanisms might reduce arterial stiffness and potentially improve cardiovascular structure and function in early stage chronic kidney disease?**

Work presented in this thesis specifically addresses the role of MR mediated effects on arterial stiffness and ventricular structure and function. Other mechanisms undoubtedly contribute to the complex biology of arterial stiffness. Thus treatment strategies which target

alternative mechanisms might also improve arterial stiffness in early CKD. Examples might include:

1. Disorders of mineral-bone metabolism: elevated serum phosphate levels mediate renal damage and accelerate renal decline. In rat models of uraemia, high dietary phosphate was associated with calcium and phosphate deposition in the renal tubules and interstitium with interstitial oedema and fibrosis evident on histology (Haut et al., 1980). More recently, serum phosphate levels (still within the normal reference range) have emerged as a powerful risk factor for cardiovascular mortality and arterial stiffness in the general population and in ESKD (Dhingra et al., 2007; Kestenbaum et al., 2005). Deposition of calcium-phosphate mineral in the vasculature promotes osteogenic transformation of vascular smooth muscle cells and the development of vascular medial calcification. The extent of calcification correlates with severity of arterial stiffness and is attenuated in the aorta and coronary arteries with treatment by non-calcium-based phosphate binders like sevelamer hydrochloride (Cozzolino et al., 2003). Phosphate might also exert direct fibrotic effects on myocardium and is an independent determinant of LV mass in ESKD. Indeed, treatment with a daily dialysis regimen and associated reductions in phosphate levels independently reduced LV mass over 12 months compared to conventional dialysis regimens (Achinger and Ayus, 2006).

In this thesis it has been postulated that improvements in cardiac structure and function with spironolactone might reflect preceding reductions in arterial stiffness. Thus an alternative strategy using treatments which lower serum phosphate by either dietary or pharmacological means could be examined in a randomised controlled trial to test the hypothesis that they also improve arterial stiffness in early CKD.

2. There has also been interest in novel therapeutic strategies which specifically target structural components within the arterial wall known to adversely affect extracellular matrix remodelling and increase arterial stiffness. Increased levels of advanced glycation end (AGE) products in CKD are postulated to contribute to increased arterial stiffness by the formation of irreversible covalent cross-links between collagen and elastin in the arterial medial layer. Future studies could extend early data investigating the role of: i) inhibitors of advanced glycation end (AGE) products such as aminoguanidine, a nucleophilic hydrazine which decreases the formation of aortic collagen cross-linkages produced by the formation of AGEs (Brownlee et al., 1986). ii) Crosslink breakers such as alagebrium, phenyl-4,5-dimethylthazolium chloride (ALT-711 a thiazolium derivative) which specifically inhibits the irreversible cross-links between glycation products . In vivo efficacy of ALT-711 has been confirmed by experiments performed in rats, showing that the increased arterial stiffness associated with diabetes can be reversed by a short term treatment with this cross-link breaker (Wolffenbuttel et al., 1998).

3. Matrix metalloproteinases (MMP) inhibitors might offer an alternative strategy in reducing arterial remodelling in CKD. Matrix metalloproteinases are endopeptidase enzymes which increase arterial stiffness by degradation of elastic laminae and induction of calcium deposition. In CKD, MMP levels increase progressively with advancing stages of disease (Chung et al., 2009). Potential therapeutic strategies might include attempts to up-regulate endogenous tissue inhibitors (TIMPS) or the use of exogenous agents to inhibit MMP expression or activity. Doxycycline, a non-specific inhibitor can modulate cellular proliferation, migration and matrix remodelling through its ability to inhibit MMPs. It has been used to improve elastic fibre integrity in the thoracic aorta in Marfan syndrome (Chung

et al., 2007) and in animal models of lung disease lymphangioleiomyomatosis (Moir et al., 2011).

### **9.3 Un-answered experimental considerations**

#### **9.3.1 What is the mechanism of action of salt in aldosterone mediated cardiovascular injury?**

A significant contribution of work in this thesis was designed from background experimental studies examining the effects of exogenous aldosterone in animal models. When combined with a high salt diet, aldosterone consistently induced severe myocardial and renal vascular damage characterised by cardiac hypertrophy, fibrosis, proteinuria and vascular inflammatory injury independent of blood pressure elevation. Furthermore, end-organ damage was markedly reduced by the addition of MR antagonists (spironolactone, eplerenone) and / or adrenalectomy without significant changes in blood pressure. These data have led investigators to conclude that aldosterone mediates its adverse effects by a humoral response rather than as a result of haemodynamic changes secondary to hypertension (Lacolley et al., 2001; Rocha & Stier, Jr., 2001).

A consistent feature of these models was the need for concomitant high salt intake. Indeed hypertension and cardiac fibrosis were not observed with exogenous aldosterone and a low-salt diet despite increases in plasma aldosterone and severely reduced urinary sodium / potassium ratio (Brilla & Weber, 1992). The reverse is also true; adrenalectomy reversed fibrotic responses in angiotensin II / salt loaded rats, indicating a key role for MR signalling (Rocha et al., 2002a). Thus synergistic activity between activated MRs and co-factors such as salt appears to be important in mediating its pathophysiological effects.

Clinical conditions such as heart failure and liver disease which currently use MRBs are characterised by sodium overload. A small pilot study in ESKD examined changes in serum aldosterone in response to changes in extracellular fluid status at various stages of dialysis (Bomback et al., 2009a). Compared to healthy volunteers, patients on dialysis showed much less effective suppression of aldosterone levels during extracellular volume expansion. Thus inappropriately high aldosterone levels (aldosterone escape) relative to sodium and volume status might explain why MRBs proved efficacious in the CRIB-II study and in the small number of studies examining their effects on reducing proteinuria in CKD.

An alternative hypothesis is that the high salt load associated with disordered aldosterone homeostasis might sensitise the MR and cardiovascular system to the effects of mineralocorticoids. Further studies examining salt manipulation and its effect on aldosterone levels and markers of cardiovascular injury in CKD are warranted. This might be examined in a double blind, randomised crossover trial in which euvolaemic patients with CKD stage 2-4 are allocated to high, normal and low salt intakes (with appropriate wash out periods). Comparative fluid status could be assessed using established non-invasive techniques such as multiple frequency bioimpedance spectroscopy and 24 hour urinary sodium excretion (a surrogate marker for extracellular volume). Plasma and urinary components of the RAAS could be measured along with markers of vascular inflammation, endothelial dysfunction and arterial stiffness with different salt intakes.

### **9.3.2 What is the role of physiological glucocorticoids in mineralocorticoid receptor activation?**

The action of endogenous glucocorticoids at the MR in cardiomyocytes continues to be debated. Further studies are clearly justified to elucidate the underlying molecular mechanisms by which glucocorticoid-MR complexes mediate effects on the cardiovascular system. At present the working hypothesis is that over 90% of MRs in cardiomyocytes are occupied (but not activated) by endogenous glucocorticoids which act as MR antagonist. This reflects high intracellular levels of glucocorticoids (relative to mineralocorticoids) and low levels of 11 $\beta$ -HSD2. However with tissue damage, ROS generation, oxidative stress and low levels or inhibition of 11 $\beta$ -HSD2, the cellular redox state is altered (reduced intracellular NADH). This appears to modulate the action of the glucocorticoid so that it activates the MR and therefore mimics the action of aldosterone (Funder, 2005; Mihailidou et al., 2009). The molecular mechanisms responsible for these apparent opposing effects are unresolved.

Chronic kidney disease is characterised by increased oxidative stress and declining levels of 11 $\beta$ -HSD2 with advancing stages of renal disease (Quinkler et al., 2005). Hence, it is likely that MR-mediated cardiac injury in CKD is to some extent mediated by glucocorticoid-induced MR activation. The adverse effects mediated by the glucocorticoid-MR complex might also explain why cardiac mortality is increased in patients with normal endogenous plasma aldosterone levels (Tomaschitz et al., 2010). In clinical studies of heart failure, the efficacy of MRBs might reflect blockade of the action of both aldosterone and glucocorticoids. Furthermore, it has been postulated that occupancy of the MR by spironolactone may also induce expression of protective factors over and above its role in excluding aldosterone and glucocorticoids (Chun et al., 2002).



The role of the glucocorticoid receptor (GR) in mediating cardiovascular injury also warrants further examination. In a recent experimental study, the potent synthetic glucocorticoid dexamethasone (which has a low affinity for the MR) produced similar myocardial injury and cardiomyocyte apoptosis to aldosterone and cortisol. Furthermore, spironolactone prevented the deleterious actions of all three hormones (Mihailidou et al., 2009). At present it remains unclear if spironolactone acts at both the MR and GR (despite a lower affinity for this receptor) while little is known about the distribution of these receptors and their signalling ligands in cardiomyocytes and fibroblasts.

Some of these questions could be addressed by experimental work using primary cultures of adult rat cardiomyocytes (Lewis et al., 2004). It would be interesting to examine the basal expression levels of the MR, GR and 11 $\beta$ HSD type 1 and 2 in rat cardiomyocytes. The presence of functional GRs and MRs in cardiomyocytes could be examined by competitive binding experiments using increasing concentrations of titrated dexamethasone and aldosterone in the presence and absence of unlabelled ligand or specific MR (eplerenone) and GR antagonists (/RU38486). Also of interest are the specific effects of glucocorticoids and mineralocorticoids on the regulation of established genes involved in the development and modulation of cardiac fibrosis including: Angiotensin II receptor 1, matrix metalloproteinase 2 and 9 and macrophage chemoattractant protein 1 in rat primary cultures. In the longer term, a proof of concept study could be performed using in vivo cardiac biopsies of patients with left ventricular hypertrophy. This might define the expression of MR / GR and marker genes of hypertrophy, inflammation and fibrosis.

## 9.4 Conclusion

The initial results from the CRIB-II study, future planned studies and on-going development of newer selective therapeutic agents will allow for continued investigation of the role of a variety of MRBs in chronic kidney disease. There is potential for these agents to be an effective therapeutic strategy in CKD in much the same way as we have seen in heart failure and after myocardial infarction. In the words of the eminent Professor Bertram Pitt “we can be cautiously optimistic that use of mineralocorticoid receptor blockers in addition to an ACE inhibitor or angiotensin receptor blocker will reduce the mortality and morbidity associated with chronic kidney disease, as well as prevent its progression to end-stage renal disease with all of its health-care and health-cost consequences”.

## **APPENDICIES**

### **PUBLICATIONS ARISING FROM THIS THESIS**

## REFERENCES

- Achinger, S. G. and Ayus, J. C. (2006) Left ventricular hypertrophy: is hyperphosphatemia among dialysis patients a risk factor? **J.Am.Soc.Nephrol.**, 17 (12 Suppl 3): p. S255-S261
- Adeney, K. L., Siscovick, D. S., Ix, J. H. et al (2009) Association of serum phosphate with vascular and valvular calcification in moderate CKD. **J.Am.Soc.Nephrol.**, 20 (2): 381-387
- Agabiti-Rosei, E., Mancia, G., O'Rourke, M. F. et al (2007) Central blood pressure measurements and antihypertensive therapy: a consensus document. **Hypertension**, 50 (1): 154-160
- Airaksinen, K. E., Salmela, P. I., Linnaluoto, M. K. et al (1993) Diminished arterial elasticity in diabetes: association with fluorescent advanced glycosylation end products in collagen. **Cardiovasc.Res.**, 27 (6): 942-945
- ALLHAT Collaborative (2000) Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). ALLHAT Collaborative Research Group. **JAMA**, 283 (15): 1967-1975
- Amann, K., Breitbach, M., Ritz, E. et al (1998) Myocyte/capillary mismatch in the heart of uremic patients. **J.Am.Soc.Nephrol.**, 9 (6): 1018-1022
- Amann, K., Neuss, R., Ritz, E. et al (1995) Changes of vascular architecture independent of blood pressure in experimental uremia. **Am.J.Hypertens.**, 8 (4 Pt 1): 409-417
- Amundsen, B. H., Helle-Valle, T., Edvardsen, T. et al (2006) Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging. **J.Am.Coll.Cardiol.**, 47 (4): 789-793
- Aoki, J., Ikari, Y., Nakajima, H. et al (2005) Clinical and pathologic characteristics of dilated cardiomyopathy in hemodialysis patients. **Kidney Int.**, 67 (1): 333-340
- Aoun, S., Blacher, J., Safar, M. E. et al (2001) Diabetes mellitus and renal failure: effects on large artery stiffness. **J.Hum.Hypertens.**, 15 (10): 693-700
- Armstrong WF, R. T. (2010) **Feigenbaum's Echocardiography**. 7th edn. Philadelphia: Lippincott Williams & Wilkins.

Arriza, J. L., Weinberger, C., Cerelli, G. et al (1987) Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. **Science**, 237 (4812): 268-275

Avolio, A. P., Chen, S. G., Wang, R. P. et al (1983) Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. **Circulation**, 68 (1): 50-58

Axel, L. and Dougherty, L. (1989) MR imaging of motion with spatial modulation of magnetization. **Radiology**, 171 (3): 841-845

Baigent C (2010) "Study of Heart And Renal Protection". In: **American Society of Nephrology**.

Baigent, C., Burbury, K. and Wheeler, D. (2000) Premature cardiovascular disease in chronic renal failure. **Lancet**, 356 (9224): 147-152

Bakris, G. L., Siomos, M., Richardson, D. et al (2000) ACE inhibition or angiotensin receptor blockade: impact on potassium in renal failure. VAL-K Study Group. **Kidney Int.**, 58 (5): 2084-2092

Bakris, G. L. and Weir, M. R. (2000) Angiotensin-converting enzyme inhibitor-associated elevations in serum creatinine: is this a cause for concern? **Arch.Intern.Med.**, 160 (5): 685-693

Baksi, A. J., Treibel, T. A., Davies, J. E. et al (2009) A meta-analysis of the mechanism of blood pressure change with aging. **J.Am.Coll.Cardiol.**, 54 (22): 2087-2092

Baltabaeva, A., Marciniak, M., Bijmens, B. et al (2008) Regional left ventricular deformation and geometry analysis provides insights in myocardial remodelling in mild to moderate hypertension. **Eur.J.Echocardiogr.**, 9 (4): 501-508

Barna, I., Keszei, A. and Dunai, A. (1998) Evaluation of Meditech ABPM-04 ambulatory blood pressure measuring device according to the British Hypertension Society protocol. **Blood Press Monit.**, 3 (6): 363-368

Bellenger, N. G., Burgess, M. I., Ray, S. G. et al (2000) Comparison of left ventricular ejection fraction and volumes in heart failure by echocardiography, radionuclide ventriculography and cardiovascular magnetic resonance; are they interchangeable? **Eur.Heart J.**, 21 (16): 1387-1396

Berger, R., Huelsman, M., Strecker, K. et al (2002) B-type natriuretic peptide predicts sudden death in patients with chronic heart failure. **Circulation**, 105 (20): 2392-2397

Bianchi, S., Bigazzi, R. and Campese, V. M. (2006) Long-term effects of spironolactone on proteinuria and kidney function in patients with chronic kidney disease. **Kidney Int.**, 70 (12): 2116-2123

- Bianchi, S., Bigazzi, R. and Campese, V. M. (2010) Intensive versus conventional therapy to slow the progression of idiopathic glomerular diseases. **Am.J.Kidney Dis.**, 55 (4): 671-681
- Blacher, J., Asmar, R., Djane, S. et al (1999a) Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. **Hypertension**, 33 (5): 1111-1117
- Blacher, J., Demuth, K., Guerin, A. P. et al (1998) Influence of biochemical alterations on arterial stiffness in patients with end-stage renal disease. **Arterioscler.Thromb.Vasc.Biol.**, 18 (4): 535-541
- Blacher, J., Guerin, A. P., Pannier, B. et al (1999b) Impact of aortic stiffness on survival in end-stage renal disease. **Circulation**, 99 (18): 2434-2439
- Blanco-Rivero, J., Cachofeiro, V., Lahera, V. et al (2005) Participation of prostacyclin in endothelial dysfunction induced by aldosterone in normotensive and hypertensive rats. **Hypertension**, 46 (1): 107-112
- Bland, J. M. and Altman, D. G. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. **Lancet**, 1 (8476): 307-310
- Blasi, E. R., Rocha, R., Rudolph, A. E. et al (2003) Aldosterone/salt induces renal inflammation and fibrosis in hypertensive rats. **Kidney Int.**, 63 (5): 1791-1800
- Bomback, A. S. and Klemmer, P. J. (2007) The incidence and implications of aldosterone breakthrough. **Nat.Clin.Pract.Nephrol.**, 3 (9): 486-492
- Bomback, A. S., Kshirsagar, A. V., Amamoo, M. A. et al (2008a) Change in proteinuria after adding aldosterone blockers to ACE inhibitors or angiotensin receptor blockers in CKD: a systematic review. **Am.J.Kidney Dis.**, 51 (2): 199-211
- Bomback, A. S., Kshirsagar, A. V., Amamoo, M. A. et al (2008b) Change in proteinuria after adding aldosterone blockers to ACE inhibitors or angiotensin receptor blockers in CKD: a systematic review. **Am.J.Kidney Dis.**, 51 (2): 199-211
- Bomback, A. S., Kshirsagar, A. V., Ferris, M. E. et al (2009a) Disordered aldosterone-volume relationship in end-stage kidney disease. **J.Renin.Angiotensin.Aldosterone.Syst.**, 10 (4): 230-236
- Bomback, A. S., Kshirsagar, A. V. and Klemmer, P. J. (2009b) Renal aspirin: will all patients with chronic kidney disease one day take spironolactone? **Nat.Clin.Pract.Nephrol.**, 5 (2): 74-75
- Brenner, B. M., Cooper, M. E., de, Z. D. et al (2001) Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. **N.Engl.J.Med.**, 345 (12): 861-869
- Briet, M., Bozec, E., Laurent, S. et al (2006) Arterial stiffness and enlargement in mild-to-moderate chronic kidney disease. **Kidney Int.**, 69 (2): 350-357

Briet, M. and Schiffrin, E. L. (2010) Aldosterone: effects on the kidney and cardiovascular system. **Nat.Rev.Nephrol.**, 6 (5): 261-273

Brilla, C. G. and Weber, K. T. (1992) Reactive and reparative myocardial fibrosis in arterial hypertension in the rat. **Cardiovasc.Res.**, 26 (7): 671-677

Brilla, C. G., Zhou, G., Matsubara, L. et al (1994) Collagen metabolism in cultured adult rat cardiac fibroblasts: response to angiotensin II and aldosterone. **J.Mol.Cell Cardiol.**, 26 (7): 809-820

British Heart Foundation (2011). **British Heart Foundation Heart Stats [online]**

Brown, D. W., Giles, W. H. and Croft, J. B. (2000) Left ventricular hypertrophy as a predictor of coronary heart disease mortality and the effect of hypertension. **Am.Heart J.**, 140 (6): 848-856

Brown, N. J. (2008) Aldosterone and vascular inflammation. **Hypertension**, 51 (2): 161-167

Brownlee, M., Vlassara, H., Kooney, A. et al (1986) Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. **Science**, 232 (4758): 1629-1632

Cachofeiro, V., Goicochea, M., de Vinuesa, S. G. et al (2008) Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. **Kidney Int.Suppl.** (111): p. S4-S9

Camargo, M. J., von, L. N., Campbell, W. G., Jr. et al (1993) Control of blood pressure and end-organ damage in maturing salt-loaded stroke-prone spontaneously hypertensive rats by oral angiotensin II receptor blockade. **J.Hypertens.**, 11 (1): 31-40

Casas, J. P., Chua, W., Loukogeorgakis, S. et al (2005) Effect of inhibitors of the renin-angiotensin system and other antihypertensive drugs on renal outcomes: systematic review and meta-analysis. **Lancet**, 366 (9502): 2026-2033

Catena, C., Colussi, G., Lapenna, R. et al (2007) Long-term cardiac effects of adrenalectomy or mineralocorticoid antagonists in patients with primary aldosteronism. **Hypertension**, 50 (5): 911-918

Chadban, S. J., Briganti, E. M., Kerr, P. G. et al (2003) Prevalence of kidney damage in Australian adults: The AusDiab kidney study. **J.Am.Soc.Nephrol.**, 14 (7 Suppl 2): p. S131-S138

Chan, M. Y., Wong, H. B., Ong, H. Y. et al (2008) Prognostic value of left atrial size in chronic kidney disease. **Eur.J.Echocardiogr.**, 9 (6): 736-740

Chapman, N., Dobson, J., Wilson, S. et al (2007) Effect of spironolactone on blood pressure in subjects with resistant hypertension. **Hypertension**, 49 (4): 839-845

- Chen, C. H., Fetics, B., Nevo, E. et al (2001) Noninvasive single-beat determination of left ventricular end-systolic elastance in humans. **J.Am.Coll.Cardiol.**, 38 (7): 2028-2034
- Chen, C. H., Nakayama, M., Nevo, E. et al (1998) Coupled systolic-ventricular and vascular stiffening with age: implications for pressure regulation and cardiac reserve in the elderly. **J.Am.Coll.Cardiol.**, 32 (5): 1221-1227
- Chen, C. H., Nevo, E., Fetics, B. et al (1997) Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. **Circulation**, 95 (7): 1827-1836
- Chrysostomou, A. and Becker, G. (2001) Spironolactone in addition to ACE inhibition to reduce proteinuria in patients with chronic renal disease. **N.Engl.J.Med.**, 345 (12): 925-926
- Chun, T. Y., Bloem, L. and Pratt, J. H. (2002) Spironolactone increases integrin beta3 gene expression in kidney and heart muscle cells. **Mol.Cell Endocrinol.**, 194 (1-2): 175-182
- Chung, A. W., Au, Y. K., Sandor, G. G. et al (2007) Loss of elastic fiber integrity and reduction of vascular smooth muscle contraction resulting from the upregulated activities of matrix metalloproteinase-2 and -9 in the thoracic aortic aneurysm in Marfan syndrome. **Circ.Res.**, 101 (5): 512-522
- Chung, A. W., Yang, H. H., Kim, J. M. et al (2009) Upregulation of matrix metalloproteinase-2 in the arterial vasculature contributes to stiffening and vasomotor dysfunction in patients with chronic kidney disease. **Circulation**, 120 (9): 792-801
- Cockcroft, D. W. and Gault, M. H. (1976) Prediction of creatinine clearance from serum creatinine. **Nephron**, 16 (1): 31-41
- Connell, J. M. and Davies, E. (2005) The new biology of aldosterone. **J.Endocrinol.**, 186 (1): 1-20
- Coresh, J., Selvin, E., Stevens, L. A. et al (2007) Prevalence of chronic kidney disease in the United States. **JAMA**, 298 (17): 2038-2047
- Cosentino, F. and Luscher, T. F. (1998) Endothelial dysfunction in diabetes mellitus. **J.Cardiovasc.Pharmacol.**, 32 Suppl 3 p. S54-S61
- Cozzolino, M., Staniforth, M. E., Liapis, H. et al (2003) Sevelamer hydrochloride attenuates kidney and cardiovascular calcifications in long-term experimental uremia. **Kidney Int.**, 64 (5): 1653-1661
- Cruz, C. S., Cruz, A. A. and Marcilio de Souza, C. A. (2003) Hyperkalaemia in congestive heart failure patients using ACE inhibitors and spironolactone. **Nephrol.Dial.Transplant.**, 18 (9): 1814-1819



Culleton, B. F., Larson, M. G., Wilson, P. W. et al (1999) Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. **Kidney Int.**, 56 (6): 2214-2219

Dahlof, B., Pennert, K. and Hansson, L. (1992) Reversal of left ventricular hypertrophy in hypertensive patients. A metaanalysis of 109 treatment studies. **Am.J.Hypertens.**, 5 (2): 95-110

Dahlof, B., Sever, P. S., Poulter, N. R. et al (2005) Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomised controlled trial. **Lancet**, 366 (9489): 895-906

Davis, B. R., Cutler, J. A., Gordon, D. J. et al (1996) Rationale and design for the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). ALLHAT Research Group. **Am.J.Hypertens.**, 9 (4 Pt 1): 342-360

de Denuis S., Tardif, J. C., White, M. et al (2006) Quantification of the risk and predictors of hyperkalemia in patients with left ventricular dysfunction: a retrospective analysis of the Studies of Left Ventricular Dysfunction (SOLVD) trials. **Am.Heart J.**, 152 (4): 705-712

Del, V. L., Procaccio, M., Viganò, S. et al (2007) Mechanisms of disease: The role of aldosterone in kidney damage and clinical benefits of its blockade. **Nat.Clin.Pract.Nephrol.**, 3 (1): 42-49

Devereux, R. B., Alonso, D. R., Lutas, E. M. et al (1986) Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. **Am.J.Cardiol.**, 57 (6): 450-458

Devereux, R. B., Dahlof, B., Gerds, E. et al (2004) Regression of hypertensive left ventricular hypertrophy by losartan compared with atenolol: the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) trial. **Circulation**, 110 (11): 1456-1462

Dhaun, N., Macintyre, I. M., Melville, V. et al (2009) Blood pressure-independent reduction in proteinuria and arterial stiffness after acute endothelin-a receptor antagonism in chronic kidney disease. **Hypertension**, 54 (1): 113-119

Dhingra, R., Sullivan, L. M., Fox, C. S. et al (2007) Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. **Arch.Intern.Med.**, 167 (9): 879-885

Doherty, N. E. and Siegel, R. J. (1985) Cardiovascular manifestations of systemic lupus erythematosus. **Am.Heart J.**, 110 (6): 1257-1265

Drazner, M. H. (2011) The progression of hypertensive heart disease. **Circulation**, 123 (3): 327-334

Duprez, D. A., De Buyzere, M. L., Rietzschel, E. R. et al (1998) Inverse relationship between aldosterone and large artery compliance in chronically treated heart failure patients. **Eur.Heart J.**, 19 (9): 1371-1376

Edvardsen, T., Gerber, B. L., Garot, J. et al (2002) Quantitative assessment of intrinsic regional myocardial deformation by Doppler strain rate echocardiography in humans: validation against three-dimensional tagged magnetic resonance imaging. **Circulation**, 106 (1): 50-56

Edwards, C. R., Stewart, P. M., Burt, D. et al (1988) Localisation of 11 beta-hydroxysteroid dehydrogenase--tissue specific protector of the mineralocorticoid receptor. **Lancet**, 2 (8618): 986-989

Endemann, D. H. and Schiffrin, E. L. (2004) Endothelial dysfunction. **J.Am.Soc.Nephrol.**, 15 (8): 1983-1992

Fagart, J., Hillisch, A., Huyet, J. et al (2010) A new mode of mineralocorticoid receptor antagonism by a potent and selective nonsteroidal molecule. **J.Biol.Chem.**, 285 (39): 29932-29940

Farquharson, C. A. and Struthers, A. D. (2000) Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. **Circulation**, 101 (6): 594-597

Farquharson, C. A. and Struthers, A. D. (2002) Aldosterone induces acute endothelial dysfunction in vivo in humans: evidence for an aldosterone-induced vasculopathy. **Clin.Sci.(Lond)**, 103 (4): 425-431

Fellstrom, B. C., Jardine, A. G., Schmieder, R. E. et al (2009) Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. **N.Engl.J.Med.**, 360 (14): 1395-1407

Finn, J. P., Nael, K., Deshpande, V. et al (2006) Cardiac MR imaging: state of the technology. **Radiology**, 241 (2): 338-354

Foley, R. N. and Parfrey, P. S. (1998) Cardiovascular disease and mortality in ESRD. **J.Nephrol.**, 11 (5): 239-245

Foley, R. N., Parfrey, P. S., Harnett, J. D. et al (1995a) Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. **Kidney Int.**, 47 (1): 186-192

Foley, R. N., Parfrey, P. S., Harnett, J. D. et al (1995b) The prognostic importance of left ventricular geometry in uremic cardiomyopathy. **J.Am.Soc.Nephrol.**, 5 (12): 2024-2031

Foley, R. N., Parfrey, P. S. and Sarnak, M. J. (1998) Clinical epidemiology of cardiovascular disease in chronic renal disease. **Am.J.Kidney Dis.**, 32 (5 Suppl 3): p. S112-S119

- Fox, K. M. (2003) Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). **Lancet**, 362 (9386): 782-788
- Fraser, R. (2003) Studying genes and the development of cardiac hypertrophy: convenient intermediate phenotypes in man. **J.Hypertens.**, 21 (5): 873-874
- Fullerton, M. J. and Funder, J. W. (1994) Aldosterone and cardiac fibrosis: in vitro studies. **Cardiovasc.Res.**, 28 (12): 1863-1867
- Funder, J. W. (2004) Aldosterone, mineralocorticoid receptors and vascular inflammation. **Mol.Cell Endocrinol.**, 217 (1-2): 263-269
- Funder, J. W. (2005) RALES, EPHESUS and redox. **J.Steroid Biochem.Mol.Biol.**, 93 (2-5): 121-125
- Ganau, A., Devereux, R. B., Roman, M. J. et al (1992) Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. **J.Am.Coll.Cardiol.**, 19 (7): 1550-1558
- Ganong WF (2005) **Review of Medical Physiology**. 22nd edn. California: McGraw-Hill Professional, Lange Medical Books.
- Ghali, J. K., Liao, Y., Simmons, B. et al (1992) The prognostic role of left ventricular hypertrophy in patients with or without coronary artery disease. **Ann.Intern.Med.**, 117 (10): 831-836
- Go, A. S., Chertow, G. M., Fan, D. et al (2004) Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. **N.Engl.J.Med.**, 351 (13): 1296-1305
- Goodfield, N. E., Bhandari, S., Plant, W. D. et al (1995) Cardiac involvement in Wegener's granulomatosis. **Br.Heart J.**, 73 (2): 110-115
- Greene, E. L., Kren, S. and Hostetter, T. H. (1996) Role of aldosterone in the remnant kidney model in the rat. **J.Clin.Invest**, 98 (4): 1063-1068
- Groenink, M., de, R. A., Mulder, B. J. et al (1998) Changes in aortic distensibility and pulse wave velocity assessed with magnetic resonance imaging following beta-blocker therapy in the Marfan syndrome. **Am.J.Cardiol.**, 82 (2): 203-208
- Grothues, F., Smith, G. C., Moon, J. C. et al (2002) 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.**, 90 (1): 29-34
- Guerin, A. P., Blacher, J., Pannier, B. et al (2001) Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. **Circulation**, 103 (7): 987-992

Gunnett, C. A., Lund, D. D., McDowell, A. K. et al (2005) Mechanisms of inducible nitric oxide synthase-mediated vascular dysfunction. **Arterioscler.Thromb.Vasc.Biol.**, 25 (8): 1617-1622

Hage, F. G., Venkataraman, R., Zoghbi, G. J. et al (2009) The scope of coronary heart disease in patients with chronic kidney disease. **J.Am.Coll.Cardiol.**, 53 (23): 2129-2140

Haut, L. L., Alfrey, A. C., Guggenheim, S. et al (1980) Renal toxicity of phosphate in rats. **Kidney Int.**, 17 (6): 722-731

Hayashi, M., Tsutamoto, T., Wada, A. et al (2003) Immediate administration of mineralocorticoid receptor antagonist spironolactone prevents post-infarct left ventricular remodeling associated with suppression of a marker of myocardial collagen synthesis in patients with first anterior acute myocardial infarction. **Circulation**, 107 (20): 2559-2565

Hayashi, S. Y., Rohani, M., Lindholm, B. et al (2006) Left ventricular function in patients with chronic kidney disease evaluated by colour tissue Doppler velocity imaging. **Nephrol.Dial.Transplant.**, 21 (1): 125-132

Hene, R. J., Boer, P., Koomans, H. A. et al (1982) Plasma aldosterone concentrations in chronic renal disease. **Kidney Int.**, 21 (1): 98-101

Herzog, C. A., Ma, J. Z. and Collins, A. J. (1998) Poor long-term survival after acute myocardial infarction among patients on long-term dialysis. **N.Engl.J.Med.**, 339 (12): 799-805

Herzog, C. A., Mangrum, J. M. and Passman, R. (2008) Sudden cardiac death and dialysis patients. **Semin.Dial.**, 21 (4): 300-307

Hill, J. A., Karimi, M., Kutschke, W. et al (2000) Cardiac hypertrophy is not a required compensatory response to short-term pressure overload. **Circulation**, 101 (24): 2863-2869

Hillebrand, U., Schillers, H., Riethmuller, C. et al (2007) Dose-dependent endothelial cell growth and stiffening by aldosterone: endothelial protection by eplerenone. **J.Hypertens.**, 25 (3): 639-647

Himathongkam, T., Dluhy, R. G. and Williams, G. H. (1975) Potassium-aldosterone-renin interrelationships. **J.Clin.Endocrinol.Metab.**, 41 (1): 153-159

Horita, Y., Taura, K., Taguchi, T. et al (2006) Aldosterone breakthrough during therapy with angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers in proteinuric patients with immunoglobulin A nephropathy. **Nephrology (Carlton.)**, 11 (5): 462-466

Hudsmith, L. E., Petersen, S. E., Francis, J. M. et al (2005) Normal human left and right ventricular and left atrial dimensions using steady state free precession magnetic resonance imaging. **J.Cardiovasc.Magn Reson.**, 7 (5): 775-782

Hunold, P., Vogt, F. M., Heemann, U. W. et al (2003) Myocardial mass and volume measurement of hypertrophic left ventricles by MRI--study in dialysis patients examined before and after dialysis. **J.Cardiovasc.Magn Reson.**, 5 (4): 553-561

Ibels, L. S., Alfrey, A. C., Huffer, W. E. et al (1979) Arterial calcification and pathology in uremic patients undergoing dialysis. **Am.J.Med.**, 66 (5): 790-796

Ikeda, U., Kanbe, T., Nakayama, I. et al (1995) Aldosterone inhibits nitric oxide synthesis in rat vascular smooth muscle cells induced by interleukin-1 beta. **Eur.J.Pharmacol.**, 290 (2): 69-73

Jofre, R., Rodriguez-Benitez, P., Lopez-Gomez, J. M. et al (2006) Inflammatory syndrome in patients on hemodialysis. **J.Am.Soc.Nephrol.**, 17 (12 Suppl 3): p. S274-S280

Juurlink, D. N., Mamdani, M. M., Lee, D. S. et al (2004) Rates of hyperkalemia after publication of the Randomized Aldactone Evaluation Study. **N.Engl.J.Med.**, 351 (6): 543-551

Kass, D. A. (2002) Age-related changes in ventricular-arterial coupling: pathophysiologic implications. **Heart Fail.Rev.**, 7 (1): 51-62

Kato, T. S., Noda, A., Izawa, H. et al (2004) Discrimination of nonobstructive hypertrophic cardiomyopathy from hypertensive left ventricular hypertrophy on the basis of strain rate imaging by tissue Doppler ultrasonography. **Circulation**, 110 (25): 3808-3814

Kawaguchi, M., Hay, I., Fetcs, B. et al (2003) Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations. **Circulation**, 107 (5): 714-720

Kelly, R. P., Ting, C. T., Yang, T. M. et al (1992a) Effective arterial elastance as index of arterial vascular load in humans. **Circulation**, 86 (2): 513-521

Kelly, R. P., Tunin, R. and Kass, D. A. (1992b) Effect of reduced aortic compliance on cardiac efficiency and contractile function of in situ canine left ventricle. **Circ.Res.**, 71 (3): 490-502

Kestenbaum, B., Sampson, J. N., Rudser, K. D. et al (2005) Serum phosphate levels and mortality risk among people with chronic kidney disease. **J.Am.Soc.Nephrol.**, 16 (2): 520-528

Ketteler, M., Bongartz, P., Westenfeld, R. et al (2003) Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. **Lancet**, 361 (9360): 827-833

Ketteler, M. and Giachelli, C. (2006) Novel insights into vascular calcification. **Kidney Int.Suppl**, (105): p. S5-S9

- Khosla, N., Kalaitzidis, R. and Bakris, G. L. (2009) Predictors of hyperkalemia risk following hypertension control with aldosterone blockade. **Am.J.Nephrol.**, 30 (5): 418-424
- Kim, R. J., Wu, E., Rafael, A. et al (2000) The use of contrast-enhanced magnetic resonance imaging to identify reversible myocardial dysfunction. **N.Engl.J.Med.**, 343 (20): 1445-1453
- Kimoto, E., Shoji, T., Shinohara, K. et al (2006) Regional arterial stiffness in patients with type 2 diabetes and chronic kidney disease. **J.Am.Soc.Nephrol.**, 17 (8): 2245-2252
- Koren, M. J., Devereux, R. B., Casale, P. N. et al (1991) Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. **Ann.Intern.Med.**, 114 (5): 345-352
- Korgaonkar, S., Tilea, A., Gillespie, B. W. et al (2010) Serum potassium and outcomes in CKD: insights from the RRI-CKD cohort study. **Clin.J.Am.Soc.Nephrol.**, 5 (5): 762-769
- Koyama, J., Ray-Sequin, P. A. and Falk, R. H. (2003) Longitudinal myocardial function assessed by tissue velocity, strain, and strain rate tissue Doppler echocardiography in patients with AL (primary) cardiac amyloidosis. **Circulation**, 107 (19): 2446-2452
- Lacolley, P., Safar, M. E., Lucet, B. et al (2001) Prevention of aortic and cardiac fibrosis by spironolactone in old normotensive rats. **J.Am.Coll.Cardiol.**, 37 (2): 662-667
- Lam, C. S., Roger, V. L., Rodeheffer, R. J. et al (2007) Cardiac structure and ventricular-vascular function in persons with heart failure and preserved ejection fraction from Olmsted County, Minnesota. **Circulation**, 115 (15): 1982-1990
- Lang, R. M., Bierig, M., Devereux, R. B. et al (2005) Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. **J.Am.Soc.Echocardiogr.**, 18 (12): 1440-1463
- Lariviere, R., Day, R. and Schiffrin, E. L. (1993) Increased expression of endothelin-1 gene in blood vessels of deoxycorticosterone acetate-salt hypertensive rats. **Hypertension**, 21 (6 Pt 2): 916-920
- Laurent, S., Caviezel, B., Beck, L. et al (1994) Carotid artery distensibility and distending pressure in hypertensive humans. **Hypertension**, 23 (6 Pt 2): 878-883
- Laurent, S., Cockcroft, J., Van, B. L. et al (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. **Eur.Heart J.**, 27 (21): 2588-2605
- Lemarie, C. A., Paradis, P. and Schiffrin, E. L. (2008) New insights on signaling cascades induced by cross-talk between angiotensin II and aldosterone. **J.Mol.Med.**, 86 (6): 673-678
- Levey, A. S., Beto, J. A., Coronado, B. E. et al (1998) Controlling the epidemic of cardiovascular disease in chronic renal disease: what do we know? What do we need to learn?

Where do we go from here? National Kidney Foundation Task Force on Cardiovascular Disease. **Am.J.Kidney Dis.**, 32 (5): 853-906

Levey, A. S., Bosch, J. P., Lewis, J. B. et al (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. **Ann.Intern.Med.**, 130 (6): 461-470

Levey, A. S., Coresh, J., Greene, T. et al (2006) Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. **Ann.Intern.Med.**, 145 (4): 247-254

Levey, A. S. and Stevens, L. A. (2010) Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. **Am.J.Kidney Dis.**, 55 (4): 622-627

Levy, D., Garrison, R. J., Savage, D. D. et al (1990) Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. **N.Engl.J.Med.**, 322 (22): 1561-1566

Lewis, C. J., Gong H., Brown M. J. et al (2004) Overexpression of beta 1-adrenoceptors in adult rat ventricular myocytes enhances CGP 12177A cardiostimulation: implications for 'putative' beta 4-adrenoceptor pharmacology. **Br J Pharmacol.**, 141 (5): 813-824

Lewis, J., Agodoa, L., Cheek, D. et al (2001) Comparison of cross-sectional renal function measurements in African Americans with hypertensive nephrosclerosis and of primary formulas to estimate glomerular filtration rate. **Am.J.Kidney Dis.**, 38 (4): 744-753

Lilitkarntakul, P., Dhaun, N., Melville, V. et al (2011) Blood pressure and not uraemia is the major determinant of arterial stiffness and endothelial dysfunction in patients with chronic kidney disease and minimal co-morbidity. **Atherosclerosis**, 216 (1): 217-225

London, G. M. (2000) Alterations of arterial function in end-stage renal disease. **Nephron**, 84 (2): 111-118

London, G. M. (2003) Cardiovascular disease in chronic renal failure: pathophysiologic aspects. **Semin.Dial.**, 16 (2): 85-94

London, G. M., Marchais, S. J., Guerin, A. P. et al (2004) Arterial stiffness: pathophysiology and clinical impact. **Clin.Exp.Hypertens.**, 26 (7-8): 689-699

London, G. M., Marchais, S. J., Metivier, F. et al (2000) Cardiovascular risk in end-stage renal disease: vascular aspects. **Nephrol.Dial.Transplant.**, 15 Suppl 5 97-104

London, G. M., Pannier, B., Guerin, A. P. et al (2001) Alterations of left ventricular hypertrophy in and survival of patients receiving hemodialysis: follow-up of an interventional study. **J.Am.Soc.Nephrol.**, 12 (12): 2759-2767

- Maceira, A. M., Prasad, S. K., Khan, M. et al (2006) Normalized left ventricular systolic and diastolic function by steady state free precession cardiovascular magnetic resonance. **J.Cardiovasc.Magn Reson.**, 8 (3): 417-426
- Mak, G. J., Ledwidge, M. T., Watson, C. J. et al (2009) Natural history of markers of collagen turnover in patients with early diastolic dysfunction and impact of eplerenone. **J.Am.Coll.Cardiol.**, 54 (18): 1674-1682
- Mall, G., Huther, W., Schneider, J. et al (1990) Diffuse intermyocardiocytic fibrosis in uraemic patients. **Nephrol.Dial.Transplant.**, 5 (1): 39-44
- Mann, J. F., Gerstein, H. C., Pogue, J. et al (2001) Renal insufficiency as a predictor of cardiovascular outcomes and the impact of ramipril: the HOPE randomized trial. **Ann.Intern.Med.**, 134 (8): 629-636
- Mark, P. B., Johnston, N., Groenning, B. A. et al (2006) Redefinition of uremic cardiomyopathy by contrast-enhanced cardiac magnetic resonance imaging. **Kidney Int.**, 69 (10): 1839-1845
- Masoudi, F. A., Gross, C. P., Wang, Y. et al (2005) Adoption of spironolactone therapy for older patients with heart failure and left ventricular systolic dysfunction in the United States, 1998-2001. **Circulation**, 112 (1): 39-47
- Matsushita, K., Selvin, E., Bash, L. D. et al (2009) Change in estimated GFR associates with coronary heart disease and mortality. **J.Am.Soc.Nephrol.**, 20 (12): 2617-2624
- Matsushita, K., van, d., V, Astor, B. C. et al (2010) Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. **Lancet**, 375 (9731): 2073-2081
- McCullough, P. A., Agrawal, V., Danielewicz, E. et al (2008) Accelerated atherosclerotic calcification and Monckeberg's sclerosis: a continuum of advanced vascular pathology in chronic kidney disease. **Clin.J.Am.Soc.Nephrol.**, 3 (6): 1585-1598
- McEniery, C. M., Wilkinson, I. B. and Avolio, A. P. (2007) Age, hypertension and arterial function. **Clin.Exp.Pharmacol.Physiol**, 34 (7): 665-671
- McRobbie DW, M. E. G. M. P. M. (2007) **MRI From Picture to Proton**. 2nd edn. Cambridge: Cambridge University Press.
- Meier-Kriesche, H. U., Schold, J. D., Srinivas, T. R. et al (2004) Kidney transplantation halts cardiovascular disease progression in patients with end-stage renal disease. **Am.J.Transplant.**, 4 (10): 1662-1668
- Menon, V., Greene, T., Wang, X. et al (2005) C-reactive protein and albumin as predictors of all-cause and cardiovascular mortality in chronic kidney disease. **Kidney Int.**, 68 (2): 766-772



- Mercure, C., Yogi, A., Callera, G. E. et al (2008) Angiotensin(1-7) blunts hypertensive cardiac remodeling by a direct effect on the heart. **Circ.Res.**, 103 (11): 1319-1326
- Mihailidou, A. S. and Funder, J. W. (2005) Nongenomic effects of mineralocorticoid receptor activation in the cardiovascular system. **Steroids**, 70 (5-7): 347-351
- Mihailidou, A. S., Loan Le, T. Y., Mardini, M. et al (2009) Glucocorticoids activate cardiac mineralocorticoid receptors during experimental myocardial infarction. **Hypertension**, 54 (6): 1306-1312
- Min, L. J., Mogi, M., Li, J. M. et al (2005) Aldosterone and angiotensin II synergistically induce mitogenic response in vascular smooth muscle cells. **Circ.Res.**, 97 (5): 434-442
- Mitchell, G. F., Parise, H., Benjamin, E. J. et al (2004) Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. **Hypertension**, 43 (6): 1239-1245
- Mitsnefes, M. M., Daniels, S. R., Schwartz, S. M. et al (2000) Severe left ventricular hypertrophy in pediatric dialysis: prevalence and predictors. **Pediatr.Nephrol.**, 14 (10-11): 898-902
- Moir, L., Ng, H., Poniris, M. et al (2011) Doxycycline inhibits matrix metalloproteinase-2 secretion from TSC2-null mouse embryonic fibroblasts and lymphangioliomyomatosis cells. **Br.J.Pharmacol.**,
- Mor-Avi, V., Lang, R. M., Badano, L. P. et al (2011) Current and evolving echocardiographic techniques for the quantitative evaluation of cardiac mechanics: ASE/EAE consensus statement on methodology and indications endorsed by the Japanese Society of Echocardiography. **J.Am.Soc.Echocardiogr.**, 24 (3): 277-313
- Mori, Y., Matsubara, H., Nose, A. et al (2001) Safety and availability of doxazosin in treating hypertensive patients with chronic renal failure. **Hypertens.Res.**, 24 (4): 359-363
- Mottram, P. M., Haluska, B., Leano, R. et al (2004) Effect of aldosterone antagonism on myocardial dysfunction in hypertensive patients with diastolic heart failure. **Circulation**, 110 (5): 558-565
- Mottram, P. M., Haluska, B. A., Leano, R. et al (2005) Relation of arterial stiffness to diastolic dysfunction in hypertensive heart disease. **Heart**, 91 (12): 1551-1556
- Mourad, J. J., Pannier, B., Blacher, J. et al (2001) Creatinine clearance, pulse wave velocity, carotid compliance and essential hypertension. **Kidney Int.**, 59 (5): 1834-1841
- MRC/BHF (2002) MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. **Lancet**, 360 (9326): 7-22

Myerson, S. G., Bellenger, N. G. and Pennell, D. J. (2002) Assessment of left ventricular mass by cardiovascular magnetic resonance. **Hypertension**, 39 (3): 750-755

N'Gankam, V., Uehlinger, D., Dick, B. et al (2002) Increased cortisol metabolites and reduced activity of 11beta-hydroxysteroid dehydrogenase in patients on hemodialysis. **Kidney Int.**, 61 (5): 1859-1866

Nagueh, S. F., Appleton, C. P., Gillebert, T. C. et al (2009) Recommendations for the evaluation of left ventricular diastolic function by echocardiography. **J.Am.Soc.Echocardiogr.**, 22 (2): 107-133

Nariai, T., Fujita, K., Mori, M. et al (2011) SM-368229, a novel selective and potent non-steroidal mineralocorticoid receptor antagonist with strong urinary Na<sup>+</sup> excretion activity. **J.Pharmacol.Sci.**, 115 (3): 346-353

Naruse, M., Tanabe, A., Sato, A. et al (2002) Aldosterone breakthrough during angiotensin II receptor antagonist therapy in stroke-prone spontaneously hypertensive rats. **Hypertension**, 40 (1): 28-33

Nasir, K., Rosen, B. D., Kramer, H. J. et al (2007) Regional left ventricular function in individuals with mild to moderate renal insufficiency: the Multi-Ethnic Study of Atherosclerosis. **Am.Heart J.**, 153 (4): 545-551

National Collaborating Centre for Chronic Conditions (2008), **Chronic kidney disease: national clinical guideline for early identification and management in adults in primary and secondary care.** London, Royal College of Physicians

National Institute for Health and Clinical Excellence (2008), **Chronic Kidney Disease: Early identification and management of chronic kidney disease in adults in primary and secondary care.** London

National Kidney Foundation (2002) K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. **Am.J.Kidney Dis.**, 39 (2 Suppl 1): S1-266

O'Brien, E., Coats, A., Owens, P. et al (2000) Use and interpretation of ambulatory blood pressure monitoring: recommendations of the British hypertension society. **BMJ**, 320 (7242): 1128-1134

O'Rourke, M. F., Avolio, A., Safar, M. et al (2010) Changes in the central arterial pressure pulse with aging. **J.Am.Coll.Cardiol.**, 55 (19): p. 2183

O'Rourke, M. F. and Safar, M. E. (2005) Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy. **Hypertension**, 46 (1): 200-204

Ommen, S. R., Nishimura, R. A., Appleton, C. P. et al (2000) Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling

pressures: A comparative simultaneous Doppler-catheterization study. **Circulation**, 102 (15): 1788-1794

Palmer, B. R., Pilbrow, A. P., Frampton, C. M. et al (2008) Plasma aldosterone levels during hospitalization are predictive of survival post-myocardial infarction. **Eur.Heart J.**, 29 (20): 2489-2496

Pannier, B., Guerin, A. P., Marchais, S. J. et al (2005) Stiffness of capacitive and conduit arteries: prognostic significance for end-stage renal disease patients. **Hypertension**, 45 (4): 592-596

Parfrey, P. S. (2008) Inhibitors of the renin angiotensin system: proven benefits, unproven safety. **Ann.Intern.Med.**, 148 (1): 76-77

Parfrey, P. S., Foley, R. N., Harnett, J. D. et al (1996) Outcome and risk factors for left ventricular disorders in chronic uraemia. **Nephrol.Dial.Transplant.**, 11 (7): 1277-1285

Patrianakos, A. P., Karakitsos, D. N., de, G. E. et al (2006) Alteration of proximal aorta biophysical properties in patients with end stage renal disease. **Heart**, 92 (2): 228-232

Peng, X., Haldar, S., Deshpande, S. et al (2003) Wall stiffness suppresses Akt/eNOS and cytoprotection in pulse-perfused endothelium. **Hypertension**, 41 (2): 378-381

Pennell, D. J., Sechtem, U. P., Higgins, C. B. et al (2004) Clinical indications for cardiovascular magnetic resonance (CMR): Consensus Panel report. **Eur.Heart J.**, 25 (21): 1940-1965

Peterson, J. C., Adler, S., Burkart, J. M. et al (1995) Blood pressure control, proteinuria, and the progression of renal disease. The Modification of Diet in Renal Disease Study. **Ann.Intern.Med.**, 123 (10): 754-762

Petrie, M. C., Padmanabhan, N., McDonald, J. E. et al (2001) Angiotensin converting enzyme (ACE) and non-ACE dependent angiotensin II generation in resistance arteries from patients with heart failure and coronary heart disease. **J.Am.Coll.Cardiol.**, 37 (4): 1056-1061

Pfeffer, M. A., Braunwald, E., Moye, L. A. et al (1992) Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. **N.Engl.J.Med.**, 327 (10): 669-677

Pfeffer, M. A., McMurray, J. J., Velazquez, E. J. et al (2003) Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. **N.Engl.J.Med.**, 349 (20): 1893-1906

Pitt, B. (2009) Pharmacotherapy: Cardiovascular effects of aldosterone blockade in CKD. **Nat.Rev.Cardiol.**, 6 (11): 679-680

- Pitt, B., Anker, S. D., Bushinsky, D. A. et al (2011) Evaluation of the efficacy and safety of RLY5016, a polymeric potassium binder, in a double-blind, placebo-controlled study in patients with chronic heart failure (the PEARL-HF) trial. **Eur.Heart J.**, 32 (7): 820-828
- Pitt, B., Reichek, N., Willenbrock, R. et al (2003a) Effects of eplerenone, enalapril, and eplerenone/enalapril in patients with essential hypertension and left ventricular hypertrophy: the 4E-left ventricular hypertrophy study. **Circulation**, 108 (15): 1831-1838
- Pitt, B., Remme, W., Zannad, F. et al (2003b) Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. **N.Engl.J.Med.**, 348 (14): 1309-1321
- Pitt, B., Zannad, F., Remme, W. J. et al (1999) The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. **N.Engl.J.Med.**, 341 (10): 709-717
- Poggio, E. D., Wang, X., Greene, T. et al (2005) Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. **J.Am.Soc.Nephrol.**, 16 (2): 459-466
- Pu, Q., Neves, M. F., Viridis, A. et al (2003) Endothelin antagonism on aldosterone-induced oxidative stress and vascular remodeling. **Hypertension**, 42 (1): 49-55
- Qin, W., Rudolph, A. E., Bond, B. R. et al (2003) Transgenic model of aldosterone-driven cardiac hypertrophy and heart failure. **Circ.Res.**, 93 (1): 69-76
- Quaschnig, T., Krane, V., Metzger, T. et al (2001) Abnormalities in uremic lipoprotein metabolism and its impact on cardiovascular disease. **Am.J.Kidney Dis.**, 38 (4 Suppl 1): p. S14-S19
- Quinkler, M., Zehnder, D., Lепенies, J. et al (2005) Expression of renal 11beta-hydroxysteroid dehydrogenase type 2 is decreased in patients with impaired renal function. **Eur.J.Endocrinol.**, 153 (2): 291-299
- Rahman, M., Pressel, S., Davis, B. R. et al (2005) Renal outcomes in high-risk hypertensive patients treated with an angiotensin-converting enzyme inhibitor or a calcium channel blocker vs a diuretic: a report from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). **Arch.Intern.Med.**, 165 (8): 936-946
- Rakhit, D. J., Zhang, X. H., Leano, R. et al (2007) Prognostic role of subclinical left ventricular abnormalities and impact of transplantation in chronic kidney disease. **Am.Heart J.**, 153 (4): 656-664
- Ravis, W. R., Reid, S., Sica, D. A. et al (2005) Pharmacokinetics of eplerenone after single and multiple dosing in subjects with and without renal impairment. **J.Clin.Pharmacol.**, 45 (7): 810-821

- Redfield, M. M., Jacobsen, S. J., Borlaug, B. A. et al (2005) Age- and gender-related ventricular-vascular stiffening: a community-based study. **Circulation**, 112 (15): 2254-2262
- REIN Study (1997) Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). **Lancet**, 349 (9069): 1857-1863
- Reinders, A., Reggiori, F. and Shennan, A. H. (2006) Validation of the DINAMAP ProCare blood pressure device according to the international protocol in an adult population. **Blood Press Monit.**, 11 (5): 293-296
- Remuzzi, G., Cattaneo, D. and Perico, N. (2008) The aggravating mechanisms of aldosterone on kidney fibrosis. **J.Am.Soc.Nephrol.**, 19 (8): 1459-1462
- Rickard, A. J., Morgan, J., Tesch, G. et al (2009) Deletion of mineralocorticoid receptors from macrophages protects against deoxycorticosterone/salt-induced cardiac fibrosis and increased blood pressure. **Hypertension**, 54 (3): 537-543
- Robert, V., Van, T. N., Cheav, S. L. et al (1994) Increased cardiac types I and III collagen mRNAs in aldosterone-salt hypertension. **Hypertension**, 24 (1): 30-36
- Rocha, R., Chander, P. N., Khanna, K. et al (1998) Mineralocorticoid blockade reduces vascular injury in stroke-prone hypertensive rats. **Hypertension**, 31 (1 Pt 2): 451-458
- Rocha, R., Chander, P. N., Zuckerman, A. et al (1999) Role of aldosterone in renal vascular injury in stroke-prone hypertensive rats. **Hypertension**, 33 (1 Pt 2): 232-237
- Rocha, R., Martin-Berger, C. L., Yang, P. et al (2002a) Selective aldosterone blockade prevents angiotensin II/salt-induced vascular inflammation in the rat heart. **Endocrinology**, 143 (12): 4828-4836
- Rocha, R., Rudolph, A. E., Friedrich, G. E. et al (2002b) Aldosterone induces a vascular inflammatory phenotype in the rat heart. **Am.J.Physiol Heart Circ.Physiol**, 283 (5): p. H1802-H1810
- Rocha, R. and Stier, C. T., Jr. (2001) Pathophysiological effects of aldosterone in cardiovascular tissues. **Trends Endocrinol.Metab**, 12 (7): 308-314
- Rogerson, F. M. and Fuller, P. J. (2000) Mineralocorticoid action. **Steroids**, 65 (2): 61-73
- Safar, M. E., Blacher, J., Pannier, B. et al (2002a) Central pulse pressure and mortality in end-stage renal disease. **Hypertension**, 39 (3): 735-738
- Safar, M. E., Henry, O. and Meaume, S. (2002b) Aortic pulse wave velocity: an independent marker of cardiovascular risk. **Am.J.Geriatr.Cardiol.**, 11 (5): 295-298

- Salveti, M., Muiesan, M. L., Paini, A. et al (2007) Myocardial ultrasound tissue characterization in patients with chronic renal failure. **J.Am.Soc.Nephrol.**, 18 (6): 1953-1958
- Sarnak, M. J., Levey, A. S., Schoolwerth, A. C. et al (2003) Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. **Circulation**, 108 (17): 2154-2169
- Sasayama, S., Ross, J., Jr., Franklin, D. et al (1976) Adaptations of the left ventricle to chronic pressure overload. **Circ.Res.**, 38 (3): 172-178
- Sato, A., Hayashi, K., Naruse, M. et al (2003) Effectiveness of aldosterone blockade in patients with diabetic nephropathy. **Hypertension**, 41 (1): 64-68
- Satoh, M., Nakamura, M., Saitoh, H. et al (2002) Aldosterone synthase (CYP11B2) expression and myocardial fibrosis in the failing human heart. **Clin.Sci.(Lond)**, 102 (4): 381-386
- Savoia, C., Touyz, R. M., Amiri, F. et al (2008) Selective mineralocorticoid receptor blocker eplerenone reduces resistance artery stiffness in hypertensive patients. **Hypertension**, 51 (2): 432-439
- Schiffrin, E. L. (2006) Effects of aldosterone on the vasculature. **Hypertension**, 47 (3): 312-318
- Schiffrin, E. L., Franks, D. J. and Gutkowska, J. (1985) Effect of aldosterone on vascular angiotensin II receptors in the rat. **Can.J.Physiol Pharmacol.**, 63 (12): 1522-1527
- Schiffrin, E. L., Lipman, M. L. and Mann, J. F. (2007) Chronic kidney disease: effects on the cardiovascular system. **Circulation**, 116 (1): 85-97
- Schillaci, G., Pirro, M., Mannarino, M. R. et al (2006) Relation between renal function within the normal range and central and peripheral arterial stiffness in hypertension. **Hypertension**, 48 (4): 616-621
- Schillaci, G., Verdecchia, P., Porcellati, C. et al (2000) Continuous relation between left ventricular mass and cardiovascular risk in essential hypertension. **Hypertension**, 35 (2): 580-586
- Schjoedt, K. J., Andersen, S., Rossing, P. et al (2004) Aldosterone escape during blockade of the renin-angiotensin-aldosterone system in diabetic nephropathy is associated with enhanced decline in glomerular filtration rate. **Diabetologia**, 47 (11): 1936-1939
- Schlaich, M. P., Schobel, H. P., Hilgers, K. et al (2000) Impact of aldosterone on left ventricular structure and function in young normotensive and mildly hypertensive subjects. **Am.J.Cardiol.**, 85 (10): 1199-1206
- Schrier, R. W. (2010) Aldosterone 'escape' vs 'breakthrough'. **Nat.Rev.Nephrol.**, 6 (2): p. 61

- Schupp, N., Kolkhof, P., Queisser, N. et al (2011) Mineralocorticoid receptor-mediated DNA damage in kidneys of DOCA-salt hypertensive rats. **FASEB J.**, 25 (3): 968-978
- Schwarz, U., Buzello, M., Ritz, E. et al (2000) Morphology of coronary atherosclerotic lesions in patients with end-stage renal failure. **Nephrol.Dial.Transplant.**, 15 (2): 218-223
- Sengupta, P. P., Tajik, A. J., Chandrasekaran, K. et al (2008) Twist mechanics of the left ventricle: principles and application. **JACC.Cardiovasc.Imaging**, 1 (3): 366-376
- Shlipak, M. G., Sarnak, M. J., Katz, R. et al (2005) Cystatin C and the risk of death and cardiovascular events among elderly persons. **N.Engl.J.Med.**, 352 (20): 2049-2060
- Shoji, T., Emoto, M., Shinohara, K. et al (2001) Diabetes mellitus, aortic stiffness, and cardiovascular mortality in end-stage renal disease. **J.Am.Soc.Nephrol.**, 12 (10): 2117-2124
- Silvestre, J. S., Robert, V., Heymes, C. et al (1998) Myocardial production of aldosterone and corticosterone in the rat. Physiological regulation. **J.Biol.Chem.**, 273 (9): 4883-4891
- Simpson SA, T. J. (1953) The nature of the circulating hormones of the adrenal cortex in man. **Arch.Middx.Hosp.**, 3 (4): 209-218
- Simpson, H. J., Gandy, S. J., Houston, J. G. et al (2010) Left ventricular hypertrophy: reduction of blood pressure already in the normal range further regresses left ventricular mass. **Heart**, 96 (2): 148-152
- Sleight, P., Yusuf, S., Pogue, J. et al (2001) Blood-pressure reduction and cardiovascular risk in HOPE study. **Lancet**, 358 (9299): 2130-2131
- Snell, R. (1992) **Clinical Anatomy**. 4th edn. Boston: Little, Brown and Company.
- Stenvinkel, P., Ketteler, M., Johnson, R. J. et al (2005) IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia--the good, the bad, and the ugly. **Kidney Int.**, 67 (4): 1216-1233
- Stevens, L. A., Coresh, J., Feldman, H. I. et al (2007a) Evaluation of the modification of diet in renal disease study equation in a large diverse population. **J.Am.Soc.Nephrol.**, 18 (10): 2749-2757
- Stevens, P. E., O'Donoghue, D. J., de, L. S. et al (2007b) Chronic kidney disease management in the United Kingdom: NEOERICA project results. **Kidney Int.**, 72 (1): 92-99
- Stier, C. T., Jr., Benter, I. F., Ahmad, S. et al (1989) Enalapril prevents stroke and kidney dysfunction in salt-loaded stroke-prone spontaneously hypertensive rats. **Hypertension**, 13 (2): 115-121
- Stier, C. T., Jr., Chander, P. N. and Rocha, R. (2002) Aldosterone as a mediator in cardiovascular injury. **Cardiol.Rev.**, 10 (2): 97-107

- Stier, C. T., Jr., Mahboubi, K., DiPippo, V. A. et al (1992) The antiproteinuric action of enalapril in stroke-prone spontaneously hypertensive rats is unrelated to alterations in urinary prostaglandins. **J.Pharmacol.Exp.Ther.**, 260 (3): 1410-1415
- Struthers, A. D. (2004) Aldosterone blockade in cardiovascular disease. **Heart**, 90 (10): 1229-1234
- Takemoto, Y., Barnes, M. E., Seward, J. B. et al (2005) Usefulness of left atrial volume in predicting first congestive heart failure in patients > or = 65 years of age with well-preserved left ventricular systolic function. **Am.J.Cardiol.**, 96 (6): 832-836
- The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators (1993) Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators. **Lancet**, 342 (8875): 821-828
- The RALES Investigators (1996) Effectiveness of spironolactone added to an angiotensin-converting enzyme inhibitor and a loop diuretic for severe chronic congestive heart failure (the Randomized Aldactone Evaluation Study [RALES]). **Am.J.Cardiol.**, 78 (8): 902-907
- The SOLVD Investigators (1991) Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. The SOLVD Investigators. **N.Engl.J.Med.**, 325 (5): 293-302
- Tomaschitz, A., Pilz, S., Ritz, E. et al (2011) Association of plasma aldosterone with cardiovascular mortality in patients with low estimated GFR: the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study. **Am.J.Kidney Dis.**, 57 (3): 403-414
- Tomaschitz, A., Pilz, S., Ritz, E. et al (2010) Plasma aldosterone levels are associated with increased cardiovascular mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. **Eur.Heart J.**, 31 (10): 1237-1247
- Tonelli, M., Isles, C., Curhan, G. C. et al (2004) Effect of pravastatin on cardiovascular events in people with chronic kidney disease. **Circulation**, 110 (12): 1557-1563
- Tonelli, M., Wiebe, N., Culeton, B. et al (2006) Chronic kidney disease and mortality risk: a systematic review. **J.Am.Soc.Nephrol.**, 17 (7): 2034-2047
- Toussaint, N. D., Lau, K. K., Strauss, B. J. et al (2008) Associations between vascular calcification, arterial stiffness and bone mineral density in chronic kidney disease. **Nephrol.Dial.Transplant.**, 23 (2): 586-593
- Touyz, R. M. and Schiffrin, E. L. (2000) Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. **Pharmacol.Rev.**, 52 (4): 639-672



- Ueno, H., Koyama, H., Tanaka, S. et al (2008) Skin autofluorescence, a marker for advanced glycation end product accumulation, is associated with arterial stiffness in patients with end-stage renal disease. **Metabolism**, 57 (10): 1452-1457
- United States Renal Data System (2010), **National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases**, Annual Data Report. Bethesda, Md, National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases.
- Urheim, S., Edvardsen, T., Torp, H. et al (2000) Myocardial strain by Doppler echocardiography. Validation of a new method to quantify regional myocardial function. **Circulation**, 102 (10): 1158-1164
- Vaidya, V. S., Ozer, J. S., Dieterle, F. et al (2010) Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. **Nat.Biotechnol.**, 28 (5): 478-485
- Vallance, P., Leone, A., Calver, A. et al (1992) Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. **Lancet**, 339 (8793): 572-575
- van der Meer, R. W., Diamant, M., Westenberg, J. J. et al (2007) Magnetic resonance assessment of aortic pulse wave velocity, aortic distensibility, and cardiac function in uncomplicated type 2 diabetes mellitus. **J.Cardiovasc.Magn Reson.**, 9 (4): 645-651
- Van Vliet, B. N., Chafe, L. L. and Montani, J. P. (2003) Characteristics of 24 h telemetered blood pressure in eNOS-knockout and C57Bl/6J control mice. **J.Physiol**, 549 (Pt 1): 313-325
- Van, B. W., De, B. D., Verbeke, F. et al (2007) The glomerular filtration rate in an apparently healthy population and its relation with cardiovascular mortality during 10 years. **Eur.Heart J.**, 28 (4): 478-483
- Venugopal, S. K., Devaraj, S., Yuhanna, I. et al (2002) Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. **Circulation**, 106 (12): 1439-1441
- Vickery, S., Webb, M. C., Price, C. P. et al (2008) Prognostic value of cardiac biomarkers for death in a non-dialysis chronic kidney disease population. **Nephrol.Dial.Transplant.**, 23 (11): 3546-3553
- Wang, A. Y., Lam, C. W., Chan, I. H. et al (2010) Sudden cardiac death in end-stage renal disease patients: a 5-year prospective analysis. **Hypertension**, 56 (2): 210-216
- Wang, A. Y., Lam, C. W., Wang, M. et al (2007) Prognostic value of cardiac troponin T is independent of inflammation, residual renal function, and cardiac hypertrophy and dysfunction in peritoneal dialysis patients. **Clin.Chem.**, 53 (5): 882-889
- Wang, M., Yip, G., Yu, C. M. et al (2005a) Independent and incremental prognostic value of early mitral annulus velocity in patients with impaired left ventricular systolic function. **J.Am.Coll.Cardiol.**, 45 (2): 272-277

- Wang, M. C., Tsai, W. C., Chen, J. Y. et al (2005b) Stepwise increase in arterial stiffness corresponding with the stages of chronic kidney disease. **Am.J.Kidney Dis.**, 45 (3): 494-501
- Wanner, C., Krane, V., Marz, W. et al (2005) Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. **N.Engl.J.Med.**, 353 (3): 238-248
- Wanner, C. and Metzger, T. (2002) C-reactive protein a marker for all-cause and cardiovascular mortality in haemodialysis patients. **Nephrol.Dial.Transplant.**, 17 Suppl 8 29-32
- Weber, K. T. and Brilla, C. G. (1991b) Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. **Circulation**, 83 (6): 1849-1865
- Wehling, M. (1997) Specific, nongenomic actions of steroid hormones. **Annu.Rev.Physiol**, 59 365-393
- Wei, L., Struthers, A. D., Fahey, T. et al (2010) Spironolactone use and renal toxicity: population based longitudinal analysis. **BMJ**, 340 p. c1768
- Weidemann, F., Breunig, F., Beer, M. et al (2005) The variation of morphological and functional cardiac manifestation in Fabry disease: potential implications for the time course of the disease. **Eur.Heart J.**, 26 (12): 1221-1227
- Wen, C. P., Cheng, T. Y., Tsai, M. K. et al (2008) All-cause mortality attributable to chronic kidney disease: a prospective cohort study based on 462 293 adults in Taiwan. **Lancet**, 371 (9631): 2173-2182
- Wilcox, C. S. (2002) New insights into diuretic use in patients with chronic renal disease. **J.Am.Soc.Nephrol.**, 13 (3): 798-805
- Wilkinson, I. B., Franklin, S. S. and Cockcroft, J. R. (2004) Nitric oxide and the regulation of large artery stiffness: from physiology to pharmacology. **Hypertension**, 44 (2): 112-116
- Wilkinson, I. B., Hall, I. R., MacCallum, H. et al (2002a) Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. **Arterioscler.Thromb.Vasc.Biol.**, 22 (1): 147-152
- Wilkinson, I. B., MacCallum, H., Cockcroft, J. R. et al (2002b) Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity in vivo. **Br.J.Clin.Pharmacol.**, 53 (2): 189-192
- Williams, B., Lacy, P. S., Thom, S. M. et al (2006) Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. **Circulation**, 113 (9): 1213-1225
- Williams, G. H. (2005) Aldosterone biosynthesis, regulation, and classical mechanism of action. **Heart Fail.Rev.**, 10 (1): 7-13

- Willum-Hansen, T., Staessen, J. A., Torp-Pedersen, C. et al (2006) Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. **Circulation**, 113 (5): 664-670
- Wilson, P., Morgan, J., Funder, J. W. et al (2009) Mediators of mineralocorticoid receptor-induced profibrotic inflammatory responses in the heart. **Clin.Sci.(Lond)**, 116 (9): 731-739
- Wolffenbittel, B. H., Boulanger, C. M., Crijsns, F. R. et al (1998) Breakers of advanced glycation end products restore large artery properties in experimental diabetes. **Proc.Natl.Acad.Sci.U.S.A**, 95 (8): 4630-4634
- Yasmin, McEniery, C. M., Wallace, S. et al (2005) Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. **Arterioscler.Thromb.Vasc.Biol.**, 25 (2): p. 372
- Yip, G. W., Zhang, Y., Tan, P. Y. et al (2002) Left ventricular long-axis changes in early diastole and systole: impact of systolic function on diastole. **Clin.Sci.(Lond)**, 102 (5): 515-522
- Young, A. A. (1999) Model tags: direct three-dimensional tracking of heart wall motion from tagged magnetic resonance images. **Med.Image Anal.**, 3 (4): 361-372
- Young, M. and Funder, J. W. (2000) Aldosterone and the heart. **Trends Endocrinol.Metab**, 11 (6): 224-226
- Young, M., Head, G. and Funder, J. (1995) Determinants of cardiac fibrosis in experimental hypermineralocorticoid states. **Am.J.Physiol**, 269 (4 Pt 1): p. E657-E662
- Young, M. J., Moussa, L., Dilley, R. et al (2003) Early inflammatory responses in experimental cardiac hypertrophy and fibrosis: effects of 11 beta-hydroxysteroid dehydrogenase inactivation. **Endocrinology**, 144 (3): 1121-1125
- Yu, C. M., Sanderson, J. E., Marwick, T. H. et al (2007) Tissue Doppler imaging a new prognosticator for cardiovascular diseases. **J.Am.Coll.Cardiol.**, 49 (19): 1903-1914
- Yusuf, S., Sleight, P., Pogue, J. et al (2000) Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. **N.Engl.J.Med.**, 342 (3): 145-153
- Zannad, F., Alla, F., Dousset, B. et al (2000) Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evaluation study (RALES). Rales Investigators. **Circulation**, 102 (22): 2700-2706
- Zannad, F., McMurray, J. J., Krum, H. et al (2011) Eplerenone in patients with systolic heart failure and mild symptoms. **N.Engl.J.Med.**, 364 (1): 11-21

Zile, M. R. and Brutsaert, D. L. (2002) New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment. **Circulation**, 105 (12): 1503-1508

Zimmerli, L. U., Mark, P. B., Steedman, T. et al (2007) Vascular function in patients with end-stage renal disease and/or coronary artery disease: a cardiac magnetic resonance imaging study. **Kidney Int.**, 71 (1): 68-73

Zoccali, C., Benedetto, F. A., Maas, R. et al (2002) Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. **J.Am.Soc.Nephrol.**, 13 (2): 490-496

Zoccali, C., Benedetto, F. A., Mallamaci, F. et al (2004a) Left ventricular mass monitoring in the follow-up of dialysis patients: prognostic value of left ventricular hypertrophy progression. **Kidney Int.**, 65 (4): 1492-1498

Zoccali, C., Mallamaci, F., Benedetto, F. A. et al (2001) Cardiac natriuretic peptides are related to left ventricular mass and function and predict mortality in dialysis patients. **J.Am.Soc.Nephrol.**, 12 (7): 1508-1515

Zoccali, C., Mallamaci, F. and Tripepi, G. (2004b) Inflammatory proteins as predictors of cardiovascular disease in patients with end-stage renal disease. **Nephrol.Dial.Transplant.**, 19 Suppl 5 p. V67-V72

## **APPENDIX**