EFFECTS OF PHOSPHATE BINDING WITH SEVELAMER CARBONATE ON CARDIOVASCULAR STRUCTURE AND FUNCTION IN PATIENTS WITH EARLY CHRONIC KIDNEY DISEASE

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ABSTRACT

Serum phosphate has recently emerged as a cardiovascular risk factor in several populations, including patients with chronic kidney disease. Much of the adverse cardiovascular risk profile seen in chronic kidney disease can be attributed to structural heart disease, which appears to be driven by an increase in arterial stiffness. There is strong evidence linking phosphate to vascular calcification, which in turn causes arterial stiffening. In the following studies, phosphate is shown to be an independent predictor of renal function decline in patients with stage 2–4 chronic kidney disease. In addition, phosphate is shown to be independently associated with left ventricular mass, a predictor of cardiovascular morbidity and mortality. In the final study, the cardiovascular effects of reducing phosphate exposure with sevelamer carbonate, an oral, non-calcium-based phosphate binder, are assessed in a randomised, double blind, placebo-controlled trial of 120 patients with stage 3 chronic kidney disease. Although no demonstrable effects were seen on arterial stiffness, left ventricular mass, or left ventricular function, adherence to study medication was low given the high pill burden. Testing of this hypothesis may therefore require introduction of a therapy that effectively lowers phosphate exposure through a more acceptable dosing regimen.

DEDICATION

To my family

ACKNOWLEDGEMENTS

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EXTENT OF PERSONAL CONTRIBUTION

All work presented in this thesis was carried out by myself, with the following exceptions. The idea and protocol for the randomised controlled trial presented in this thesis was originally devised by Dr Charles Ferro, Dr Jonathan Townend and Dr Richard Steeds, with further development by myself. Application for approval by the research ethics committee and authorisation by the Medicines and Healthcare Regulatory Authority was sought by Dr Charles Ferro and myself and included the use of information provided by Genzyme Corporation. Approval from the study sponsor (University Hospitals Birmingham NHS Foundation Trust) was sought by Dr Charles Ferro and myself.

All cardiac magnetic resonance images were acquired by Dr Richard Steeds and myself with the assistance of Dr William Moody and Dr Nicola Edwards. Dual energy x-ray absorptiometry scans were analysed by Dr Nicola Crabtree. Assays for high sensitive C-reactive protein were performed by Dr Nadezhda Wall and myself. Assays for N-terminal pro-brain natriuretic peptide were performed by Professor Leong Ng at Leicester Royal Infirmary. Assays for fibroblast growth factor 23 and soluble klotho were performed by Dr Daniel Zehnder at the University of Warwick. Assays for vitamin D were performed under the supervision of Dr Anne Dawnay at University College London Hospitals NHS Foundation Trust. Statistical advice was provided by Dr Peter Nightingale at Queen Elizabeth Hospital Birmingham.

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95% CI	95% confidence interval
AASI	ambulatory arterial stiffness index
ABPI	ankle-brachial pressure index
ACEI	angiotensin converting enzyme inhibitors
ACR	albumin: creatinine ratio
ADMA	asymmetrical dimethylarginine
AGE	advanced glycation end products
AIx	augmentation index
AIx_{75}	augmentation index adjusted to heart rate of 75/min
ARB	angiotensin receptor blockers
ARMORR	AcceleRated Mortality of Renal Replacement (study)
BMD	bone mineral density
bmp	bone morphogenetic protein
BP	blood pressure
cbfa1	core-binding factor α1
CARDIA	Coronary Artery Risk Development In young Adults (study)
CHF	congestive heart failure
CKD	chronic kidney disease
CKD-EPI	chronic kidney disease epidemiology collaboration
CMR	cardiovascular magnetic resonance imaging
CORD	Calcification Outcome in Renal Disease (study)
DEXA	dual energy x-ray absorptiometry

DNA	deoxyribonucleic acid
ECM	extracellular matrix
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
ESKD	end stage kidney disease
FGF-23	fibroblast growth factor 23
GFR	glomerular filtration rate
hsCRP	high sensitive C-reactive protein
IMPROVE-CKD	IMpact of Phosphate Reduction On Vascular Endpoints in Chronic Kidney Disease (study)
K/DOQI	kidney/dialysis outcome quality initiative
LVH	left ventricular hypertrophy
LV	left ventricular
MBD	mineral bone disorder
MDRD	modified diet in renal disease
MGP	matrix G1a protein
MI	myocardial infarction
MMP	matrix metalloproteinases
MR	mineralocorticoid receptor
MRI	magnetic resonance imaging
NHANES III	Third National Health and Nutrition Examination Survey
NPT-2a	sodium-phosphate co-transporter 2a
PRIMO	Paricalcitol Capsule Benefits in Renal Failure-Induced Cardiac Morbidity (study)

PTH	parathyroid hormone
PWA	pulse wave analysis
PWV	pulse wave velocity
PWV_{adj}	pulse wave velocity adjusted to mean arterial pressure
RAAS	renin-angiotensin-aldosterone system
REIN	Ramipril Efficacy In Nephropathy (study)
SAE	serious adverse event
SCD	sudden cardiac death
USRDS	United States Renal Data System
VSMC	vascular smooth muscle cells

1 BACKGROUND: CHRONIC KIDNEY DISEASE AND INCREASED ARTERIAL STIFFNESS

1.1 The Kidneys

The kidneys are two retroperitoneal organs that lie within the paravertebral gutters. Each kidney receives blood from the renal arteries, each a branch of the abdominal aorta, and drains blood into the renal vein, which is connected to the inferior vena cava. The functional unit of the kidney is the nephron, which facilitates the key removal of waste products of metabolism from the body. This is achieved through the filtration of blood at the glomerulus, followed by selective reabsorption of water and electrolytes in the proximal tubule and concentration of the filtrate in the loop of Henle and collecting duct, which finally results in the formation of urine. Urine is excreted via the ureters, which drain into the urinary bladder. In conjunction with removal of waste products, the kidney has several important homeostatic functions including the regulation of electrolyte and fluid balance, maintenance of acid-base balance, and regulation of blood pressure. This is partially mediated through renal production of the hormone renin by the juxtaglomerular apparatus, which regulates salt and water retention via the renin-angiotensin-aldosterone system (RAAS). The kidney also produces the hormone erythropoietin and activates vitamin D via 1-α-hydroxylation of the relatively inactive 25-hydroxyvitamin D (calcidiol) to produce 1,25-dihydroxyvitamin D (calcitriol).

1.2 Chronic Kidney Disease

Chronic kidney disease (CKD) is common, affecting 13% of the population of the developed world (Coresh *et al.*, 2007). Over the last decade the prevalence has risen, reflecting an increase in the prevalence of diabetes and hypertension, the two biggest causes of end stage kidney disease (ESKD) in Western society (Coresh *et al.*, 2007). Other leading causes of ESKD are shown in Table 1-1. Chronic kidney disease is categorised in five stages (Table 1-2) according to glomerular filtration rate (GFR) and markers of renal damage. The degree of renal dysfunction can be estimated using the Cockcroft-Gault formula (Cockcroft and Gault, 1976), which approximates creatinine clearance from age, weight and serum creatinine, the 4-variable Modification of Diet in Renal Disease (MDRD) equation (Levey *et al.*, 1999), or the recently-introduced chronic kidney disease epidemiology collaboration (CKD-EPI) formula (Levey *et al.*, 2009), both of which determine estimated GFR (eGFR) from age, race, gender and serum creatinine. Although the MDRD equation has been extensively adopted, it has been criticised for underestimating true GFR at near-normal levels of kidney function (Stevens *et al.*, 2007). The CKD-EPI formula has been proposed as a more accurate alternative method for estimating true GFR, particularly at GFR >60 ml/min/1.73m^2. Determination of GFR using radionuclear isotopes remains the gold-standard technique.

Examination of epidemiological data indicates that the number of patients with early stage CKD greatly exceeds those with ESKD requiring renal replacement therapy in the form of dialysis or renal transplantation. In 2003 the prevalence of ESKD in the

US was only 0.2% compared to a prevalence of 10.8% for CKD stages 1–4 (Coresh *et al.*, 2003).

Cause	Percentage
Diabetes mellitus	43.8
Hypertension	28.1
Glomerulonephritis	6.5
Cystic kidney disease	2.3
Other urologic causes	1.4
Other causes	12.7
Unknown	5.2

Table 1-1. Causes of end stage kidney disease.

Data are percentages. 2009 data taken from the US Renal Data System 2011 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda.

Stage	Description	GFR (ml/min/1.73m^2)
1	Kidney damage* with normal or increased GFR	≥90
2	Kidney damage* with mildly decreased GFR	60–89
3	Moderately decreased GFR	30–59
4	Severely decreased GFR	15–29
5	Kidney failure	<15 or dialysis

Table 1-2. Stages of chronic kidney disease.

*Evidence of functional or structural kidney abnormalities for ≥3 months.

GFR, glomerular filtration rate.

1.3 Chronic Kidney Disease and Cardiovascular Risk

The epidemiological association between cardiovascular disease and CKD is well established, with several observational studies demonstrating an increased risk of cardiovascular disease in patients with CKD (Di Angelantonio *et al.*, 2010; Go *et al.*, 2004; Kurth *et al.*, 2009; McCullough *et al.*, 2007; Van Biesen *et al.*, 2007). In a study of over 1.1 million adults in the United States who had serum creatinine measured between 1996 and 2000, there was an independent graded inverse association between the risk of death or cardiovascular disease and eGFR at levels of eGFR below 60 ml/min/1.73m^2 (Go *et al.*, 2004). Similarly, in a pooled analysis of four community-based longitudinal studies that comprised 22634 patients, CKD (defined as GFR between 15 and 60 ml/min/1.73m^2) was an independent risk factor for the composite outcome of myocardial infarction (MI), fatal coronary heart disease,

stroke and death (Weiner *et al.*, 2004). A recent meta-analysis of over 105,000 patients from 14 studies indicated an increased risk of all-cause and cardiovascular mortality with eGFRs below 60 ml/min/1.73m^2 (Matsushita *et al.*, 2010); the hazard ratios increased with decreasing eGFR. The level of eGFR at which the risk of mortality from cardiovascular disease begins to rise is unknown. A longitudinal study of 8913 randomly selected, apparently healthy subjects followed up over a 10-year period suggested that increased cardiovascular mortality occurs even earlier at eGFR levels of <90 ml/min/1.73m^2 independent of other risk factors; this is a level of renal function that is commonly regarded as being near-normal (Van Biesen *et al.*, 2007). It is evident that patients with early stage CKD are far more likely to die from cardiovascular disease than progress to ESKD requiring dialysis or transplantation (Go *et al.*, 2004; Keith *et al.*, 2004). Whilst patients with ESKD have a cardiovascular risk of between 10 and 100 times that of healthy controls, they represent only 0.2% of the general population; the burden of cardiovascular disease caused by early stage CKD, which represents over 10% of the population, is therefore much greater in public health terms.

The pathophysiological mechanisms that underlie the increased risk of cardiovascular disease in CKD are not fully understood. The increased cardiovascular risk cannot be explained by “traditional” risk factors alone, many of which (including cholesterol and obesity) are inversely associated with survival in CKD. This phenomenon, which has been described as “reverse causality”, “reverse epidemiology” and “confounding by disease”, may reflect survival bias amongst the subset of CKD patients that reach ESKD (Baigent and Landray, 2007; Kalantar-

Zadeh *et al.*, 2003). Although approximately 50% of all ESKD deaths are due to cardiovascular disease, data from the United States Renal Data System (USRDS) indicates only 13% of these are attributable to vasculo-occlusive atherosclerotic coronary disease (MI), with the majority being attributed to sudden cardiac death (SCD), arrhythmia and congestive heart failure (CHF) (Ganesh *et al.*, 2001; U.S. Renal Data System, USRDS 2011 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States", 2011). Heart failure is a major cause of morbidity and mortality in CKD with incident rates 3–4 times higher than in non-CKD subjects (Foley *et al.*, 2005; U.S. Renal Data System, USRDS 2011 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States", 2011). Thus, it appears that structural heart disease, leading to CHF and SCD, rather than occlusive arterial disease, is the leading cause of cardiovascular mortality in CKD.

Left ventricular systolic dysfunction affects 15% of incident dialysis patients and is an independent predictor of death in this population (Foley *et al.*, 1995). Our research group has previously demonstrated the presence of subclinical LV systolic dysfunction in early CKD. In a study comparing 40 normotensive stage 2 and 3 non-diabetic CKD patients free from cardiovascular disease with 30 healthy controls with normal kidney function, LV peak systolic longitudinal strain and strain rate (indicators of longitudinal tissue deformation and hence LV systolic function) measured using echocardiography were reduced in the CKD patients despite the presence of apparently “normal” LV function when assessed using conventional echocardiographic criteria such as LV ejection fraction and tissue Doppler imaging

(Edwards *et al.*, 2008b). Abnormalities in these subclinical markers of systolic dysfunction have been associated with adverse cardiovascular outcomes in late stage CKD (Rakhit *et al.*, 2007).

In contrast to LV systolic dysfunction, abnormalities in LV structure are evident in almost all patients commencing dialysis. In a prospective study of 433 incident haemodialysis patients in whom echocardiography was performed at the time of initiation of dialysis, 74% had established LV hypertrophy (LVH) (Foley *et al.*, 1995). Abnormalities in LV structure and diastolic function have also been demonstrated in subjects with early stage CKD. In a cross-sectional observational study comparing echocardiographic findings of 117 normotensive patients with stage 2 and 3 non-diabetic CKD to 40 healthy control subjects, patients with CKD had delayed ventricular relaxation and increased LV end diastolic stiffness and pressure (evidenced by elevated left atrial volumes) compared to controls (Edwards *et al.*, 2008a). Furthermore, ventricular-arterial coupling was preserved, with an increase in arterial stiffness being demonstrated alongside the increase in LV stiffness. In an echocardiographic study of 603 patients post-MI, the presence of renal impairment was associated with increased LV mass and larger LA volumes, indicative of underlying diastolic dysfunction (Verma *et al.*, 2007). It is therefore apparent that structural and functional heart disease occurs early in the course of CKD and may be an important driver of the adverse cardiovascular outcome observed in this population. It is well established that the presence of LVH is associated with adverse cardiovascular outcomes in CKD (Boner *et al.*, 2005; Weiner *et al.*, 2005). There is increasing evidence, however, that no such biological dichotomy exists, and that LV

mass analysed as a continuous variable has a graded relationship with cardiovascular risk (Schillaci *et al.*, 2000). These findings suggest that any therapies that are effective in slowing or preventing the development of heart disease should be initiated during the early stages of CKD, long before LVH has developed. Furthermore, such therapies may provide useful insights into understanding the pathophysiological mechanisms that promote the development of increased LV mass.

Over the last decade evidence has emerged suggesting that the development of structural heart disease in CKD is driven by an underlying increase in arterial stiffness. This is mediated by the process of arteriosclerosis, a disease of the arterial medial layer that is characterized by increased collagen content, medial calcification and hyperplasia and hypertrophy of vascular smooth muscle cells (VSMC) that culminates in arterial wall hypertrophy and increased arterial stiffening. This differs from atherosclerosis, which is a disease of the arterial intima characterised by calcified fibro-atheromatous plaque formation and vascular occlusion (Schwarz *et al.*, 2000). Although associations have been established between the degree of arterial stiffness and atheromatous plaque burden (van Popele *et al.*, 2001), recent studies have failed to demonstrate a significant influence of traditional atherosclerotic risk factors on the development of arteriosclerosis (Cecelja and Chowienczyk, 2009), suggesting that alternative pathologies are driving this process. Whilst endothelial dysfunction and intimal disease are known to contribute to arterial stiffening (Moody *et al.*, 2012), the relationship between arteriosclerosis and atheromatous disease remains poorly understood; this is compounded by the fact that both pathological

processes are often present in any one individual. Arterial stiffness is increased in the early stages of CKD when kidney function is only mildly impaired (Edwards *et al.*, 2008a; Wang *et al.*, 2005), and cross-sectional studies have demonstrated increasing arterial stiffness at each advancing stage of CKD (Wang *et al.*, 2005).

1.4 The Clinical Importance of Increased Arterial Stiffness

A major function of the aorta and large arteries is to buffer oscillatory changes in blood pressure (BP) that result from intermittent ventricular ejection. The healthy elastic aorta acts as a reservoir throughout the cardiac cycle, expanding in systole to accommodate blood ejected by the left ventricle and recoiling in diastole to maintain diastolic pressure and facilitate coronary perfusion, which predominantly occurs in diastole. This highly distensible system dampens the aortic pressure waveform and ensures that most organs and tissues receive near steady flow with no exposure to peak systolic pressures; this mechanism is so efficient that there is almost no drop in mean arterial pressure from the ascending aorta to the peripheral arteries (Avolio *et al.*, 2009). Loss of arterial distensibility results in a more rigid aorta that is less able to buffer the pulsatile ejection of the left ventricle, resulting in greater pressure augmentation in systole, higher pulse pressures and greater shear stress (Davies *et al.*, 2008). An often cited explanation for the elevated systolic pressure that accompanies an increase in arterial stiffness (Franklin *et al.*, 1997) is the more rapid return of reflected waves of ventricular contraction from the distal vasculature. According to this hypothesis, in young, healthy, compliant arteries, reflected waves return to the ascending aorta in diastole, thus augmenting diastolic pressure and

coronary blood flow, whilst in aged stiffer arteries the reflected waves return earlier in systole, augmenting systolic and pulse pressures and increasing ventricular afterload. Although an attractive concept, there is now abundant evidence, recently published as a meta-analysis, showing that reflected waves arrive in systole irrespective of age (Baksi *et al.*, 2009). These data, together with information available from techniques that separate reflected waves from reservoir pressure, strongly suggest that the “cushioning effect” or Windkessel model appears to be the more important physiological explanation (Wang *et al.*, 2003). Regardless of the underlying mechanism, as arterial stiffness increases, the myocardium, brain and kidneys are exposed to higher systolic pressures and greater pressure fluctuations resulting in myocardial hypertrophy and fibrosis, cerebral and renal microvascular damage and an increased risk of stroke and development or progression of renal impairment (Figure 1-1) (O'Rourke and Safar, 2005). Furthermore, lower diastolic pressure reduces diastolic coronary perfusion and promotes subendocardial ischaemia and ventricular stiffening (London *et al.*, 1996); it also places greater reliance on systolic coronary perfusion, conferring heightened vulnerability of the myocardium to any decline in systolic function (Saeki *et al.*, 1995).

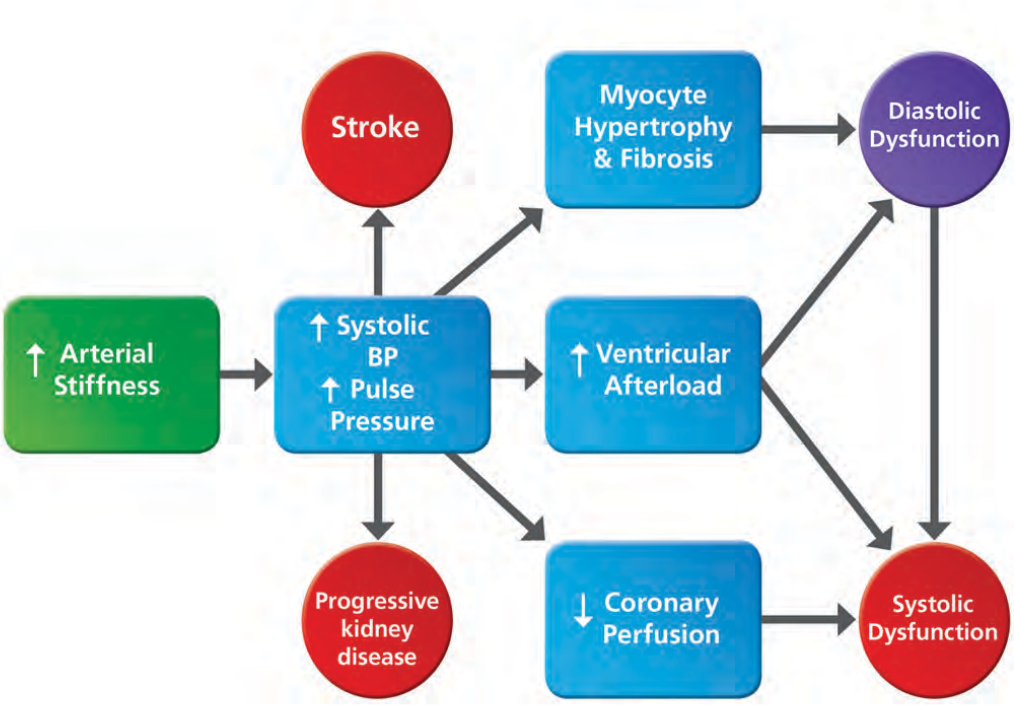

Figure 1-1. Consequences of increased arterial stiffness.

Cardiac function is physiologically matched with arterial function through ventricular-arterial coupling to ensure maximum cardiac work and efficiency (Chen *et al.*, 1998). As arterial stiffness increases, the left ventricle generates greater end-systolic pressures that enhance ventricular systolic wall stress and increase ventricular stiffness. These compensatory adaptations maintain cardiac performance with enhanced contractility at rest, but at a price: cardiac reserve is reduced, diastolic function is impaired and the cardiovascular response to alterations in pressure and volume load is blunted leading to haemodynamic instability (Chen *et al.*, 1998).

Myocardial oxygen consumption also increases, promoting subendocardial ischaemia (Watanabe *et al.*, 1993). Increased ventricular stiffening further impairs diastolic coronary perfusion through increased compression of the coronary microvasculature (Davies *et al.*, 2006). Whilst this is a common feature of advanced disease characterised by LVH, it is often present despite normal ventricular wall thickness (Chen *et al.*, 1998). Reduced proximal aortic distensibility is closely linked to indices of LV stiffness in CKD (Edwards *et al.*, 2008a) and also correlates with reduced exercise capacity despite normal or enhanced LV systolic function (Hundley *et al.*, 2001). It has been suggested that increased arterial stiffness arising from early CKD may underlie many cases of heart failure with normal ejection fraction, otherwise known as diastolic heart failure (Edwards *et al.*, 2008a). Impaired renal function is a common finding in patients with diastolic heart failure (Rusinaru *et al.*, 2011), and it is possible that diastolic dysfunction may represent a cardiac manifestation of underlying CKD.

Increased arterial stiffness, systolic pressure and pulse pressure are characteristic features of ageing in Western populations (Franklin *et al.*, 2001) and are independent risk factors for mortality and development of CHF (Haider *et al.*, 2003). Increased arterial stiffness has been proven to be a powerful independent predictor of all-cause mortality and cardiovascular events in patients with ESKD (Blacher *et al.*, 1999), hypertension (Boutouyrie *et al.*, 2002; Laurent *et al.*, 2001) and diabetes mellitus (Cruickshank *et al.*, 2002) as well as in older adults (Meaume *et al.*, 2001; Sutton-Tyrrell *et al.*, 2005) and the general population (Mattace-Raso *et al.*, 2006; Willum-Hansen *et al.*, 2006). A recent meta-analysis of 17 longitudinal studies that

measured aortic pulse wave velocity (PWV), an index of arterial stiffness, in 15877 individuals from a variety of population groups demonstrated an adjusted increase in total cardiovascular events, cardiovascular mortality and all-cause mortality of 14%, 15% and 15% for every 1 m/s increase in PWV respectively (Vlachopoulos *et al.*, 2010).

1.5 Mechanisms of Arterial Stiffness in Chronic Kidney Disease

1.5.1 Increasing Arterial Stiffness with Age

The biomechanical properties of the arteries are largely dependent on the relative quantities of collagen and elastin, the main scaffolding proteins of the extracellular matrix (ECM). Numerous studies have shown that the large arteries stiffen with age with overproduction of abnormal collagen fibres and relative loss of elastin from the ECM (Greenwald, 2007). The elastin lamellae become sparser with signs of fragmentation and calcification, while collagen molecules progressively acquire cross-links. A major unresolved question is whether these changes are truly time-dependent or reflect cumulative exposure to the risk factors described below. Similarly, the possible effect of the age-related decline in GFR on arterial stiffness remains unknown. Studies of populations living hunter-gatherer or subsistence farming lifestyles, such as the Bushmen of the Kalahari desert in Southern Africa, have shown such individuals to have low systolic blood pressures that do not rise with age (Kaminer and Lutz, 1960). When members of such populations migrate to urban areas and adopt industrialised lifestyles together with Western diets, their systolic BP and pulse pressure begin to rise with ageing, suggesting exposure to

environmental factors may be more important than ageing itself (Cooper *et al.*, 1997; He *et al.*, 1991; Poulter *et al.*, 1990).

1.5.2 Alterations in the Extracellular Matrix

Evidence of altered ECM structure in CKD comes from studies of subtotally nephrectomised rats in which aortic wall thickness was significantly greater than in controls (Amann *et al.*, 1997). Extracellular matrix volume was increased, elastic fibres were smaller and collagen "islands" were evident. Vascular smooth muscle cells were larger and greater in number with ultrastructural changes suggesting increased secretory activity. No studies have yet examined changes in arterial microstructure in human CKD, but the coronary arteries of CKD patients show increased medial thickness and lower luminal area than those of non-CKD controls (Schwarz *et al.*, 2000).

The mechanisms underlying these ECM changes in CKD are currently undetermined but matrix metalloproteinases (MMP) have been implicated. These endopeptidase enzymes regulate the ECM and are produced by vascular and inflammatory cells. Increased MMP production enhances collagen and elastin turnover through enzymatic cross-link degradation (Jacob, 2003), causing unravelling and weakening of the ECM. There are data supporting this mechanism in hypertensive patients (Yasmin *et al.*, 2005) but also accumulating evidence of a role for MMP in the development of arterial stiffness in CKD, including the presence of increased vascular MMP activity in ESKD patients compared to healthy controls (Chung *et al.*, 2009), suggesting a possible area for future therapeutic intervention.

1.5.3 The Role of Advanced Glycation End Products

Irreversible covalent cross-linking of collagen and elastin with carbohydrates or carbonyl compounds through non-enzymatic glycation results in the formation of advanced glycation end products (AGE) (Reiser *et al.*, 1992). Such post-synthetic glycation occurs in abundance in impaired glucose tolerance and diabetes mellitus and to a lesser extent with ageing, although it is unclear whether or not this is inevitable or a consequence of exposure to factors such as oxidative stress and inflammation (Konova *et al.*, 2004). Affected collagen is stiffer and less susceptible to slow hydrolytic degradation; glycation may also influence arterial stiffening through generation of reactive oxidant species and nitric oxide deactivation, promoting endothelial dysfunction (Bucala *et al.*, 1991). Hypertensives and older patients treated with AGE cross-link breakers demonstrate significant reductions in arterial stiffness and endothelial dysfunction (Kass *et al.*, 2001; Zieman *et al.*, 2007).

Levels of circulating AGE correlate directly with serum creatinine in diabetic and non-diabetic CKD (Makita *et al.*, 1991; Schwedler *et al.*, 2002). Advanced glycation end products accumulate in ESKD as demonstrated by skin autofluorescence and are independently associated with increased arterial stiffness (Ueno *et al.*, 2008) and mortality. A weak association between skin autofluorescence and arterial stiffness, measured through determination of aortic PWV, has also been demonstrated in over 1700 stage 3 CKD patients, although following adjustment for age, gender, haemoglobin and renal function, this relationship only persisted in diabetic patients (McIntyre *et al.*, 2011). Although there is some suggestion that AGE are implicated

in the development of arterial stiffness in CKD, this finding is not universal (Schwedler *et al.*, 2002). Further studies are required to determine whether dietary AGE restriction or the use of AGE cross-link breakers might be an effective method of reducing arterial stiffness in CKD.

1.5.4 Endothelial Dysfunction

Endothelial dysfunction, characterised by impaired endothelium-dependent vasodilator activity or enhanced endothelium-dependent vasoconstriction, is strongly associated with increased arterial stiffness in healthy individuals (McEniery *et al.*, 2006). Numerous studies have confirmed the presence of endothelial dysfunction in ESKD (Luksha *et al.*, 2011; van Guldener *et al.*, 1998; van Guldener *et al.*, 1997). Abnormal endothelial function has also been demonstrated in patients with non-diabetic CKD (eGFR range 14–54 ml/min/1.73m^2) compared to healthy controls (Thambyrajah *et al.*, 2000), and has been implicated as a contributor to the increased cardiovascular risk in this population (Stam *et al.*, 2006; Yilmaz *et al.*, 2011). Although this may reflect relatively high levels of systemic inflammation, oxidative stress and the high prevalence of risk factors such as hypertension, reduced renal clearance of uraemic toxins such as asymmetrical dimethylarginine (ADMA), a product of protein turnover, may also contribute (Vallance *et al.*, 1992). Asymmetrical dimethylarginine, together with its structural isomer symmetrical dimethylarginine, inhibits nitric oxide synthesis dose-dependently *in vitro* and increases basal vascular tone and BP in humans (Bode-Boger *et al.*, 2006; Vallance *et al.*, 1992). High plasma ADMA concentrations are associated with, and predict progression of, increased carotid intima-media thickness (Zoccali *et al.*, 2002a) and

are also linked to LVH (Zoccali *et al.*, 2002b) in ESKD patients. A recent study has also demonstrated the potential role of low vitamin D status as a contributor to endothelial dysfunction in older adults (Jablonski *et al.*, 2011). This is no doubt that any direct underlying relationship between CKD and endothelial dysfunction is potentially confounded by the presence of co-morbidities such as diabetes mellitus and hypertension, which themselves promote abnormal endothelial function; the study of kidney donors before and after uninephrectomy provides a unique opportunity to assess the effect of an acute drop in eGFR on endothelial function in the absence of these confounders (Moody *et al.*, 2012).

Endothelin peptides are synthesised by endothelial cells and are powerful vasoconstrictors acting on VSMC. They are implicated in the pathogenesis of several cardiovascular conditions and the progression of CKD (Dhaun *et al.*, 2006). Infusion of endothelin-1 in healthy humans to increase plasma levels to those seen in ESKD is associated with significant increases in PWV, central systolic pressure and pulse pressure (Vuurmans *et al.*, 2003). Short-term endothelin-A receptor antagonism in non-diabetic CKD was associated with reductions in proteinuria and arterial stiffness that appeared to be independent of BP-lowering (Dhaun *et al.*, 2009). Their long-term effects on cardiovascular risk reduction in CKD are yet to be determined, but there is emerging evidence that endothelin receptor antagonists may be of value in treating resistant hypertension, perhaps by direct effects on arterial stiffness (Weber *et al.*, 2009).

Although it is accepted that endothelial dysfunction promotes arterial stiffening, a study of cultured endothelial cells *in vitro* suggests that stiff arteries themselves further reduce nitric oxide bioavailability through diminished expression of endothelial nitric oxide synthase (Peng *et al.*, 2003); arterial stiffness may therefore be self-perpetuating. Novel agents such as ghrelin appear to improve endothelial dysfunction (Tesauro *et al.*, 2009) and offer potentially promising avenues for further investigation in CKD.

1.5.5 Chronic Inflammation

Although commonly regarded as a risk factor for atheroma, a clear association between inflammation and arterial stiffness exists, as demonstrated by studies of conditions characterised by chronic systemic inflammation (Cachofeiro *et al.*, 2008; Maki-Petaja *et al.*, 2006) and by studies of inflammatory markers and arterial stiffness in the healthy population (Yasmin *et al.*, 2004). More specifically, aortic inflammation, as assessed using positron emission tomography imaging, has recently been shown to influence arterial stiffness (Joly *et al.*, 2009). The long-term use of immunosuppressive agents in inflammatory disorders is associated with a reduction in surrogate markers of cardiovascular risk (Choi *et al.*, 2002) and such agents require further cautious investigation.

Systemic inflammation and oxidative stress promote endothelial dysfunction by decreasing nitric oxide availability through reductions in nitric oxide synthase activity and an increase in production of peroxynitrite, a cytotoxic oxidant that leads to oxidation of low density lipoprotein and uncoupling of endothelial nitric oxide

synthase (Koppenol *et al.*, 1992). Pro-inflammatory cytokines also damage endothelial cells by increasing the release of acid sphingomyelinase, which depresses endothelial cell signalling and function. Low glutathione levels further increase activation of sphingomyelinase (Liu and Hannun, 1997). Replenishment of glutathione using N-acetylcysteine, a cheap and well-tolerated food supplement, has been shown to improve endothelial function and cardiovascular outcomes in two small studies of patients with ESKD (Tepel *et al.*, 2003; Wittstock *et al.*, 2009), and further assessment of its potential in reducing arterial stiffness is warranted. Inflammatory degradation of ECM elastin has been shown to accelerate arterial calcification in animal CKD models (Aikawa *et al.*, 2009), prompting potential exploration of the role of immunosuppression and selective inhibition of elastase enzymes as therapeutic interventions in arterial stiffness reduction.

1.5.6 The Renin-Angiotensin-Aldosterone System

Angiotensin II is a powerful vasoconstrictor but also promotes inflammation by stimulating VSMC to generate intracellular superoxides and inflammatory cytokines (Kranzhofer *et al.*, 1999); furthermore, in vitro experiments demonstrate induction of vascular remodelling through VSMC hypertrophy and proliferation, increased collagen synthesis and increased production of MMP (Takagishi *et al.*, 1995). This ECM remodelling can be controlled by angiotensin converting enzyme inhibitors (ACEI) (Ahimastos *et al.*, 2005). Interestingly, ACEI and angiotensin II receptor blockers (ARB) also inhibit AGE formation *in vitro* dose-dependently, possibly by reducing generation of reactive oxygen species and reactive carbonyl compounds (Miyata *et al.*, 2002). In a rat model of cystic renal disease, which was associated

with increased aortic stiffness and aortic calcification when compared to control animals, treatment with the ACEI perindopril was associated with reduced arterial stiffening and calcification of the elastic media (Ng *et al.*, 2011).

Short- and long-term inhibition of the RAAS with ACEI and ARB is associated with reductions in arterial stiffness but is almost invariably accompanied by BP reduction (Ahimastos *et al.*, 2005; Mitchell *et al.*, 2007). The relative importance of BP-lowering is difficult to distinguish from the direct tissue effects outlined above. In a longitudinal study of haemodialysis patients treated with ACEI, BP-lowering combined with decreased arterial stiffness was associated with reduced all-cause and cardiovascular mortality (Guerin *et al.*, 2001). Such mortality benefits were absent in subjects with unaltered arterial stiffness despite BP reduction, lending some support to the theory that RAAS inhibition reduces risk through a BP-independent mechanism in ESKD.

Aldosterone levels, which frequently remain elevated despite treatment with ACEI and ARB, are correlated with arterial stiffness in hypertensive men independently of BP (Blacher *et al.*, 1997). Aldosterone increases arterial stiffness independently of wall stress in subtotally nephrectomised rats given high-salt diets (Lacolley *et al.*, 2002) and these effects are inhibited by the mineralocorticoid receptor (MR) antagonist eplerenone. Such MR activation is associated with endothelial dysfunction and activation of VSMC genes involved in vascular fibrosis, inflammation and calcification (Brown, 2008). Aldosterone reduces endothelial nitric oxide by increasing nicotinamide adenine dinucleotide phosphate oxidase activity and

promoting formation of reactive oxygen species (Struthers and MacDonald, 2004). Additionally, aldosterone upregulates expression and sensitivity of vascular angiotensin receptors in rats (Ullian *et al.*, 1992). The MR antagonist spironolactone inhibits angiotensin II-mediated VSMC proliferation *in vitro*, reduces aortic and myocardial collagen accumulation (Lacolley *et al.*, 2001) and reduces oxidative stress and endothelial dysfunction (Virdis *et al.*, 2002). In subtotally nephrectomised rats, spironolactone reduces proteinuria, arterial pressure and cardiac hypertrophy (Greene *et al.*, 1996). Another MR antagonist, eplerenone, was shown to improve endothelial function and reduce formation of reactive oxygen species in an animal model of diet-induced atherosclerosis (Rajagopalan *et al.*, 2002). These studies highlight the importance of aldosterone in the development of cardiovascular and renal injury in animal models of CKD.

There are limited data demonstrating the influence of RAAS on arterial stiffness in human CKD. A pilot study of 25 stage 2 and 3 CKD patients demonstrated that ARB treatment improved small artery compliance (Garg *et al.*, 2005). A recent study comparing monotherapy with either enalapril or candesartan against combined therapy with both enalapril and candesartan in patients with stage 3–4 CKD demonstrated additive reductions in BP with combined therapy, as well as significant reductions in arterial stiffness measured using PWV and augmentation index (AIx) (Frimodt-Moller *et al.*, 2012). The direct renin inhibitor aliskiren was shown to reduce ankle-brachial PWV over 12 weeks in a small cohort of Japanese patients, although this study did not include a control group (Kanaoka *et al.*, 2012). In a randomised placebo-controlled trial, the addition of spironolactone to ACEI or ARB therapy in

patients with stage 2 and 3 CKD significantly reduced arterial stiffness and LV mass, supporting the hypothesis that aldosterone is a major mediator of arterial stiffness and LVH in CKD (Edwards *et al.*, 2009). There was, however, a significant reduction in BP such that a BP-lowering effect on arterial stiffness could not be excluded. Follow-up studies of this patient group have also demonstrated improvements in echocardiographic parameters of systolic and diastolic function amongst those taking spironolactone (Edwards *et al.*, 2010). Larger studies are required to determine whether such treatment is associated with improved clinical outcomes.

A meta-analysis of RAAS blockade with either ACEI or ARB in CKD revealed a significant reduction in cardiovascular outcomes and incidence of heart failure when compared to placebo, although no reduction in cardiovascular or all-cause mortality was noted (Balamuthusamy *et al.*, 2008). The mechanism by which this improvement in cardiovascular outcome arises is undetermined, but an assessment of arterial stiffness parameters in such patients may prove to be highly informative.

1.5.7 Diet

Observational studies highlight the importance of environmental factors such as high-salt diets in the development of hypertension and arterial stiffness (Safar *et al.*, 2000). Dietary sodium enhances age-related vascular changes by promoting VSMC hypertrophy and increased VSMC tone; it also increases collagen cross-linking and facilitates aldosterone-induced oxidative stress and inflammation (Safar *et al.*, 2000). In the presence of aldosterone, small increases in plasma sodium concentration decrease nitric oxide release and increase endothelial cell stiffness *in vitro*

(Oberleithner *et al.*, 2007). Restricting dietary sodium in hypertensive patients effectively reduces arterial stiffness (Gates *et al.*, 2004). The Western diet is relatively rich in dietary oxidants, AGE and bioavailable phosphate (Ferro *et al.*, 2009). These substances undergo renal metabolism or excretion and therefore accumulate in CKD. The influence of dietary factors such as sodium, phosphate and pro-oxidant compounds on arterial structure and function in subjects with and without CKD is remarkably poorly understood and is a fertile area for future research.

1.5.8 Vascular Calcification

Vascular calcification is highly prevalent in CKD and ESKD and is closely linked with increased arterial stiffness. Chronic kidney disease is characterised by deranged handling of phosphate and calcium, and phosphate has been identified as a key mediator of soft tissue calcification. Before the mechanisms by which phosphate promotes vascular calcification are examined in detail, an overview of normal phosphate regulation and development of abnormal phosphate handling in CKD is given.

1.6 Normal Phosphate Regulation

Phosphorus has an essential role in many cellular functions, such as signal transduction and energy exchange, and helps to regulate enzyme and receptor activities. It is also an important structural component of deoxyribonucleic acid (DNA) and cell membranes. Most organic phosphorus is in the form of phosphate (PO_4); over 80% of total body phosphorus is stored in bones and teeth as

hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), with the remainder being found in the intracellular compartment and in serum as phosphate anions ($H_2PO_4^-$ and HPO_4^{2-}) (Tonelli *et al.*, 2010; Uribarri, 2007). Serum phosphate represents <1% of total body phosphorus and is largely non-protein-bound; the normal laboratory range (0.8 mmol/l–1.8 mmol/l) reflects inorganic phosphate content (Uribarri, 2007). Although serum phosphate represents a small proportion of total body phosphorus, derangements in serum phosphate level can indicate the presence of significant underlying abnormalities in phosphate homeostasis. Serum phosphate levels display a circadian rhythm, with the lowest levels (typically 1.1 ± 0.1 mmol/l) occurring late morning and the peak (1.5 ± 0.1 mmol/l) occurring shortly after midnight (Portale *et al.*, 1987). In health, phosphate levels are closely regulated and maintained within a normal physiological range through adjustments in gastrointestinal absorption, renal excretion and resorption or formation of bone (Hruska *et al.*, 2008), with the skeleton acting as a phosphate reservoir. Approximately 1000–1500 mg of phosphate is consumed in the Western diet per day (Tonelli *et al.*, 2010). Three-quarters of this is absorbed from the gastrointestinal tract along the entire length of the small intestine and the remainder is excreted in the faeces. Despite a wide variation in the dietary intake of phosphate, total body phosphate levels are maintained within the physiological range by hormonal regulation. Gastrointestinal phosphate absorption is both passive and active, involving paracellular diffusion along an electrochemical gradient or active transport via the sodium-phosphate co-transporter type 2b (NPT-2b) located on enterocytes of the small intestine (Feild *et al.*, 1999). Active absorption of phosphate from the gastrointestinal tract via this co-transporter is stimulated by 1,25-dihydroxyvitamin D (Ramirez *et al.*, 1986). An estimated 300 mg

of phosphate undergoes resorption from bone into the blood pool each day, with an equal amount being deposited back into bone to maintain equilibrium. The kidney is an important regulator of phosphate homeostasis, enabling removal of any excess phosphate in the urine. Most phosphate in serum is filtered at the glomerulus, but approximately 85% is reabsorbed in the renal tubules via the sodium-phosphate co-transporter 2a (NPT-2a) situated on cells of the proximal renal tubule; the remaining phosphate is excreted in the urine (Tenenhouse, 2007). Phosphate reabsorption by NPT-2a is increased by hypophosphataemia, 1,25-dihydroxyvitamin D, volume depletion and chronic hypocalcaemia, and reduced by hyperphosphataemia, parathyroid hormone (PTH), phosphatonins such as fibroblast growth factor 23 (FGF-23), volume expansion and chronic hypercalcaemia. The importance of gastrointestinal NPT-2b was demonstrated in a recent study of heterozygote NPT-2b knockout mice (Ohi *et al.*, 2011); these animals had hypophosphataemia and reduced urinary phosphate excretion compared to wild type mice, and expression of renal NPT-2a was increased.

Two of the principal hormones that regulate renal phosphate handling are PTH, produced by the parathyroid glands, and FGF-23, a 251-amino acid that is secreted by osteocytes and osteoblasts in bone. Secretion of PTH by the parathyroid glands increases in response to a phosphate load or hypocalcaemia. Parathyroid hormone increases reabsorption of calcium by the kidney but also increases renal phosphate excretion. It also stimulates 1-α-hydroxylase to increase activation of vitamin D, thus promoting gastrointestinal absorption of phosphate and calcium. Production of FGF-23 is stimulated by 1,25-dihydroxyxvitamin D and also increases in response to a

dietary phosphate load; this was first demonstrated in human dietary studies (Antoniucci *et al.*, 2006; Burnett *et al.*, 2006; Ferrari *et al.*, 2005) and more recently in a larger cross-sectional study of 1261 health professionals (Gutierrez *et al.*, 2011), although the underlying regulatory pathways are currently undetermined (Wolf, 2012). Serum phosphate levels are maintained within the normal range as both PTH and FGF-23 inhibit renal tubular expression of NPT-2a (Shimada *et al.*, 2004), reducing phosphate reabsorption by the kidney and increasing urinary phosphate excretion. FGF-23 also suppresses 1,25-dihydroxyvitamin D by inhibiting renal expression of the activating enzyme 1-α-hydroxylase and stimulating expression of the catabolic enzyme 24-hydroxylase, thereby reducing gastrointestinal phosphate absorption (Saito *et al.*, 2003). The tubular actions of FGF-23 are mediated via FGF receptors (mainly FGFR-1); the interaction between FGF-23 and FGFR-1 is facilitated by klotho, a transmembrane protein co-receptor that increases the binding affinity of FGF-23 (Urakawa *et al.*, 2006). Klotho null mice have a premature ageing phenotype characterised by osteopenia and vascular medial calcification, highlighting the importance of FGF-23 in the regulation of normal mineral homeostasis (Kuro-o *et al.*, 1997).

1.7 Abnormal Phosphate Metabolism in Chronic Kidney Disease

Chronic kidney disease is the commonest cause of disordered phosphate metabolism. Even small reductions in GFR result in disturbed calcium and phosphate handling, suggesting that the concept of humans being born with “surplus” renal function may be incorrect. During the initial stages of CKD, increased

production of the phosphaturic hormone FGF-23, stimulated by increasing serum phosphate arising from reduced glomerular filtration and renal tubular degradation, acts as a potent stimulus that reduces reabsorption of phosphate from the proximal renal tubules via the co-transporter NPT-2a. This compensatory response serves to maintain normal phosphate balance by increasing urinary phosphate excretion in the face of declining renal capacity to excrete phosphate; serum phosphate levels therefore remain within the normal laboratory range (Gutierrez *et al.*, 2005). Increased FGF-23 also reduces renal 1,25-dihydroxyvitamin D production by reducing 1α-hydroxylase activity (Shimada *et al.*, 2004), causing a decrease in gastrointestinal absorption of calcium and consequent hypocalcaemia, although phosphate absorption is largely unaffected (Ramirez *et al.*, 1986). Renal activation of vitamin D is also impaired as a consequence of parenchymal injury, but elevated serum phosphate levels also directly inhibit 1α-hydroxylase; both result in a reduction in active vitamin D production and therefore gastrointestinal calcium absorption. Hypocalcaemia and hyperphosphataemia stimulate release of PTH from the parathyroid glands; PTH inhibits reabsorption of phosphate in the proximal renal tubules by NPT-2a, thereby increasing the urinary fractional excretion of phosphate, but also increases resorption of calcium and phosphate from bone. FGFR-1 receptors are present in the parathyroid gland indicating that FGF-23 can also affect PTH production directly (Krajisnik *et al.*, 2007). Increases in FGF-23 levels precede the rise in serum phosphate and PTH and the decline in 1,25-dihydroxyvitamin D (Gutierrez *et al.*, 2005; Isakova *et al.*, 2011b); FGF-23 is therefore a sensitive marker of the presence of disordered mineral metabolism in CKD. Evidence from animal models of CKD suggests that an increase in FGF-23 is the upstream trigger that

promotes a decrease in 1,25-dihydroxyvitamin D and a rise in PTH, as inhibition of FGF-23 is associated with high serum phosphate, normal 1,25-dihydroxyvitamin D levels and reduced urinary phosphate excretion (Hasegawa *et al.*, 2010). Expression of klotho, the FGF-23 co-receptor, declines progressively in CKD, mirroring the increase in FGF-23 production; CKD has been described as an FGF-23-resistant state (Koh *et al.*, 2001; Kuro-o, 2011). Phosphate levels begin to rise at levels of GFR <30 ml/min/1.73m^2 (Craver *et al.*, 2007; Hsu and Chertow, 2002; Levin *et al.*, 2007) as these compensatory mechanisms are overwhelmed and unable to reduce tubular phosphate reabsorption any further in the face of declining filtration rate. A positive phosphate balance is established, with dietary phosphate intake exceeding urinary phosphate excretion. Elevated serum phosphate levels further promote the release of FGF-23 and PTH; by the time ESKD is reached, hyperphosphataemia is an almost universal finding.

In addition to changes in gastrointestinal phosphate absorption and renal phosphate excretion, these hormonal changes also result in resorption of calcium and phosphate from the skeleton. At GFRs <60 ml/min/1.73m^2, 75-100% of patients have evidence of renal bone disease (Elder, 2002). The commonest form of bone disease observed in CKD is high-turnover hyperparathyroid bone disease; mixed osteodystrophy (characterised by high-turnover bone disease with defective mineralisation) and low-turnover adynamic bone disease occur less frequently (Elder, 2002). The term chronic kidney disease-mineral bone disorder (CKD-MBD) was introduced to describe the abnormalities of bone and mineral metabolism and the

presence of vascular and soft tissue calcification characterised by CKD (Moe *et al.*, 2007).

1.7.1 Vitamin D and Parathyroid Hormone

Increasing phosphate levels suppress endogenous vitamin D synthesis and increase PTH secretion. Lower levels of vitamin D are powerfully associated with increased cardiovascular risk (Moe *et al.*, 2007). Hypovitaminosis D is also associated with increased arterial calcification (Watson *et al.*, 1997), decreased cardiac contractility (Zittermann *et al.*, 2003), upregulation of the renin-angiotensin axis, hypertension and LVH (Li, 2003; Xiang *et al.*, 2005). Vitamin D therapy, used in the treatment of bone mineral disorders related to CKD, is associated with reduced cardiovascular mortality in observational studies of ESKD (Shoji *et al.*, 2004); this may be partly explained by reduced vascular calcification through suppressed cbfa1 synthesis (Drissi *et al.*, 2002). Hyperparathyroidism, commonly present in advanced CKD, may also contribute to vascular calcification. Parathyroid hormone receptors are present on VSMC and parathyroidectomy is associated with reduced calcium deposition (Rostand and Drueke, 1999). Hyperparathyroidism is strongly associated with hypertension (Smith *et al.*, 2000), increased arterial stiffness (Smith *et al.*, 2000), LVH, cardiac fibrosis (Amann *et al.*, 1994), impaired cardiac contractility (Rostand and Drueke, 1999), impaired endothelial function (Kosch *et al.*, 2000) and cardiovascular mortality (Rostand and Drueke, 1999). In spite of these associations, a recently published randomised controlled trial comparing the calcimimetic agent cinacalcet against placebo in 3883 haemodialysis patients with moderate to severe secondary hyperparathyroidism demonstrated no significant reduction in the primary

composite endpoint of death, myocardial infarction, hospitalisation for unstable angina, heart failure or peripheral vascular event after a median of 21 months of treatment, despite significant reductions in PTH levels (Chertow *et al.*, 2012).

1.7.2 Fibroblast Growth Factor-23

Interest in FGF-23 has soared recently with several studies demonstrating an association between FGF-23 levels and cardiovascular outcome. A relationship between FGF-23 levels and increased mortality has been demonstrated in haemodialysis patients (Gutierrez *et al.*, 2008; Jean *et al.*, 2009), in patients with stage 2–4 CKD (Isakova *et al.*, 2011c; Kendrick *et al.*, 2011) and in patients with prevalent coronary artery disease and normal renal function (Parker *et al.*, 2010). There is also evidence linking FGF-23 with cardiovascular events in CKD patients (Kendrick *et al.*, 2011; Seiler *et al.*, 2010) and with LV systolic dysfunction and atrial fibrillation in patients with normal renal function (Seiler *et al.*, 2011). A putative mechanism underlying this relationship was suggested from cross-sectional studies in which FGF-23 was independently associated with increased LV mass and LVH (Gutierrez *et al.*, 2009; Kirkpantur *et al.*, 2011; Mirza *et al.*, 2009b). This observation was confirmed by Faul *et al* in 3070 CKD patients (Faul *et al.*, 2011). In a series of experiments the same investigators demonstrated that FGF-23 induces LVH both *in vitro* and *in vivo*, and that treatment with an FGF-23 antagonist attenuates the development of LVH in animal CKD models (Faul *et al.*, 2011). Furthermore, FGF-23 has been linked to progression of renal dysfunction in a cohort of 177 patients with non-diabetic CKD (Fliser *et al.*, 2007); this finding was corroborated in a prospective study of 3879 patients with stage 2–4 CKD, in which FGF-23 predicted progression

to ESKD (Isakova *et al.*, 2011c). Other studies have associated FGF-23 with endothelial dysfunction in the general population (Mirza *et al.*, 2009a) and with endothelial dysfunction and vascular calcification in CKD patients (Desjardins *et al.*, 2012; Yilmaz *et al.*, 2010).

1.7.3 Phosphate in the Western Diet

Daily dietary phosphate intake is highly variable and not only dependent on the type of food consumed but also in the way it is prepared (Cupisti *et al.*, 2006). Food rich in protein, such as milk, dairy products and meat, has a high content of naturally occurring bio-available phosphate. This exists as organic cellular or protein constituents and is hydrolysed in the gastrointestinal tract to release inorganic phosphate, which is readily absorbed (Uribarri, 2007). Although "primitive" diets based on grains and legumes often have high phosphate content, they provide little bioavailable phosphate as most of this occurs in the form of non-absorbable phytates. Effectively these diets result in prolonged eating of foods with low phosphate content rather than the "binge-eating" of high phosphate foods associated with large increases in post-prandial serum phosphate levels, which are common in today's fast-food society (Bell *et al.*, 1977). In a rat model of CKD-MBD, animals fed the equivalent of a Western diet rich in bioavailable phosphate had higher urinary phosphate excretion and higher FGF-23 levels compared to animals fed a grain-based diet based on non-absorbable phytates (Moe *et al.*, 2009). These results were replicated in a controlled crossover dietary study of 9 patients with CKD and a mean eGFR of 32 ml/min/1.73m^2 fed with meat and vegetarian diets; patients receiving the meat diets had higher serum phosphate and FGF-23 levels (Moe *et al.*, 2011).

Similar findings were found in a controlled study of 16 healthy female subjects given five different diets over separate 24-hour sessions, in which urinary phosphate excretion was higher after a meat-rich diet compared to when whole grains or cheese was given (Karp *et al.*, 2007). Phosphate excretion also increased after dietary phosphate supplementation. Post-prandial increases in serum phosphate seen following an acute phosphate load also appear to be associated with transient endothelial dysfunction (Shuto *et al.*, 2009).

The widespread use of inorganic phosphate-containing additives in the Western diet, such as phosphoric acid (E338) and sodium polyphosphates, used as an acidulate and preservative in many foods, including beer, cheese, jam, cured meats and cola soft drinks, has contributed to a significant increase in our daily phosphate intake (Calvo and Park, 1996; Uribarri and Calvo, 2003). This is compounded by the lack of clear labelling of phosphate content on food packaging and in nutritional information. It is estimated that consumption of greater quantities of processed food could increase phosphate intake by up to 1000 mg/day (Bell *et al.*, 1977). Finally, the consumption of multivitamins and mineral supplements can also significantly increase daily phosphate intake (Uribarri, 2007). In health the body is able to handle a high dietary phosphate load by increasing urinary phosphate excretion (Bell *et al.*, 1977; Karp *et al.*, 2007), but in the setting of reduced glomerular filtration this compensatory mechanism is impaired and hyperphosphataemia rapidly ensues. The potential impact of food additives on phosphate levels in ESKD was demonstrated in a randomised multicentre trial of 279 patients with hyperphosphataemia (Sullivan *et al.*, 2009). Patients randomised to receive dietary education regarding avoidance of

phosphate additives had a greater decline in serum phosphate levels after 3 months compared to those receiving standard care (0.6 mg/dl [0.19 mmol/l] greater decline, 95% CI -1.0 to -0.1 mg/dl).

Important experimental evidence demonstrating the effects of dietary phosphate on cardiovascular structure was provided in a comparison of subtotally nephrectomised rats receiving high and low phosphate diets. In this rat model of CKD, rats fed the high phosphate diet had higher serum phosphate concentrations compared to rats receiving the low phosphate diet (Amann *et al.*, 2003). The phosphate rich rats also had more cardiac interstitial fibrosis and increased arterial wall thickness compared to rats fed the low phosphate diet. Sham-operated controls demonstrated no evidence of cardiac fibrosis or arterial wall thickening, irrespective of the diet given. These observations indicate that structural cardiovascular disease develops in the setting of a phosphate-rich diet and CKD, which is highly prevalent in the Western world and which impairs phosphate excretion, resulting in hyperphosphataemia. This mechanism in no way underestimates the contribution of a high sodium intake to the development of hypertension. Indeed by reducing arterial distensibility with age, phosphate exposure may well exacerbate the effect of sodium loading.

1.8 Phosphate, Vascular Calcification and Increased Arterial Stiffness

The cell biology of vascular calcification is complex, but it is clear that phosphate plays a key role in this pathophysiological process. Vascular calcification refers to deposition of calcium-phosphate mineral (hydroxyapatite) in cardiovascular tissues.

Long thought to be a passive process, there is now compelling evidence that arterial calcification is actively regulated, involving direct osteogenic gene activation together with suppression of calcification inhibitors. Vascular smooth muscle cells and osteoblasts derive from a common mesenchymal precursor cell, and VSMC appear to retain their ability to mineralise. Exposure of VSMC to high concentrations of intracellular calcium and phosphate *in vitro* results in their phenotypic switch to an osteogenic cell type with up-regulation of genes promoting matrix mineralisation and calcium deposition (Jono *et al.*, 2000). A calcium-phosphate precipitate forms in association with the ECM (Reynolds *et al.*, 2004). This osteogenic differentiation appears to be driven by upregulation of transcription factors such as core binding factor α1 (cbfa1), osteopontin, osterix and bone morphogenetic protein (bmp), which control expression of osteogenic proteins such as osteocalcin, osteonectin and alkaline phosphatase. In the media of calcified arteries cbfa1 is highly expressed in association with osteopontin and type I collagen. Upregulation of these transcription factors is observed in VSMC *in vitro* and *in vivo*, although mineralisation is only seen to occur after induction of hyperphosphataemia, which is prevalent in advanced CKD (Mathew *et al.*, 2008). The type 3 sodium-dependent phosphate co-transporter, Pit-1, facilitates movement of inorganic phosphate into VSMC, which stimulates cbfa1 expression dose-dependently (Jono *et al.*, 2000). Mineralisation of the ECM is completely inhibited by antagonism of Pit-1, suggesting that calcification is an active cellular process dependent on phosphate uptake (Reynolds *et al.*, 2004). This concept is supported by the finding that inhibition of phosphate uptake in Pit-1 knockdown cells blocks the induction of the osteogenic transcription factors cbfa1 and osteopontin (Li *et al.*, 2006). Phosphate also induces VSMC death and apoptotic

body release, associated with inflammation and calcification, as well as release of matrix vesicles from living cells (Reynolds *et al.*, 2004). These vesicles contain preformed hydroxyapatite and calcify intensively. Osteogenic proteins are also expressed in VSMC following their exposure to uraemic serum in vitro (Chen *et al.*, 2002); this occurs independently of phosphate concentration, suggesting that the uraemic milieu, perhaps through oxidative stress, also directly induces vascular calcification.

Loss of inhibitors of mineralisation such as fetuin A, osteoprotegerin and matrix G1a protein (MGP) is associated with progressive arterial and ectopic soft tissue calcification. Levels of MGP are inversely correlated with severity of coronary artery calcification (Jono *et al.*, 2004) and fetuin A levels have been shown to inversely correlate with aortic PWV IN ESKD (Kuzniar *et al.*, 2008). Fetuin A appears to decline in parallel with renal function (Cottone *et al.*, 2010). In a cross-sectional study of 312 patients with ESKD, fetuin A levels were lower than in healthy controls and were associated with increased cardiovascular and all-cause mortality (Ketteler *et al.*, 2003). A subsequent larger analysis of data from 822 non-diabetic patients with stage 3 and 4 CKD, however, demonstrated no such relationship with mortality (Ix *et al.*, 2007). The down-regulation of these inhibitors *in vivo* in ESKD may allow calcification to occur at relatively low phosphate concentrations, as suggested by the association of modestly elevated serum phosphate (still within the reference range) with a greater prevalence of coronary, aortic and valvular calcification independent of serum vitamin D and PTH levels in studies of CKD patients (Adeney *et al.*, 2009).

Vascular medial calcification is prominent in CKD and plays an important role in the pathogenesis of arterial stiffness. The extent of arterial calcification correlates with severity of arterial stiffness independent of age and BP in ESKD and early stage CKD (Guerin *et al.*, 2000; Raggi *et al.*, 2007; Toussaint *et al.*, 2008), as well as in the general population (McEniery *et al.*, 2009). The presence of arterial calcification is a strong predictor of all cause and cardiovascular mortality in ESKD (Chiu *et al.*, 2010; London *et al.*, 2003). In the prospective, longitudinal, multicentre observational Coronary Artery Risk Development In Young Adults (CARDIA) study of 3015 young adults with normal renal function followed up for 15 years, higher serum phosphate levels (>3.9 mg/dl; equivalent to >1.26 mmol/l) at baseline were associated with greater levels of coronary artery calcification at follow-up in multivariate analyses (Foley *et al.*, 2009b). Higher serum phosphate was also shown to be an independent predictor of development of coronary artery calcification over a 6-year period in a longitudinal study of community-dwelling adults (Tuttle and Short, 2009). Higher serum phosphate levels have been reported amongst ESKD patients with coronary artery calcification in another cross-sectional study (Goodman *et al.*, 2000). There is weak evidence for a link between phosphate and arterial stiffness. The studies reporting this association are small with some variability in association depending on the technique used to assess arterial stiffness. In a cross-sectional study of 48 patients with CKD and a GFR ranging from 17 to 55 ml/min/1.73m^2, serum phosphate correlated with PWV (r=0.35, P=0.02) in univariate analysis; this association was not, however, significant in multivariable analyses (Toussaint *et al.*, 2008). In another cross-sectional study of 1370 participants of the Multi-Ethnic Study of Atherosclerosis, of which 440 had CKD, subjects with phosphate levels >4 mg/dl

(>1.28 mmol/l) had a 4.6 times greater risk for a high ankle-brachial pressure index (ABPI, a measure of peripheral arterial stiffness) compared to those with phosphate levels <3 mg/dl (0.96 mmol/l) after multivariable adjustment, although an increase in pulse pressure or large and small arterial elasticity was not demonstrated (Ix *et al.*, 2009). A cross-sectional analysis of 581 patients from the third National Health and Nutrition Examination Survey (NHANES III) also used ABPI as a measure of arterial stiffness to demonstrate an increasing proportion of patients with high ABPI across increasing quartiles of serum phosphate (Kendrick *et al.*, 2010). A more recent study of 42 patients with nephrotic syndrome demonstrated increasing age-adjusted PWV across tertiles of serum phosphate, although this was a retrospective analysis (Sexton *et al.*, 2013).

Arterial stiffness and calcification appear to be driven by the characteristic renal mineral bone disorder comprising hyperphosphataemia, hypocalcaemia and hyperparathyroidism together with reduced bone mineral density (BMD). The presence of CKD-MBD is strongly linked with the extent of arterial calcification, a powerful predictor of mortality (Moe *et al.*, 2007). Several studies have shown that CKD-MBD is related to adverse outcomes in dialysis patients (Moe *et al.*, 2007). In the largest of these involving over 40,000 patients, the population attributable risk for disorders of mineral metabolism was 17.5%, owing largely to the high prevalence of hyperphosphataemia (Block *et al.*, 2004). Many observational studies have reported an association between low BMD and cardiovascular morbidity and mortality in the general population (Kado *et al.*, 2000; van der Klift *et al.*, 2002; von der Recke *et al.*, 1999). There is also an inverse relationship between bone mineral content and

vascular calcification and several longitudinal studies have shown that individuals with the greatest rate of bone loss demonstrate the fastest progression of vascular calcification (Hak *et al.*, 2000; Kiel *et al.*, 2001; Schulz *et al.*, 2004).

The results of the studies described above indicate that phosphate is intimately linked with the development of vascular calcification in CKD, which in turn may lead to increased arterial stiffness and adverse cardiovascular outcome. The evidence implicating phosphate with increased arterial stiffness is, however, less convincing.

1.9 Phosphate as a Cardiovascular Risk Factor

Observational studies have shown serum phosphate to be an independent predictor of mortality in CKD (Kestenbaum *et al.*, 2005) and ESKD patients (Block *et al.*, 1998; Ganesh *et al.*, 2001). The first report to demonstrate an independent relationship between serum phosphate and adverse outcome was a cross-sectional study of haemodialysis patients (Block *et al.*, 1998). The adjusted risk of overall death for patients with a serum phosphate >6.5 mg/dl (2.1 mmol/l) was 1.27 times greater compared to those with serum phosphate levels between 2.4 and 6.5 mg/dl (0.77 and 2.1 mmol/l). A follow-up study of 12833 haemodialysis patients confirmed this relationship and also showed serum phosphate to be independently associated specifically with cardiovascular death (Ganesh *et al.*, 2001). Every 1 mg/dl (0.32 mmol/l) increase in serum phosphate was associated with a 9% greater increase in risk of death from coronary artery disease and a 6% increased risk of SCD. In a retrospective analysis of 3490 patients with pre-dialysis CKD, an adjusted survival

model estimated a 23% increase in the risk of death for every 1 mg/dl (0.32 mmol/l) increase in serum phosphate (Kestenbaum *et al.*, 2005). Furthermore, the adjusted hazard ratio for death rose to 1.9 in patients with serum phosphate >5 mg/dl (>1.6 mmol/l) compared to those with serum phosphate <2.5 mg/dl (<0.8 mmol/l). Phosphate remained an independent predictor of mortality even when within the normal laboratory range. The importance of phosphate as an independent predictor of mortality in CKD was recently confirmed in a meta-analysis of 47 cohort studies that included 327644 patients with early and late stage CKD (Palmer *et al.*, 2011). The relative risk for all-cause mortality was 1.18 (95% confidence interval 1.12–1.25) for every 1 mg/dl (0.32 mmol/l) increase in serum phosphate. Serum phosphate is also a predictor of mortality after renal transplantation (Connolly *et al.*, 2009; Sampaio *et al.*, 2011), with higher serum phosphate levels at time of transplantation being associated with an increased risk of all-cause mortality. Furthermore, data from two longitudinal studies suggests higher phosphate levels measured at the time of transplantation or six to twelve months post-transplantation are associated with an increased risk of graft failure (Benavente *et al.*, 2012; Sampaio *et al.*, 2011).

Although elevated serum phosphate, calcium and PTH and vitamin D deficiency are all associated with increased all-cause and cardiovascular mortality in CKD, a systematic review has indicated that the mortality risk for phosphate is greater than for these other parameters (Covic *et al.*, 2009b) of CKD-MBD, highlighting the importance of phosphate as the key driver for adverse outcome in this population.

Serum phosphate levels within the reference range are also associated with cardiovascular morbidity and mortality in studies of the general population (Dhingra *et al.*, 2007; Foley *et al.*, 2008; Larsson *et al.*, 2010; Tonelli *et al.*, 2005). In a prospective analysis of over 3300 Framingham Offspring study participants free of overt cardiovascular disease and CKD, higher serum phosphate levels were associated with an increased risk of cardiovascular disease (including heart attack, stroke, angina, peripheral vascular disease and heart failure) in a continuous graded relationship (Dhingra *et al.*, 2007). An association between higher levels of serum phosphate and the risk of death and future cardiovascular events, including development of heart failure and fatal or non-fatal MI, was demonstrated in 4127 survivors of MI with normal kidney function in a *post hoc* analysis of participants of the Cholesterol And Recurrent Events (CARE) study (Tonelli *et al.*, 2005). A similar relationship between phosphate and cardiovascular mortality has been demonstrated in type II diabetics (Chonchol *et al.*, 2009). In the prospective, community-based Atherosclerosis Risk in Communities Study involving over 13,000 participants with normal renal function followed up for 13 years, serum phosphate was independently associated with an increased risk of all-cause mortality (adjusted hazard ratio 1.14 for every 0.5 mg/dl increase in serum phosphate) (Foley *et al.*, 2008). In another community study of over 2000 men with 30 years of follow-up, serum phosphate independently predicted all-cause and cardiovascular mortality (Larsson *et al.*, 2010).

In summary, there is a wealth of information from population studies linking phosphate to increased cardiovascular morbidity as well as cardiovascular and all cause mortality, both in the general population and in CKD. In addition, data from *in*

vitro and small animal work demonstrate possible mechanisms through which phosphate may influence cardiovascular risk, including the promotion of vascular calcification or development of LVH mediated by FGF-23. Hard evidence indicating that phosphate is the direct cause of adverse cardiovascular outcome is, however, lacking.

1.10 Phosphate Binders in Chronic Kidney Disease

The current management of bone-mineral disorder in CKD involves the use of dietary phosphate restriction in combination with oral phosphate binders to control hyperphosphataemia, together with administration of active vitamin D or vitamin D analogues to maintain normal PTH and calcium levels. In advanced CKD dietary phosphate restriction alone is usually insufficient to control the hyperphosphataemia observed at these levels of renal dysfunction. The use of oral phosphate binders is thus advocated by nephrologists to correct hyperphosphataemia in an attempt to prevent secondary hyperparathyroidism and CKD-MBD. The KDIGO guidelines recommend the maintenance of normal serum phosphate levels through dietary restrictions and phosphate binders in stage 3–4 CKD (KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD), 2009).

The calcium-based binders calcium carbonate and calcium acetate are the most commonly used phosphate binders worldwide and are effective at reducing serum phosphate in hyperphosphataemic patients (Qunibi *et al.*, 2011). Despite this proven

efficacy, and the strong evidence linking serum phosphate to adverse cardiovascular outcomes and mortality, there is a notable lack of randomised, placebo-controlled trials of phosphate-lowering therapy that demonstrate reductions in mortality or cardiovascular outcomes (KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD), 2009). Two observational studies have noted lower all-cause mortality rates amongst individuals receiving phosphate binder therapy in intention-to-treat multivariate analyses and propensity score matching. In an analysis of a prospectively studied cohort of 10044 incident haemodialysis patients that participated in the Accelerated Mortality of Renal Replacement (ARMORR) study, Isakova *et al.* demonstrated that treatment with phosphate binders was independently associated with reduced mortality at 1 year (Isakova *et al.*, 2009). A similar analysis performed in a historical cohort of 1188 men with non-dialysis-dependent CKD and a mean eGFR of 38 ± 17 ml/min/1.73m^2 also demonstrated a significantly reduced mortality amongst patients treated with phosphate binders (adjusted hazard ratio 0.61, P<0.001) (Kovesdy *et al.*, 2010).

Recently concerns have emerged regarding the use of calcium-based phosphate binders. These agents cause calcium loading that may result in the promotion of soft tissue and vascular calcification. Newer, non-calcium-based phosphate binders, such as sevelamer and lanthanum, have been introduced as alternative agents for reducing serum phosphate without contributing to total calcium load. Sevelamer, initially released as sevelamer hydrochloride and available from 2009 as sevelamer carbonate following concerns regarding acidosis from the hydrochloride component

(Pai and Shepler, 2009), is a non-absorbed phosphate binding crosslinked polymer that is taken with meals to reduce the gastrointestinal absorption of phosphate. The structure of sevelamer comprises multiple amines that interact with phosphate ions in the gut through covalent and ionic bonding, thus preventing gastrointestinal absorption. Randomised studies and meta-analyses comparing treatment with sevelamer against calcium-based agents in haemodialysis patients suggest that sevelamer has slightly lower efficacy in reducing serum phosphate levels (Navaneethan *et al.*, 2009; Suki *et al.*, 2007), but this is not a consistent finding. In a randomised, unblinded study comparing sevelamer with calcium-based binders in 200 haemodialysis patients treated over 52 weeks, control of hyperphosphataemia was equivalent in both groups (Chertow *et al.*, 2002). The incidence of adverse effects relating to treatment appears to be slightly higher in patients receiving sevelamer treatment, but this is offset by the lower risk of hypercalcaemia (Suki *et al.*, 2007). Studies comparing the effects of sevelamer with calcium-based phosphate binders on progression of vascular calcification have been contradictory. One of the first randomised studies comparing sevelamer with calcium-based agents in ESKD indicated a significant increase in calcium score in the coronary arteries and aorta measured using electron beam tomography in the calcium-based phosphate binder group compared to the sevelamer group (Chertow *et al.*, 2002). A number of subsequent open-label studies have supported this finding. In an open label study of 114 haemodialysis patients, sevelamer was not associated with progression of coronary artery or aortic calcification when compared to calcium carbonate after 52 weeks of treatment (Braun *et al.*, 2004). Another open-label randomised study of 42 haemodialysis patients showed patients receiving sevelamer had less progression of

aortic calcification over 24 weeks compared to those randomised to receive calcium carbonate (Takei *et al.*, 2008). In incident dialysis patients with at least mild coronary artery calcification, use of calcium-based binders was associated with more rapid progression of calcification when compared to the use of sevelamer hydrochloride over an 18-month period (Block *et al.*, 2005). There is a suggestion from a small study of 15 haemodialysis patients that sevelamer reduces PWV, but this requires confirmation with larger studies (Takenaka and Suzuki, 2005). Similar results for vascular calcification have been demonstrated with sevelamer in the pre-dialysis CKD population (Russo *et al.*, 2007) and with lanthanum carbonate in ESKD (Toussaint *et al.*, 2011). In another open-label study, however, there was no significant difference in progression of vascular calcification between haemodialysis patients receiving sevelamer and those receiving calcium after 12 months (Qunibi *et al.*, 2008). Whether the use of sevelamer is associated with reduced mortality is also unclear, and studies to date have been underpowered for this endpoint. A recently published open-label study of 212 patients with stage 3–4 CKD randomised to treatment with sevelamer or calcium carbonate demonstrated lower all-cause mortality amongst patients randomised to sevelamer (Di Iorio *et al.*, 2012). In a secondary analysis of 127 incident haemodialysis patients randomised to sevelamer or calcium-based binders over a median of 44 weeks, all-cause mortality was significantly lower amongst those receiving sevelamer (P=0.05). These findings contrast with the results of an earlier larger, multicentre, open-label randomised trial of sevelamer and calcium-based binders in 2103 ESKD patients, in which treatment with sevelamer was not associated with lower mortality rates (Suki *et al.*, 2007). In a sub-group analysis of 927 patients aged over 65 years, however, a significantly lower

mortality rate was observed in the sevelamer group (hazard ratio 0.77, 95% CI 0.61–0.96). Meta-analyses have not shown any significant difference in mortality amongst haemodialysis patients receiving sevelamer and those receiving traditional calcium-based binders (Jamal *et al.*, 2009; Navaneethan *et al.*, 2009; Tonelli *et al.*, 2007).

Lanthanum carbonate is an alternative non-calcium-based phosphate binder that appears as efficacious as calcium carbonate in reducing serum phosphate levels in ESKD but with a much lower incidence of hypercalcaemia (Finn, 2006; Hutchison *et al.*, 2005). There are limited data available regarding effects of lanthanum on vascular calcification or cardiovascular and all-cause mortality, and follow-up safety data are limited to two years (Hutchison *et al.*, 2005; Toussaint *et al.*, 2011). Two small studies have evaluated the short-term effects of lanthanum on FGF-23 levels in humans with conflicting results (Gonzalez-Parra *et al.*, 2011; Isakova *et al.*, 2011a). Findings from studies using sevelamer have been more encouraging, with evidence of a decrease in FGF-23 levels during the first 6–8 weeks of therapy (Oliveira *et al.*, 2010; Yilmaz *et al.*, 2012). These results require verification in larger blinded randomised studies with longer periods of follow-up.

1.11 The Hypothesis

In summary, CKD is characterised by alterations in phosphate handling, with reduced urinary phosphate excretion causing an increase in secretion of FGF-23 and PTH and reduction in levels of 1,25-dihydroxyvitamin D. These compensatory mechanisms result in resorption of calcium and phosphate from bones, and the

characteristic CKD-MBD. It is postulated that excess circulating phosphate is deposited within soft tissues such as the vasculature. Phosphate is an active promoter of calcification at the cellular level and induces the osteogenic transformation of VSMC and the subsequent deposition of mineral within the extracellular matrix. The resulting calcification of the vascular media is associated with increased arterial stiffness, which is an independent predictor of mortality in CKD. Stiffening of the aorta increases afterload of the left ventricle, resulting in structural changes, such as increased LV mass, and functional changes such as impaired relaxation and diastolic dysfunction, both of which are predictors of adverse cardiovascular outcome.

The results of studies determining the effects of lowering phosphate exposure on markers of vascular calcification and parameters of arterial stiffness may have profound implications for the management of cardiovascular risk in the CKD population. Reducing phosphate exposure may be achieved through either dietary modification or through pharmacological means. Modification of dietary behaviour as part of a large randomised trial is a notoriously difficult undertaking and will invariably have multiple effects on both micro- and macronutrients. Observational studies of diet, such as vegetarian versus omnivorous diets, particularly between two different populations, are full of potential confounders, perhaps most notably fat and salt intake, and any associations between diet and outcomes do not necessarily reflect a common pathway (Flegal, 1999). Furthermore, compliance with a modified diet outside of a controlled environment over the time period necessary for such modifications to be associated with detectable changes in arterial stiffness, LV mass

or LV function is likely to be poor. Possible alternative treatments to reduce phosphate exposure include the use of niacin and related compounds, which inhibit active gut phosphate absorption. There are some concerns, however, with respect to long-term efficacy, tolerability and safety (Berns, 2008); niacin has since been withdrawn from the market following safety concerns. Hyperphosphataemia in CKD is currently treated with oral phosphate binding agents. Although efficacious at reducing serum phosphate in advanced CKD, currently available phosphate binders have to be taken with meals and all have adverse effects (Tonelli *et al.*, 2010). The relatively recent introduction of non-calcium-containing phosphate binders following concerns regarding calcium-loading provides a possible vehicle for testing the above hypothesis without fears of increasing vascular calcification and thus promoting arterial stiffening. Indeed, lowering serum phosphate with newer, non-calcium-containing agents such as sevelamer carbonate may attenuate the progression of arterial calcification and arterial stiffening when compared with the traditional calcium-based phosphate binders (Takenaka and Suzuki, 2005).

It is hypothesised that treatment of patients with stage 3 CKD with the non-calcium-based oral phosphate binder, sevelamer carbonate, will limit exposure of the vasculature to phosphate and thus attenuate the progression of arterial stiffness, reduce the development of systolic hypertension, reduce LV mass and reduce diastolic dysfunction (Ferro *et al.*, 2009).

2 TECHNIQUES

The following chapter describes the common methods used in the forthcoming studies.

2.1 Cardiovascular Magnetic Resonance Imaging

2.1.1 Ventricular Mass, Volumes and Function

Cardiovascular magnetic resonance imaging was performed using a 1.5-T Siemens Symphony scanner (Siemens, Erlangen, Germany; Figure 2-1) with subjects introduced headfirst into the scanner in the supine position (Figure 2-2). Serial contiguous short axis cines were piloted from the vertical long axis and horizontal long axis images of the left and right ventricles (ECG R wave-gated with retrospective triggering, steady-state free precession imaging [True-FISP]; temporal resolution 40-50 ms, repetition time 3.2 ms, echo time 1.6 ms, flip angle 60^{o}, slice thickness 7 mm with 3 mm gap) in accordance with previously validated methodologies (Maceira *et al.*, 2006). Images were acquired in held end-expiration. Analysis was performed offline (Argus Software, Siemens, Erlangen, Germany) by a single blinded observer for the assessment of ventricular volumes (end-diastolic, end-systolic and stroke volumes), function (ejection fraction) and LV mass (Maceira *et al.*, 2006; Myerson *et al.*, 2002). The outline of the LV myocardium was traced by drawing around the epicardium and endocardium at end-diastolic and end-systolic frames for each short axis cine. The most basal slice was determined by the presence of a complete ring of myocardium at end-diastole. Verification of LV stroke

volume was performed in two ways: i) by tracing around the endocardium of the right ventricle at end-diastole and end-systole for each short axis cine to determine right ventricular stroke volume; and ii) by assessing aortic forward flow at the level of the pulmonary artery. Left ventricular measurements were accepted if LV stroke volume was within 10 ml of right ventricular stroke volume and aortic forward flow. As a second internal measure of quality control, LV mass was accepted if end-diastolic and end-systolic masses were within 10% of each other.

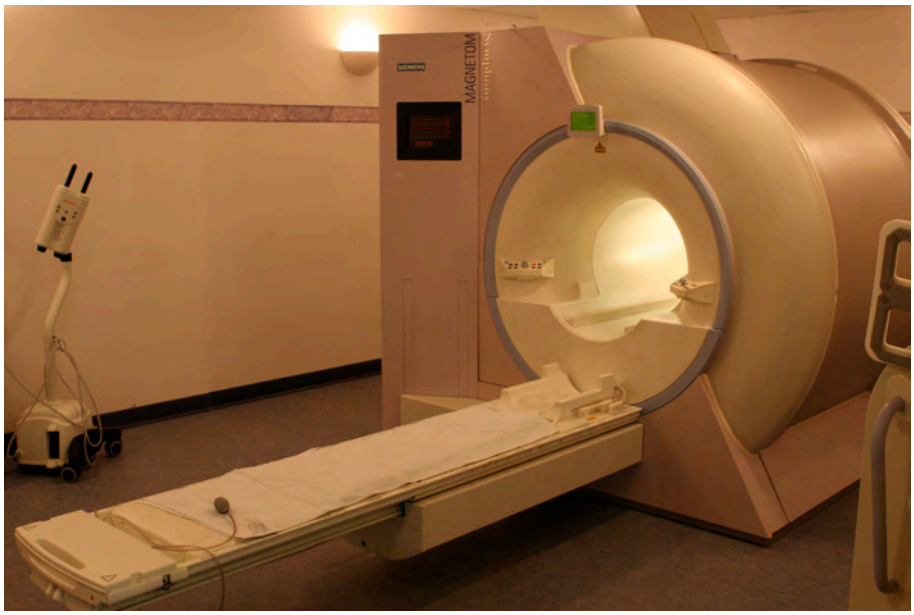

Figure 2-1. Siemens Symphony magnetic resonance scanner.

Figure 2-2. Subjects were introduced into the magnetic resonance scanner headfirst in a supine position.

Left ventricular mass was indexed to body surface area, which was calculated using the Mosteller formula (BSA in m^2)=√(weight in kg x height in cm)/3600) (Mosteller, 1987). Left ventricular hypertrophy was defined as an LV mass index greater than age and gender corrected limits according to previously published recommendations (Maceira *et al.*, 2006).

2.1.2 Aortic Distensibility

For the determination of thoracic aortic distensibility steady-state free precession (True-FISP), R-wave gated, retrospectively triggered, sagittal-oblique cine sequences were undertaken in held end-expiration with the following parameters: temporal resolution 50-60 ms, echo time 2.2 ms, flip angle 60°, field of view 300 mm and slice thickness of 5 mm (Figure 2-3). Transverse slices were then acquired at the level of

the pulmonary artery (Figure 2-4) to examine the ascending and proximal descending aorta and the level of the diaphragm to assess the distal descending aorta. Aortic distensibility ($x10^{-3}$ $mmHg^{-1}$) was calculated through measurement of cardiac cycle-dependent changes in aortic area after accounting for brachial pulse pressure and resting vessel cross-sectional area using a previously validated formula (Groenink *et al.*, 1998):

$$Aortic\ Distensibility = \frac{\Delta\ Aortic\ Area}{Minimum\ Aortic\ Area \times Pulse\ Pressure}$$

where Δ Aortic Area = (Maximum Aortic Area – Minimum Aortic Area) and Pulse Pressure was the average of three brachial pulse pressure measurements performed synchronously with a non-ferromagnetic cuff in the non-dominant arm at the time of CMR image acquisition. Area measurements were performed offline in triplicate at all three aortic levels using CMR Tools software (Imperial College, London, UK). Cardiac magnetic resonance-derived aortic distensibility allows accurate non-invasive determination of aortic stiffness with high reproducibility (Forbat *et al.*, 1995) that closely correlates with the current gold-standard of carotid-femoral PWV described in section 2.2 (Nelson *et al.*, 2009). Furthermore, CMR has the added advantage of enabling direct evaluation of regional stiffness at different aortic locations at high spatial and temporal resolution. As measurements of aortic distensibility are dependent on distending pressure (McEniery *et al.*, 2007) all values were adjusted for mean arterial pressure. This adjustment was performed by carrying out linear regression of the two variables of aortic distensibility and mean

arterial pressure to determine unstandardised residual values. These values were then added to the mean aortic distensibility to determine adjusted aortic distensibility.

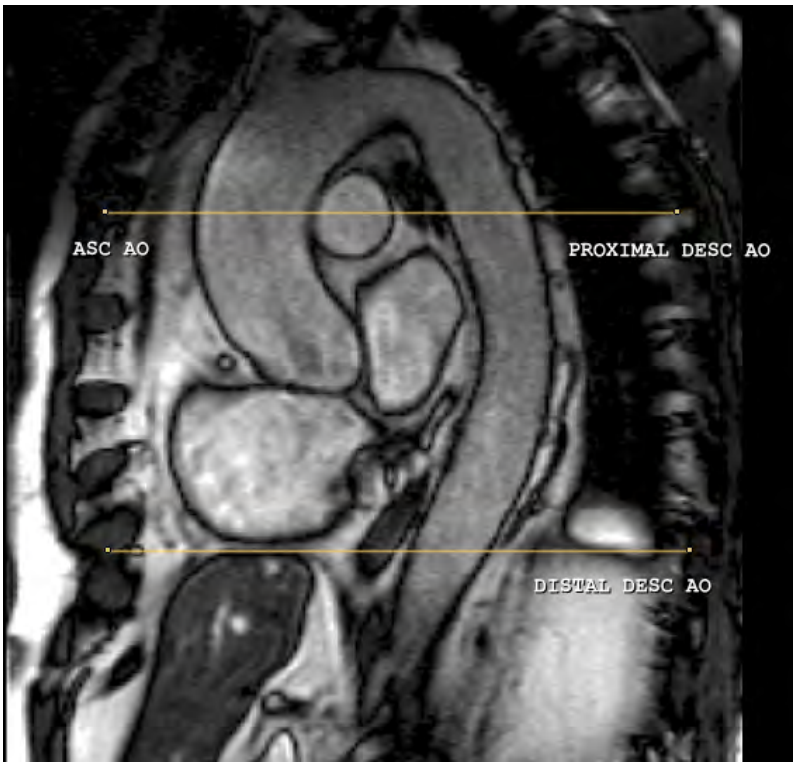

Figure 2-3. Oblique sagittal slice of the thoracic aorta acquired with cardiovascular magnetic resonance imaging.

Transverse lines indicate position of slices for determination of ascending, proximal descending and distal descending aortic distensibility.

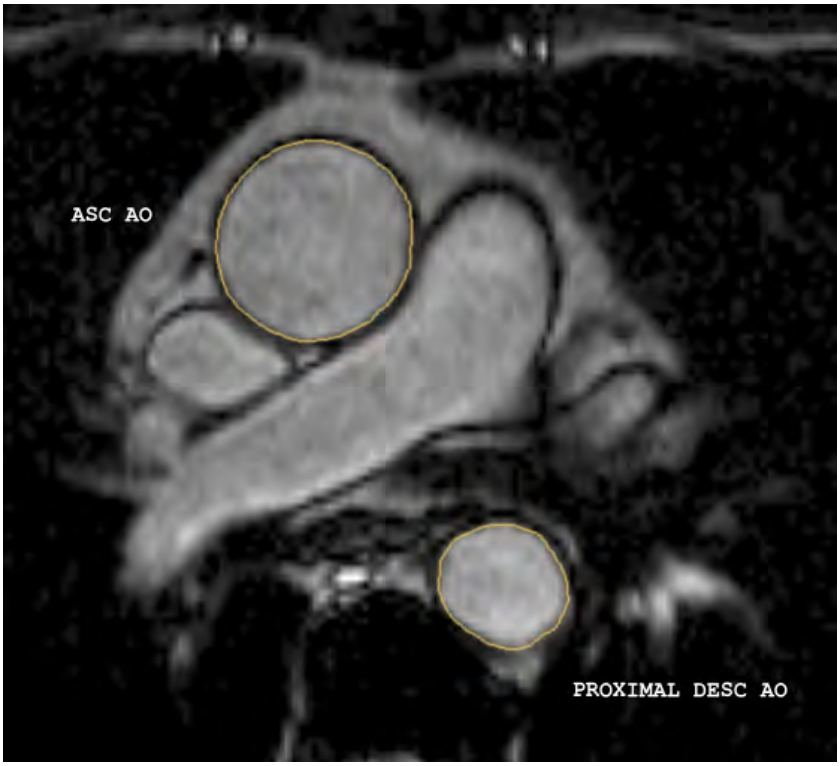

Figure 2-4. Transverse slice through the aorta at the level of the pulmonary artery. Cross-sectional areas for the ascending and proximal descending aorta have been determined.

2.1.3 Reproducibility

Interstudy reproducibility of LV mass and aortic distensibility measurements was determined by repeating CMR on the same day in 12 randomly selected subjects (10% of the overall cohort recruited for the subsequent randomised study described in section 5). Both scans were then analysed by the same observer. Intraobserver variability of LV mass and aortic distensibility was assessed through repeat analysis of 12 randomly selected scans by the same observer. Both sets of results were analysed using an intraclass correlation coefficient for variability with a two-way random effects model.

The overall reliability of interstudy measurements of LV mass index was good with an intraclass correlation coefficient of 0.918 (95% CI 0.742–0.976, P<0.0005). Intraobserver reproducibility for LV mass was also high with an intraclass correlation coefficient of 0.992 (95% CI 0.981–0.997, P<0.0005).

2.2 Assessment of Arterial Stiffness

Several non-invasive methods for determination of local or regional aortic stiffness are available (Laurent *et al.*, 2006). Determination of carotid-femoral PWV along the aorto-iliac pathway is considered the simplest, most reproducible non-invasive method for the direct assessment of regional arterial stiffness. This particular measure of arterial stiffness has been used in a number of epidemiological studies demonstrating the value of aortic stiffness in predicting future cardiovascular events and is considered the current “gold-standard” technique.

There are many different devices available for the determination of PWV (DeLoach and Townsend, 2008). The SphygmoCor system (AtCor Medical, Sydney, Australia) uses a high-fidelity micromanometer (SPC-301, Millar Instruments, Texas, USA) and the technique of applanation tonometry to sequentially record carotid (proximal) and femoral (distal) waveforms transcutaeneously; this system has been validated for the assessment of aortic stiffness against invasive measurements of central aortic pressure (Laurent *et al.*, 2006; Pauca *et al.*, 2001). In the studies described in subsequent chapters, PWV and PWA were performed using the technique of applanation tonometry and a SphygmoCor device (AtCor Medical, Sydney) (Laurent

et al., 2006). Measurements were performed in the morning following an overnight fast and subjects were advised to avoid consumption of caffeine or alcohol in the 12 hours preceding the study. Applanation tonometry was performed in a quiet room following 15 minutes of supine rest using a high-fidelity micromanometer (SPC-301, Millar Instruments, Texas; Figure 2-5). Measurements were obtained in held expiration where necessary to optimise trace quality. All measurements were made in triplicate and mean values used in analysis.

Figure 2-5. The technique of applanation tonometry using a high-fidelity micromanometer.

2.2.1 Pulse Wave Velocity

Carotid-femoral PWV was calculated by measuring the distance between the carotid and femoral arteries and dividing this by the transit time (the time delay between the occurrence of each waveform). The distance between the two recording sites was approximated through measurement of the surface distance in metres using a

standard tape measure. This has been previously performed in three ways: i) measurement of the total distance between carotid artery and femoral artery; ii) measurement of the total distance then subtracting the distance from the carotid artery to the sternal notch; and iii) measuring the distance from the sternal notch to the femoral artery and subtracting the distance from the carotid artery to the sternal notch (Van Bortel *et al.*, 2002). In all the studies described in subsequent chapters the distance calculation employed was measurement of the total distance between carotid and femoral artery with subtraction of the distance from the carotid artery to sternal notch.

The transit time was calculated using the foot-to-foot method, which determines the time difference between the onset of the pulse wave at the carotid and femoral measuring sites. The foot of the pulse wave was defined as the onset of the steep rise of the pulse wave at end diastole. Carotid and femoral arterial waveforms were sequentially recorded using the micromanometer whilst an ECG was simultaneously obtained (Figure 2-6). The transit time was calculated by determining the onset of the pulse wave from the R wave of the ECG; the time between the R wave and carotid (proximal) pulse was subtracted from the time between the R wave and femoral (distal) pulse to derive the transit time. Carotid-femoral PWV was then calculated in metres per second using the formula:

$$PWV = \frac{Distance\ (Metres)}{Transit\ Time\ (Seconds)}$$

This method is reproducible in both healthy subjects and in patients with renal impairment (intraobserver variability of 0.07±1.17 m/s and interobserver variability of -0.30±1.25 for a typical mean of 8.15 ± 3.01 m/s) (Wilkinson *et al.*, 1998; Wimmer *et al.*, 2007).

Figure 2-6. Output from pulse wave velocity.

Aortic PWV is BP dependent so values were adjusted for mean arterial pressure as described elsewhere (Ford *et al.*, 2010). Adjustment was achieved by performing a linear regression of PWV with mean arterial pressure as the determinant. Unstandardised residuals derived from this regression were then added to the mean PWV to calculate adjusted PWV (PWV_{adj}).

2.2.2 Pulse Wave Analysis

The technique of pulse wave analysis (PWA) enables assessment of the central aortic pressure waveform and determination of central aortic BP and the augmentation index (AIx; Figure 2-7). Pulse wave analysis was performed non-invasively with the high-fidelity micromanometer and the SphygmoCor system. The micromanometer was used to flatten, but not occlude, the radial artery using gentle pressure (Figure 2-8). Data were collected directly into a portable computer and after 11 seconds of data capture, an averaged peripheral waveform with corresponding central aortic waveform, central aortic pressures and AIx were generated using a validated transfer function (Figure 2-9) (Chen *et al.*, 1997). Although measurement at the carotid artery is preferred as this provides a true central pressure waveform, measurement at the radial artery is technically less challenging as the artery is well supported by surrounding bony structures. The determination of central aortic pressures from a transfer function has been validated using invasive techniques (Pauca *et al.*, 2001), although it should be noted that the use of brachial BP as a proxy for radial or carotid BP is a potential source of error. This method is reproducible in both healthy subjects and in patients with renal impairment (Savage *et al.*, 2002; Wilkinson *et al.*, 1998).

The central pressure waveform begins at the start of systole with arrival of the foot of the pulse wave and peaks at systolic pressure (P1). The inflection point (P2) represents the arrival of reflected waves from the periphery. The dicrotic notch, which occurs synchronously with closure of the aortic valve, represents the end of systole. The AIx is defined as the difference between the two systolic peaks of the

arterial pressure waveform (P1 and P2; Figure 2-7) expressed as a percentage of the pulse pressure. Results for AIx were also adjusted to a heart rate of 75 beats/minute (AIx_{75}) (Wilkinson *et al.*, 2000). Central AIx increases with age and systolic blood pressure (Baksi *et al.*, 2009) and is an independent predictor of all-cause and cardiovascular mortality in patients with ESKD (London *et al.*, 2001; Safar *et al.*, 2002).

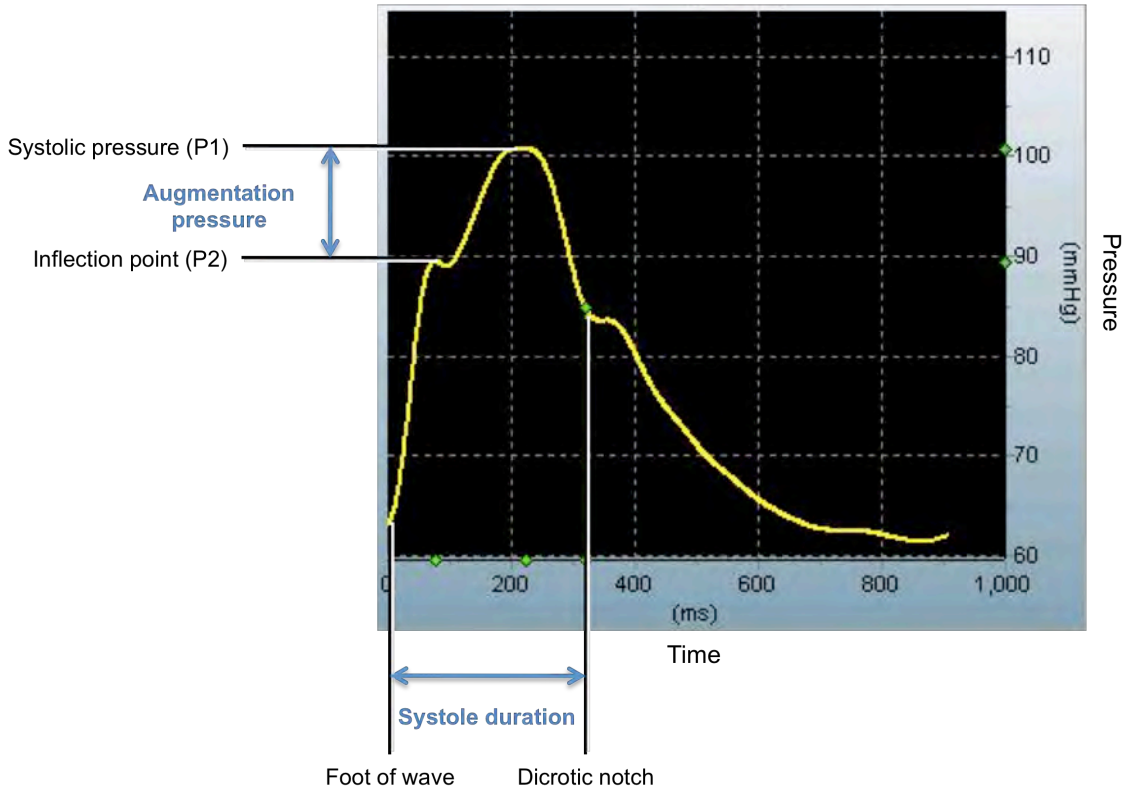

Figure 2-7. The central proximal aortic pressure waveform generated from pulse wave analysis. Systolic pressure is represented by the peak of the pulse wave (P1). The inflection point (P2) represents the arrival of reflected waves from the periphery. The pressure difference between P1 and P2 is the augmentation pressure. This is expressed as a percentage of pulse pressure to derive the augmentation index.

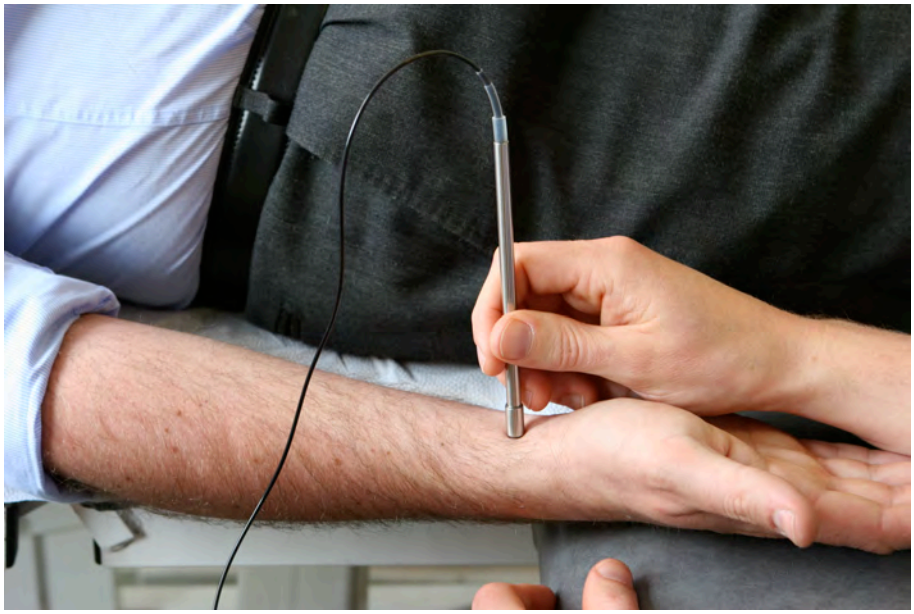

Figure 2-8. Using applanation tonometry to perform pulse wave analysis on the radial artery.

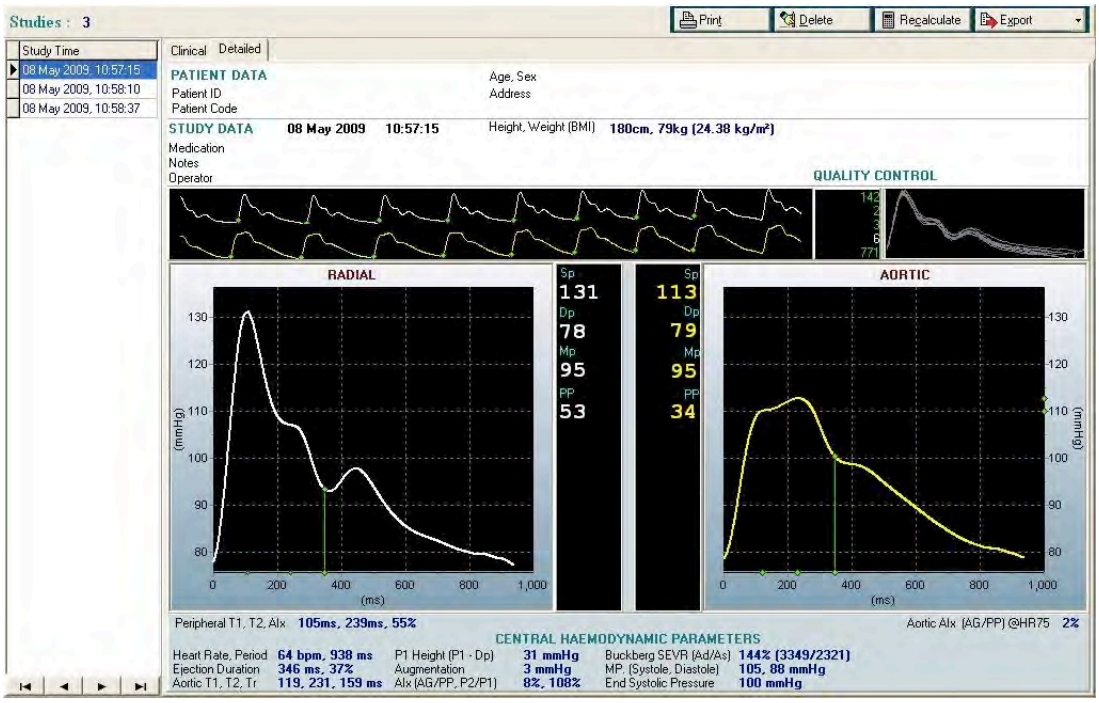

Figure 2-9. Output from pulse wave analysis.

2.3 Assessment of Blood Pressure

Office brachial BP was measured with a validated oscillometric sphygmomanometer (Dinamap Procare 200, GE Healthcare, Hatfield, United Kingdom) following 15 minutes of supine rest as per the latest British Hypertension Society and European Society of Hypertension guidelines (O'Brien *et al.*, 2003). Measurements were made in triplicate in the non-dominant arm and the first two measurements were discarded.

Twenty-four hour ambulatory BP was recorded using a validated ambulatory BP monitor (Meditech ABPM-04, PMS Instruments, Maidenhead, United Kingdom) with recordings performed every 30 minutes during the day (07:00 to 23:00) and every hour at night (23:00 to 07:00). Day-time, night-time and 24-hour averages were calculated for systolic BP, diastolic BP, pulse pressure, mean arterial pressure and heart rate.

2.4 Echocardiography

A comprehensive transthoracic echocardiogram (Vivid 7, GE Vingmed Ultrasound, Horten, Norway; Figure 2-10) was performed with the subject in the left lateral decubitus position by a single experienced blinded echocardiographer using second harmonic imaging and an M3S multi-frequency transducer (Figure 2-11). Analysis was performed offline by a single blinded observer using an EchoPAC workstation (version 108.1.4, GE Vingmed Ultrasound, Horten, Norway). All parameters were measured in triplicate and averaged as per the latest recommendations from the

American Society of Echocardiography (Lang *et al.*, 2005). Ventricular dimensions, wall thickness, chamber volumes and stroke volume were determined using standard methods. Left ventricular mass was determined by the 2-dimensional area-length formula and indexed to body surface area. Resting LV diastolic function was determined using standard techniques, including measurement of mitral inflow velocities E and A, E/A ratio, E wave deceleration, mitral inflow velocity propagation, isovolumic relaxation time and pulmonary vein systolic and diastolic flow (Nagueh *et al.*, 2009). Peak systolic (s'), early diastolic (e') and late diastolic (a') mitral annular velocities were measured at end expiration at the septal, lateral, inferior and anterior LV walls with real time pulsed wave tissue Doppler (Alam *et al.*, 1999), allowing calculation of mean e' and E/e' for further assessment of diastolic function.

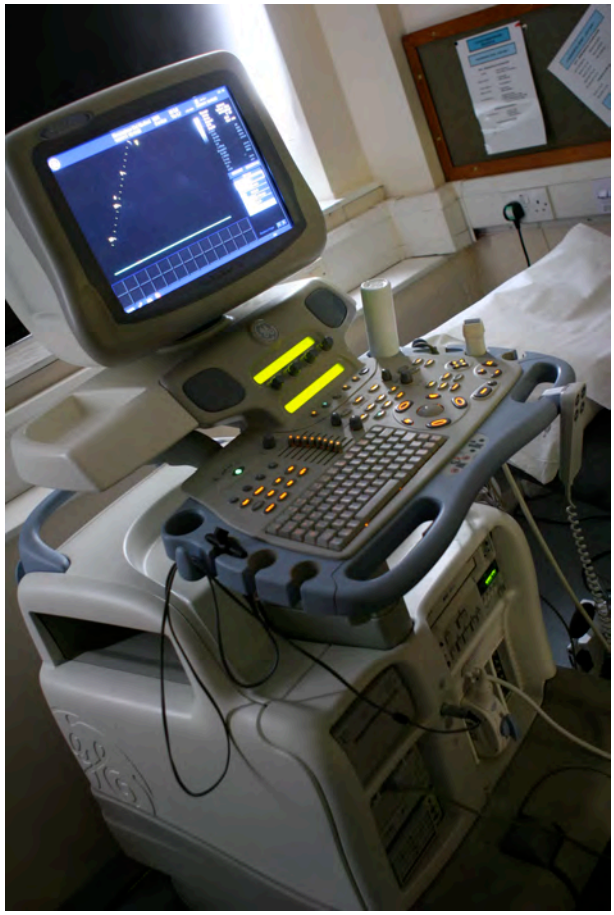

Figure 2-10. Vivid 7 echocardiography machine.

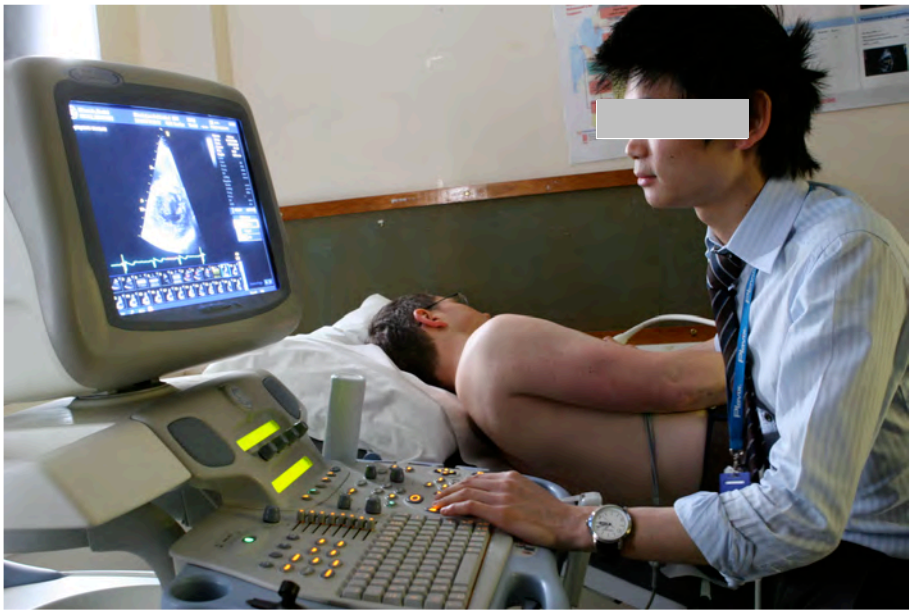

Figure 2-11. Transthoracic echocardiography in progress.

2.4.1 Strain and Strain Rate Imaging

Images for 2-dimensional LV strain and strain rate imaging were acquired in cineloop format in triplicate using gray scale harmonic imaging from the apical 4-, 2- and 3-chamber views at end-expiration at frame rates >70/second for offline analysis using commercially available software (Speqle Tracking, GE Healthcare, United Kingdom). The endocardial border was manually traced at end-systole and the software then generated a region of interest to overlie the myocardium. The thickness of this region of interest was altered according to the thickness of the myocardium. This enabled frame-to-frame tracking of ultrasonic speckles that changed position according to underlying myocardial tissue motion throughout the cardiac cycle. Tracking of the myocardium was assessed by both the software and the observer; if tracking was unsatisfactory in any myocardial segment the region of interest was

manually adjusted up to a maximum of three times to enable adequate tracking. If tracking of any segments remained suboptimal, such segments were excluded from analyses. Any views in which more than three segments failed to track were excluded from analyses. Peak longitudinal systolic (s') and early diastolic (e') velocities (Figure 2-12), peak longitudinal systolic, end systolic and post systolic strain (if present; Figure 2-13) and peak longitudinal systolic (s') and early diastolic (e') strain rate (Figure 2-14) were measured and recorded for each myocardial segment (where available) in triplicate and averaged. Values were recorded for all three segments (basal, mid and apical) that comprised the six walls of the left ventricle (anterolateral, inferoseptum, anterior, inferior, anteroseptum and inferolateral). Global peak longitudinal systolic strain was defined as the mean peak longitudinal systolic strain of all 18 myocardial segments.

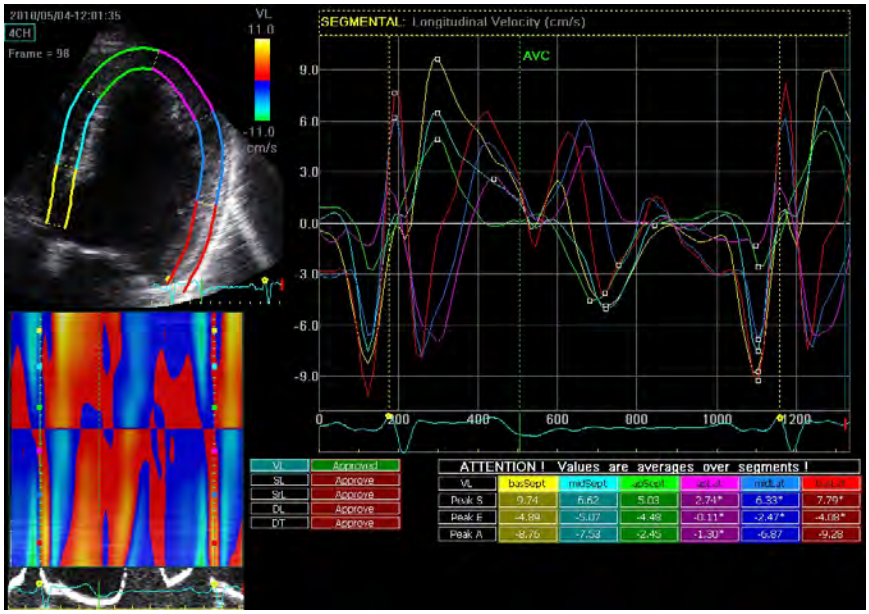

Figure 2-12. Segmental left ventricular longitudinal velocities determined using speckle tracking.

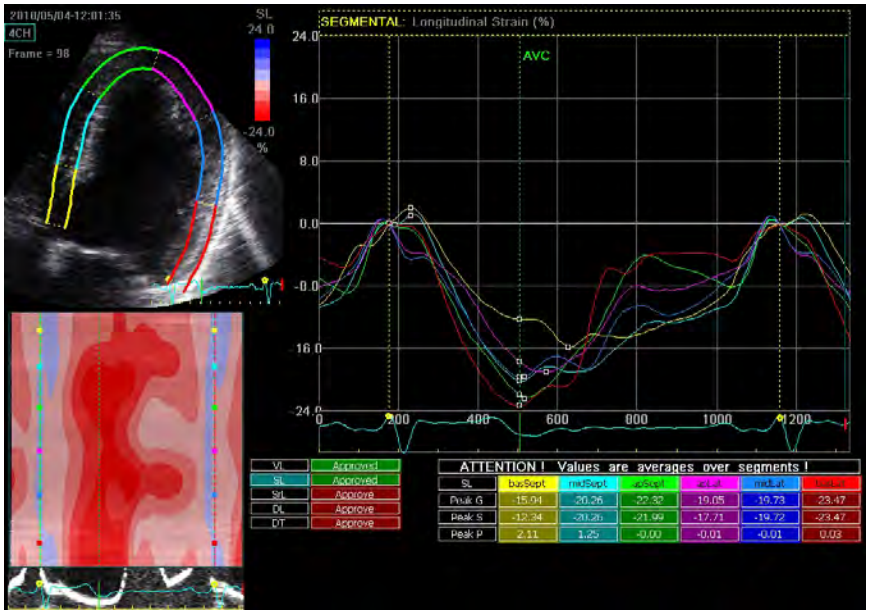

Figure 2-13. Segmental left ventricular longitudinal strain determined using speckle tracking.

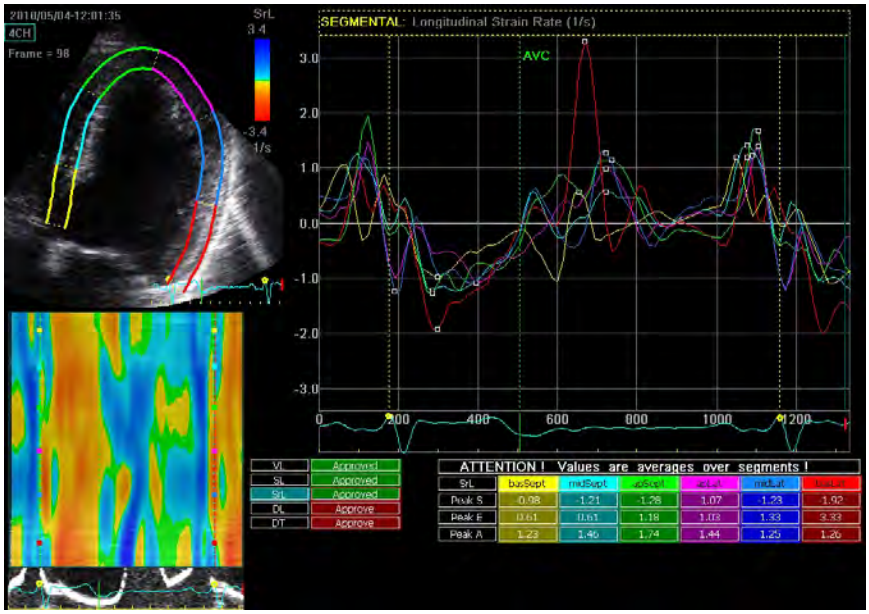

Figure 2-14. Segmental left ventricular longitudinal strain rate determined using speckle tracking.

2.4.2 Left Ventricular Rotation and Twist

The same techniques described in section 2.4.1 were adopted for acquisition and analysis of images for the assessment of LV rotation and twist. Parasternal short axis views were acquired at basal, equatorial and apical levels and stored for offline analysis. Peak rotation, time to peak rotation and systolic and diastolic rotation rates were recorded at the base and apex (Figure 2-15) for each myocardial segment. Instantaneous twist curves were automatically generated by the software through subtraction of the basal rotation curve from the apical rotation curve (Figure 2-16); peak twist and time to peak twist were recorded. Similarly instantaneous rotation rate curves were generated through subtraction of the basal rotation rate curve from the apical rotation rate curve; peak twist rate, untwist rates and timings were recorded. Basal and apical pairs were analysed in triplicate and averaged.

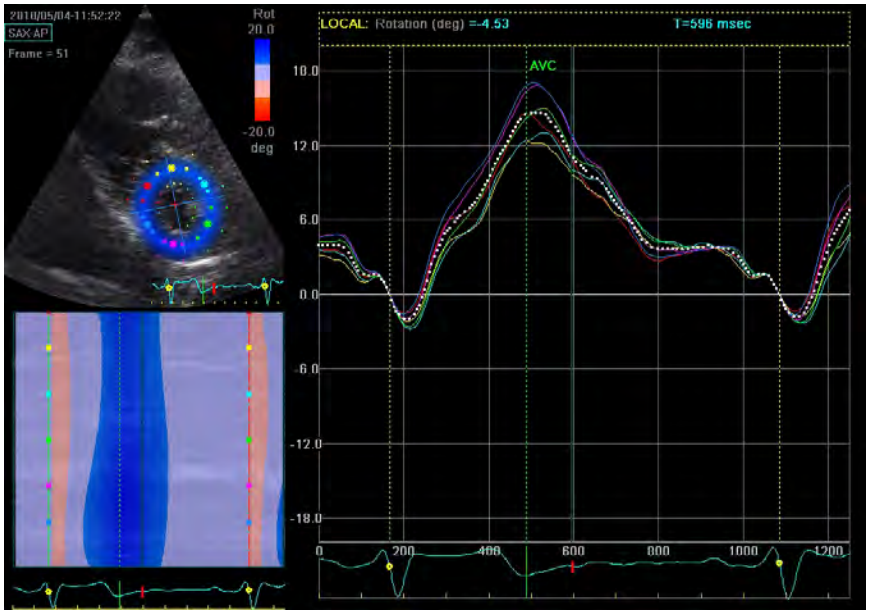

Figure 2-15. Apical rotation assessed using speckle tracking.

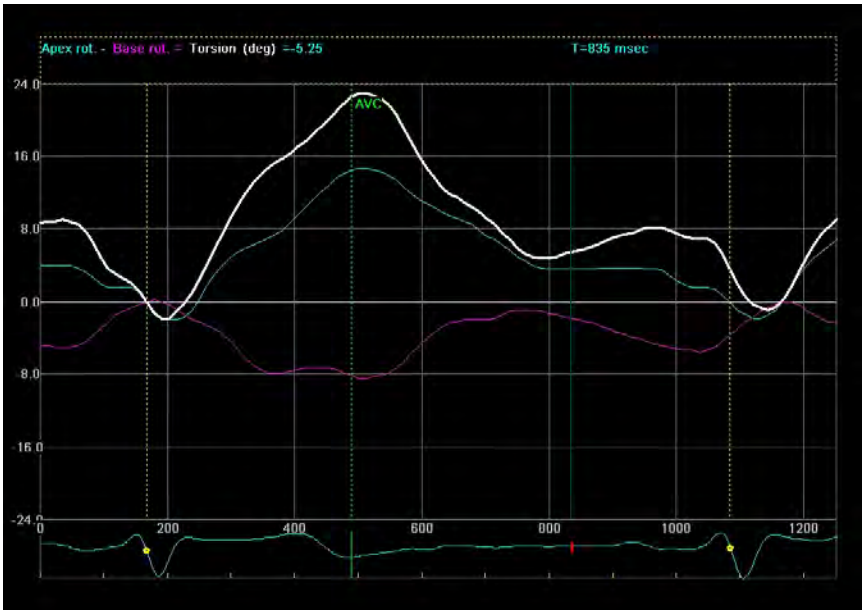

Figure 2-16. Left ventricular twist assessed using speckle tracking.

2.5 Dual Energy X-ray Absorptiometry Scanning

Dual energy x-ray absorptiometry scanning (Hologic QDR Series 4500 with Discovery Software version 11.02:03, Hologic Europe, Zaventem, Belgium) was used to assess BMD of the lumbar spine (L1–L4) and both proximal femurs (femoral neck, Ward's region, trochanteric region). An experienced bone densitometry clinical scientist blinded to clinical data reported all scans. T-scores that refer to the young adult reference mean calculated from the manufacturer's database (based on the NHANES population) were determined (Looker *et al.*, 1998). Osteopenia and osteoporosis were defined according to the World Health Organisation definitions (normal bone T-score >-1, osteopenia T-score -1 to -2.5 and osteoporosis T-score <-2.5). The BMD of each subject was standardised by calculating the difference between the observed and predicted values (sourced from the manufacturer's

reference database based on the NHANES population) divided by the square root of the estimated variance. This derived standard score, or Z-score, is a measure of the deviation from the expected population mean, adjusted for the covariance on a scale with zero mean and unit standard deviation, so that 95% of the normal population will have a Z-score between -2 and 2. The coefficient of variation for the determination of BMD is 1.1%.

2.6 Lateral Lumbar Spine Radiography

The presence and extent of abdominal aortic calcification were assessed using a lateral lumbar spine radiograph and validated semi-quantitative scoring method as previously described (Figure 2-17) (Kauppila *et al.*, 1997). This semi-quantitative technique shows good correlation with electron beam computed tomography assessment (Bellasi *et al.*, 2006). The abdominal aorta adjacent to the L1–L4 vertebra was divided into four sections using the midpoint of each intervertebral space as a boundary. The anterior and posterior aortic walls of each section were scored out of 3 according to the extent of calcification present: if less than 1/3 of the wall was calcified, a score of 1 would be given; if more than 1/3 but less than 2/3 of the wall was calcified, a score of 2 would be given; if more than 2/3 of the wall was calcified, a score of 3 was given. The maximum score possible for the entire abdominal aorta was 24. All radiographs were analysed and scored by two independent observers. Any discordance in the presence or absence of calcification between the two observers resulted in re-assessment of that radiograph and evaluation by a radiologist to reach a final consensus.

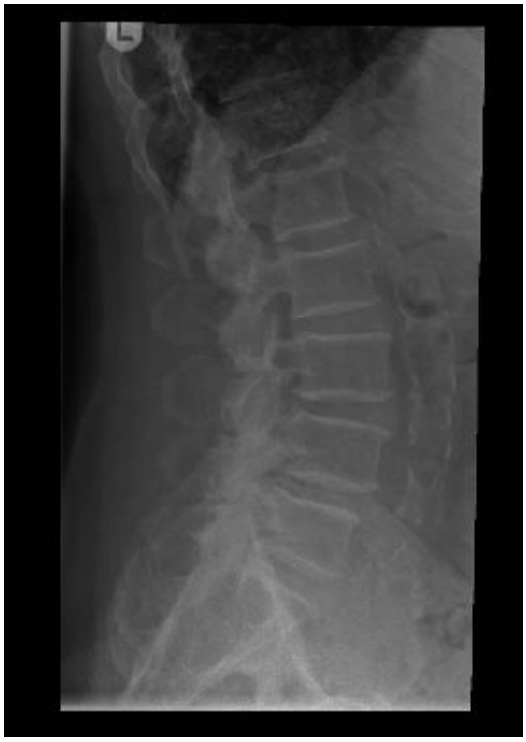

Figure 2-17. Lateral lumbar spine radiography demonstrating calcification of the anterior and posterior walls of the abdominal aorta.

2.7 Biochemical Assays

2.7.1 Assessment of Renal Function

Renal function was assessed in the following studies through measurement of eGFR using the 4-variable MDRD equation with serum creatinine recalibrated to be traceable to an isotope-derived mass spectroscopy method (Levey *et al.*, 1999). Although a less accurate method of determining “true” GFR compared to radionuclide studies, this reflects the method used by the vast majority of population studies that demonstrate an increased cardiovascular risk in patients with CKD and does not

involve use of ionising radiation. The MDRD equation has been criticised for underestimating true GFR at higher levels of kidney function (Stevens *et al.*, 2007), leading to the introduction of the CKD-EPI formula in 2009 (Levey *et al.*, 2009). As recruitment of subjects for these studies began prior to the introduction of this formula, the MDRD equation was used.

2.7.2 High Sensitive C-Reactive Protein

High sensitive CRP (hsCRP) was measured using an in-house enzyme-linked immunosorbent assay (ELISA; IBL International GMBH, Hamburg, Germany). This was performed by Dr Nadezhda Wall and myself in the School of Immunity and Infection at the University of Birmingham. 10 µl of each serum sample was diluted in buffer to a 1:1000 dilution and 100 µl added to a pre-coated 96 well plate. Five standard calibrators were diluted to a 1:100 dilution and also added to the plate in order to generate a calibration curve. Following 30 minutes of incubation at room temperature and three wash cycles, 100 µl of conjugated monoclonal anti-human CRP antibody was added before another 30 minutes of incubation at room temperature. A second wash was performed before tetramethylbenzidine solution was added. After 10 minutes of incubation at room temperature in a light-protected environment the reaction was stopped using 0.5 molar sulphuric acid and the plate was read immediately using a microplate reader at an absorbance of 450 nm. Optical density was analysed using GraphPad Prism (GraphPad Software, California, USA) and samples were plotted against the calibration curve generated from the plate standards. The minimum detectable concentration of the assay was 0.02 µg/ml

with an intra-assay coefficient of variation of 4.1–6.9% and inter-assay coefficient of variation of 5.8–6.3%.

2.7.3 N-terminal Pro-brain Natriuretic Peptide

Plasma concentrations of N-terminal pro-brain natriuretic peptide (NT-proBNP) were determined with the assistance of Professor Leong Ng and Paulene Quinn of the Pharmacology and Therapeutics Group at the University of Leicester. Levels of NT-proBNP were determined using an immunoluminometric assay that has been previously described (Hughes *et al.*, 1999). A rabbit anti-human NT-proBNP polyclonal antibody directed against a domain of the C-terminal section of NT-proBNP and labelled with the chemiluminescent label 4-(2-succinimidyloxycarbonylethyl)phenyl-10-methylacridinium 9-carboxylate fluorosulphonate was used. Samples were assayed in duplicate and the average of two measurements reported. The lower limit of detection for the assay was 0.3 pmol/l.

2.7.4 Fibroblast Growth Factor 23

The phosphatonin FGF-23, its soluble co-receptor klotho, and vitamin D were measured alongside serum phosphate, calcium and PTH in order to accurately determine calcium-phosphate status. The roles of FGF-23, klotho and vitamin D in phosphate and calcium homeostasis are described in section 1.7. Fibroblast growth factor 23 was measured using a two-site second-generation monoclonal antibody ELISA (Kainos Laboratories Inc., Tokyo, Japan) that detects biologically active intact FGF-23 (Yamazaki *et al.*, 2002). 50 µl of assay diluent and 50 µl of serum sample

were added to each well of a 96 well plate. 50 µl of each of the seven FGF-23 standards were also added to each plate in order to generate a calibration curve. The plate was incubated for 2 hours at room temperature on a plate mixer before four wash cycles were performed. 100 µl of murine monoclonal antibody conjugated to horseradish peroxidase was then added to each well before further incubation at room temperature for 1 hour on the plate mixer. After a second series of wash cycles 100 µl of tetramethylbenzidine was added to each well before 30 minutes of incubation in a light-protected environment. 100 µl of 0.5 molar sulphuric acid was then added to each well to stop the reaction before the plate was read using an ELISA plate reader (Medgenix Diagnostics, Belgium) at an absorbance of 450 nm. Reporting of absorbance data was performed using SoftMax Pro version 3.1.1. software (Molecular Devices Inc, California, USA) and measurements determined using the calibration curve plotted from the seven standards. The range of detection of the assay was 10–800 pg/ml. The FGF-23 assays were performed under the supervision of Dr Daniel Zehnder, Associate Clinical Professor, at the University of Warwick.

2.7.5 Soluble Klotho

Soluble serum α-klotho was determined using a solid phase sandwich ELISA (Immuno-Biological Laboratories Co., Japan). 100 µl of buffer was added to each well of a 96 well plate. 100 µl of each serum sample along with 8 pre-diluted standards were added to the wells. The plate was then incubated at room temperature for one hour before washing. After seven wash cycles 100 µl of conjugated anti-human klotho mouse IgG monoclonal antibody was added to each

well before a further 30 minutes of incubation at room temperature. After nine wash cycles 100 µl of tetramethylbenzidine was added before another 30 minutes of incubation at room temperature in a light-protected environment. 100 µl of 1 molar sulphuric acid was then added to each well to stop the reaction before the plate was read using the ELISA plate reader at an absorbance of 450 nm. Reporting of absorbance data was performed using SoftMax Pro version 3.1.1. software (Molecular Devices Inc, California, USA) and concentrations of soluble klotho were then determined using the calibration curve plotted from the standards. The measurement range of the assay was 150–6000 pg/ml with an intra-assay coefficient of variation of 2.7–3.5% and inter-assay coefficient of variation of 2.9–11.4%. Serum α-klotho assays were performed under the supervision of Dr Daniel Zehnder, Associate Clinical Professor, at the University of Warwick.

2.7.6 Vitamin D

All vitamin D assays were performed at University College London Hospitals NHS Foundation Trust under the supervision of Dr Anne Dawnay, Consultant Biochemist. 1,25-dihydroxyvitamin D was measured using immunoextraction followed by quantification by ELISA (Immunodiagnostic Systems Ltd, Tyne and Wear, UK) using a Griffoils Triturus automated ELISA station. The measurement range of the assay was <6–333 pmol/l with an intra-assay coefficient of variation of 9.3–10.7% and an inter-assay coefficient of variation of 17.1–19.7%. 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 were measured by liquid chromatography-tandem mass spectrometry using an ACQUITY UPLC-TQD system (Waters Corporation, Milford, Massachusetts, USA) operating in positive electrospray ionisation mode. Serum

samples underwent solid phase extraction using Oasis HLB µElution plates (Waters Corporation) and extracts were injected into the chromatography system containing a BEH phenyl column (2.1 x 50 mm, 1.7 µm, Waters Corporation) with a water-methanol gradient supplemented with 0.1% formic acid and 2 mmol/l ammonium acetate. Analytes were measured using multi-residue methods with quantifier and qualifier transitions accompanied by respective isotopic internal standards. Calibration and quality control were routinely monitored with an inter-assay coefficient of variation of <10%.

2.8 Overview of Statistical Methods

All data in this work were analysed using SPSS version 19 (SPSS Inc., Chicago, Illinois, USA). Normality of data distribution for each variable was determined using the Kolmogorov-Smirnov test and normality plots. Normally distributed variables were presented as mean ± standard deviation and analysed using parametric tests. Any non-normally distributed variables were log-transformed prior to analysis to achieve normality and the logs were analysed using parametric tests; such variables were quoted as median (interquartile range). If normality was not achieved with log transforming, non-parametric analyses were performed. Between-group comparisons were performed using independent t-tests (two groups) or a one-way analysis of variance (more than two groups). Within-group comparisons across different time points were assessed using paired samples t-tests. Between-group comparisons across different time points (week 0 and week 40) were performed using a repeated measures analysis of variance, with the time point (week 0 or week

40) as the within-subjects factor and the group (placebo, sevelamer) as the between-subjects factor, thus testing the difference in change over time between the two groups. Linear regression analysis was performed to determine the relationship between continuous variables; parametric variables were assessed using Pearson's correlation and any non-parametric variables assessed using Spearman's. Co-linearity between explanatory variables was assessed by examining the variance inflation factor, with a variance inflation factor >10 indicating relevant co-linearity. Categorical variables were presented as frequency (percentage) and analysed using the Pearson χ^2 test. A Type I error rate below 5% ($P<0.05$) was considered statistically significant.

2.9 Approvals and Authorisations

West Midlands Research Ethics Committee granted ethical approval for the human research studies conducted in this thesis (ref. 08/H1208/37). The Medicines and Healthcare products Regulatory Agency provided authorisation for the unlicensed use of sevelamer carbonate in patients with normal serum phosphate and stage 3 CKD (EudraCT number 2008-003727-23). University Hospitals Birmingham NHS Foundation Trust acted as sponsor for the study (ref. RRK3563). Written, informed consent was obtained from each participant. All studies were conducted in accordance with the *Declaration of Helsinki* and the principles of *Good Clinical Practice.*

3 RELATIONSHIP BETWEEN SERUM PHOSPHATE AND DECLINE IN RENAL FUNCTION

3.1 Introduction

3.1.1 Background

Patients with CKD have high levels of cardiovascular morbidity and mortality that cannot be fully explained by traditional cardiovascular risk factors. One "alternative" risk factor for future cardiovascular morbidity and mortality is the rate of deterioration in renal function; some studies suggest this is a better predictor of future cardiovascular risk than baseline renal function (Matsushita *et al.*, 2009; Shlipak *et al.*, 2009). Markers of decline in renal function may therefore prove to be reliable determinants of future cardiovascular risk in this population.

Serum phosphate has been identified as a predictor of progression of renal dysfunction in "advanced" (stage 4 and 5) CKD (Voormolen *et al.*, 2007), in hypertensive renal disease in African-Americans (Norris *et al.*, 2006) and, more recently, in transplant recipients (Connolly *et al.*, 2009; Moore *et al.*, 2010).

3.1.2 Objective

The aim of this study was to investigate the relationship between serum phosphate levels and markers of arterial stiffness on the progression of renal dysfunction in a well-characterised cohort of patients with early-stage CKD.

3.2 Subjects and Methods

3.2.1 Participants

This was a single-centre cohort study of participants that were recruited to take part in studies examining the effectiveness of treatments aimed at lowering cardiovascular risk in patients with CKD (Edwards *et al.*, 2009). Subjects were recruited from specialist renal clinics at the Queen Elizabeth Hospital Birmingham, United Kingdom from May 2004 to October 2007. Inclusion criteria were CKD as defined by Kidney Dialysis Outcomes Quality Initiative (K/DOQI) (K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification, 2002) not yet on dialysis, stable renal function within the last 3 months (<5 ml/min/1.73m^2 change) and no change in medication in the preceding 3 months. Patients with atrial fibrillation, known LV dysfunction (ejection fraction <55%) or with signs and symptoms of congestive heart failure were excluded. Patients on calcineurin inhibitors were excluded because of the known direct haemodynamic effects of these agents. Patients on phosphate binders and vitamin D analogues were also excluded. South Birmingham Research Ethics Committee provided approval for this study.

3.2.2 Outcome Measures

Follow-up data were prospectively collected. At outpatient review venous blood was routinely collected and analysed for phosphate, calcium, PTH and other haematological and biochemical parameters. Serum calcium levels were corrected for albumin level. All serum creatinine values were measured during normal clinical

practice and recorded for the duration of follow up. Estimated glomerular filtration rate was determined by the 4-variable MDRD formula with serum creatinine recalibrated to be traceable to an isotope derived mass spectroscopy method (Levey *et al.*, 1999). Progression of renal dysfunction was defined as the slope of eGFR plotted against time. Time was defined as time between data collection until death, start of renal replacement therapy or study termination. A combined endpoint of start of dialysis (excluding temporary dialysis) or ≥25% decline in eGFR since study entry was used. The study database was closed in September 2008. Follow-up data were censored at the start of renal replacement therapy or death.

Brachial BP was measured as described in section 2.3. Aortic PWV and PWA were used as indices of arterial stiffness and were measured using the methods described in section 2.2.

3.2.3 Statistical Analysis

All data were analysed as described in section 2.8. Aortic PWV was adjusted for mean arterial pressure as described in section 2.2.1. Linear and logistic regression analysis was used to assess the relationship between decline in renal function and parameters under investigation. In multivariate analyses variables associated with decline in kidney function as defined by K/DOQI were used, including baseline eGFR, haemoglobin and systolic BP (K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification, 2002). Analysis was also corrected for age, gender and proteinuria (Hunsicker *et al.*, 1997; Jungers *et al.*,

1995; K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification, 2002).

3.3 Results

3.3.1 Participants

A total of 257 patients were recruited. Thirty-two patients were excluded due to incomplete data collection leaving 225 subjects used in analyses. Demographic, biochemical and co-morbidity characteristics of these patients are listed in Table 3-1. Renal diagnoses and medication at recruitment are listed in Table 3-2. Patients were on average on 2.0 ± 1.2 antihypertensive agents. Median follow-up was 924 days (interquartile range 637–1176) and the median number of eGFR determinations per patient was 10 (7–14). Eleven (5%) patients died during follow-up.

Variable	n=225
Age (years)	59 ± 13
Male gender (%)	135 (60)
eGFR (ml/min/1.73m^2)	43 ± 19
eGFR decline (ml/min/1.73m^2/month)	0.11 ± 0.54
Brachial systolic BP (mmHg)	138 ± 20
Brachial diastolic BP (mmHg)	80 ± 11
Aortic systolic BP (mmHg)	127 ± 18
Aortic diastolic BP (mmHg)	81 ± 12
AIx_{75}	25 ± 10
Pulse wave velocity (m/s)	9.0 ± 2.6
Pulse wave velocity$_{adj}$ (m/s)	9.0 ± 2.5
Body mass index (kg/m^2)	28.2 ± 5.2
Diabetes mellitus (%)	27 (12)
Cardiovascular disease (%)	41 (18)
Current or ex-smokers (%)	97 (43)
Phosphate (mmol/l)	1.22 ± 0.27
Calcium (mmol/l)	2.34 ± 0.14
Parathyroid hormone (pg/ml)*	51 (32–84)
Haemoglobin (g/dl)	13.1 ± 1.7
Urinary albumin: creatinine ratio (mg/mmol)	5.4 (0.8–31.5)

Table 3-1. Baseline characteristics of the study population.

*Available in 200 patients. Data are mean ± standard deviation, frequency (percentage) or median (interquartile range). eGFR, estimated glomerular filtration rate; BP, blood pressure; AIx_{75}, augmentation index adjusted for a heart rate of 75 beats per minute.

Primary kidney disease (%)	
Multi-system disease	34
Glomerulonephritis	20
Renal vascular disease/nephrosclerosis	9
Polycystic kidney disease	6
Diabetic nephropathy	3
Other	16
Unknown	12
Medication (%)	
ACEI or ARB	79
β-blocker	27
Calcium channel blocker	32
Diuretic	43
α-blocker	16
Statin	48
Aspirin	23
Warfarin	6

Table 3-2. Renal diagnoses of the study population and medication at recruitment. ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

3.3.2 Baseline

The mean baseline eGFR was 43 ± 19 ml/min/1.73m^2 (Table 3-1). The stage of CKD was divided as follows: stage 2 16%, stage 3 59%, stage 4 20% and stage 5 5%. The mean serum phosphate concentration was 1.22 ± 0.27 mmol/l. Phosphate within the target range as defined by the 2009 Kidney Disease: Improving Global Outcomes Initiative (KDIGO) guidelines (KDIGO clinical practice guideline for the

diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD), 2009) (0.8–1.2 mmol/l) was present in 121 (54%); hyperphosphataemia was present in 102 (45%) and hypophosphataemia in 2 (1%) patients. No patients were on a low protein diet.

Serum phosphate levels were significantly inversely associated with baseline eGFR (r=-0.19, P=0.005). After correction for baseline eGFR, serum phosphate was associated with PTH (β=0.28, P<0.0001), higher albuminuria (β=0.14, P=0.04), lower haemoglobin (β=0.18, P=0.008), lower calcium (β=0.14, P=0.048) and female gender (β=0.19, P=0.005).

3.3.3 Decline in Renal Function

The mean decline in renal function was 0.11 ± 0.54 ml/min/1.73m^2/month. Serum phosphate was significantly associated with decline in renal function (Figure 3-1, Table 3-3): a 1 mmol/l increase in serum phosphate was associated with a 0.34 ml/min/1.73m^2/month steeper slope of renal function decline (P=0.01). After adjusting for risk factors known to accelerate progression of renal dysfunction (baseline eGFR, haemoglobin, systolic BP, age, gender and proteinuria), serum phosphate remained independently associated with the rate of decline (P=0.02; Table 3-3). Omission of haemoglobin from the model or inclusion of renal diagnosis or medication made no appreciable difference to the result. Further adjustment for albumin did not influence the rate of decline in renal function at 0.343 (0.064–0.623) ml/min/1.73m^2/month. When dichotomized on cut-off points defined in the KDIGO guidelines (KDIGO clinical practice guideline for the diagnosis, evaluation,

prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD), 2009), patients with a plasma phosphate concentration above 1.2 mmol/l had a 0.147 (0.000–0.304; P=0.05) ml/min/1.73m^2/month steeper decline than patients with levels below this cut-off point.

Decline	Crude β	P	Adjusted β	P
Phosphate (mmol/l)	0.340 (0.0810–0.599)	0.010	0.340 (0.057–0.624)*	0.019
Brachial SBP (mmHg)	0.005 (0.001–0.008)	0.011	0.005 (0.000–0.009)†	0.020
AIx_{75}	0.000 (-0.007–0.007)	0.913	0.000 (-0.008–0.008)‡	0.928
PWV (m/s)	0.004 (-0.027–0.034)	0.802	-0.015 (-0.054–0.024)‡	0.444
PWV_{adj} (m/s)	-0.008 (-0.039–0.024)	0.625	-0.016 (-0.055–0.022)‡	0.398

Table 3-3. Association of baseline phosphate, brachial and aortic blood pressure, augmentation index and pulse wave velocity with decline in renal function.

*Adjusted for age, gender, baseline eGFR, systolic blood pressure, proteinuria, haemoglobin and serum calcium; †adjusted for age, gender, baseline eGFR, proteinuria, haemoglobin, serum calcium and serum phosphate; ‡adjusted for age, gender, baseline eGFR, systolic blood pressure, proteinuria, haemoglobin, serum calcium and serum phosphate. The β describes the magnitude of decline in renal function in ml/min/1.73m^2/month for every unit increase in the variable shown (for example, a 1 mmol/l increase in serum phosphate is associated with a 0.34 ml/min/1.73m^2/month decline in renal function). A negative β is equivalent to an improvement in renal function. SBP, systolic blood pressure; AIx_{75}, augmentation index adjusted for a heart rate of 75 beats per minute; PWV, pulse wave velocity; PWV_{adj}, pulse wave velocity adjusted for mean arterial pressure; eGFR, estimated glomerular filtration rate.

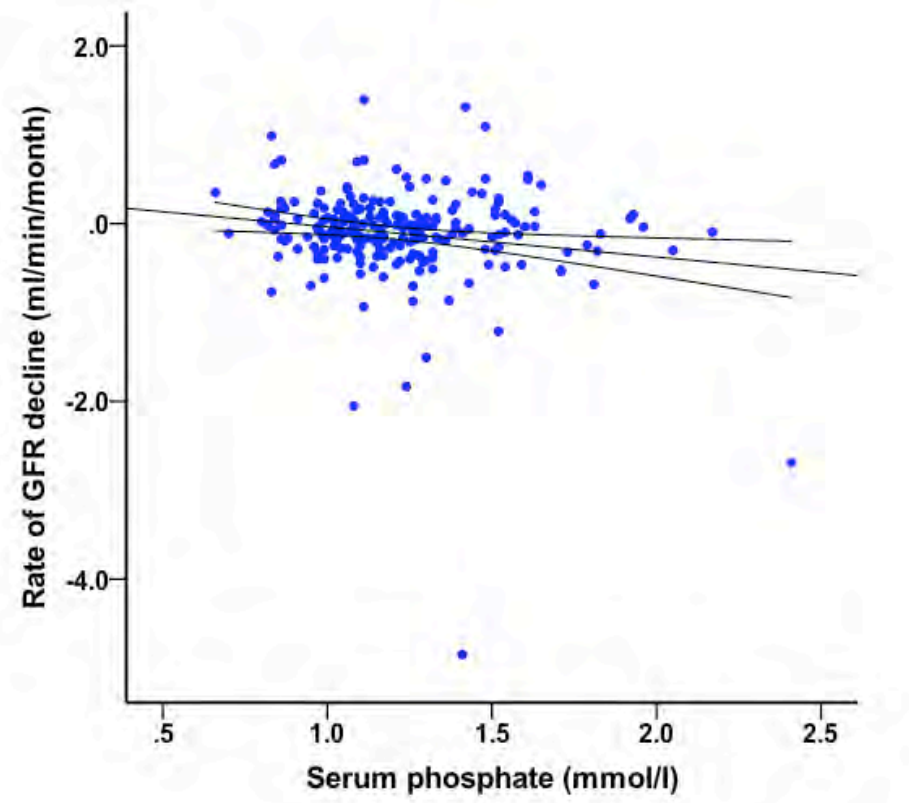

Figure 3-1. Association between serum phosphate concentration and decline in renal function. The line indicates the regression line (with 95% confidence intervals) for the relationship between decline in renal function and serum phosphate level. R=-0.170, β=-0.34 (P=0.01)

Serum calcium was not significantly associated with kidney function decline over the follow-up period (P=0.2). In the subgroup of 200 patients with PTH results, higher PTH was associated with a slightly greater decline in renal function (0.002 [0.001–0.002] ml/min/1.73m^2/month; P<0.0001).

Brachial and aortic systolic BP were associated with decline in renal function; each 1 mmHg higher systolic BP was associated with a 0.005 and 0.006 ml/min/1.73m^2/month steeper slope of renal function decline respectively (P=0.01 and P=0.004). After adjustment for risk factors known to accelerate renal decline,

higher brachial and aortic systolic BP remained independently associated with more rapid rates of decline (0.005 [0.000–0.009] and 0.006 [0.002–0.01] ml/min/1.73m^2/month [P=0.020 and 0.008] respectively; Table 3). Neither PWV nor AIx_{75} were associated with the rate of renal function decline whether unadjusted or adjusted for known risk factors (Table 3); these results were not influenced by either adjusting for brachial mean arterial pressure or aortic systolic or mean arterial pressure.

3.3.4 Combined Renal Endpoint

Comparison of characteristics of participants who did and did not reach the combined endpoint of commencement of dialysis or ≥25% decline in eGFR is shown in Table 3-4. Consistent with the results of eGFR slope, there was a significant difference in serum phosphate and brachial and aortic BP but no differences in AIx_{75} or PWV between the two groups. Serum phosphate was independently associated with the combined endpoint (β=0.211, P=0.03) after adjustment for age, gender, baseline eGFR, systolic BP, proteinuria, haemoglobin and serum calcium. Neither AIx (β=0.5, P=0.1), PWV (β=-0.02, P=0.2) nor PWV_{adj} (β=0.02, P=0.1) were associated with the combined endpoint after adjustment.

Variable	<25% decline in GFR (n=184)	≥25% decline in GFR or start of RRT (n=41)	P
Age (years)	59 ± 13	56 ± 13	0.2
Male (%)	115 (60)	26 (59)	1.0
Diabetic (%)	21 (11)	7 (16)	0.4
Number of antihypertensives	1.9 ± 1.2	2.4 ± 1.3	0.02
eGFR (ml/min/1.73m^2)	45 ± 18	33 ± 22	<0.001
ACR (mg/mmol)	4.0 (0.8–28.8)	17.8 (0.2–130.2)	0.1
Parathyroid hormone (pg/ml)*	45 (31–62)	95 (53–152)	<0.001
Calcium (mmol/l)	2.33 ± 0.14	2.35 ± 0.15	0.6
Phosphate (mmol/l)	1.19 ± 0.24	1.32 ± 0.36	0.04
Total cholesterol (mmol/l)	4.8 ± 1.1	4.8 ± 1.4	0.7
Haemoglobin (g/dl)	13.3 ± 1.7	12.2 ± 1.8	<0.001
Brachial systolic BP (mmHg)	135 ± 18	149 ± 25	0.001
Brachial diastolic BP (mmHg)	78 ± 10	86 ± 14	0.002
Aortic systolic BP (mmHg)	124 ± 16	138 ± 23	<0.001
Aortic diastolic BP (mmHg)	80 ± 10	88 ± 14	0.001
Heart rate (beats/min)	68 ± 14	70 ± 11	0.4
AIx_{75} (%)	24 ± 11	27 ± 9	0.2
Pulse wave velocity (m/s)	9.0 ± 2.5	9.0 ± 2.9	0.9
Pulse wave velocity$_{adj}$ (m/s)	9.1 ± 2.5	8.5 ± 2.7	0.2

Table 3-4. Comparison of patients reaching the combined renal end point (start of renal replacement therapy or ≥25% decline in renal function).

*Available in 200 patients. Data are mean ± standard deviation, frequency (percentage) or median (interquartile range). GFR, glomerular filtration rate; RRT, renal replacement therapy; eGFR, estimated glomerular filtration rate; ACR, urinary albumin: creatinine ratio; BP, blood pressure; AIx_{75}, augmentation index corrected for a heart rate of 75 beats per minute.

3.4 Discussion

In this observational study of 225 patients with predominantly early CKD it was demonstrated that baseline serum phosphate predicts progression of renal dysfunction over a median follow-up of 2.5 years. This association persisted following adjustment for factors known to accelerate renal function decline including age, proteinuria and systolic BP. Brachial and aortic systolic BP also independently predicted a decline in renal function, although no association was found with measures of arterial stiffness. These findings were supported by the presence of a significantly higher phosphate concentration in those patients reaching the combined renal end-point of dialysis or ≥25% decline in eGFR compared to those that did not.

3.4.1 Serum Phosphate as a Predictor of Declining Renal Function

In a rat model of renal failure, hyperphosphataemia resulting from a high-phosphate diet was associated with progression of renal dysfunction (Neves *et al.*, 2004). Serum phosphate has since been shown to predict decline in renal function in humans with more advanced (stage 4 and 5) CKD than in our cohort (Voormolen *et al.*, 2007). In our study of patients with predominantly early CKD an equivalent change in slope of renal function per equivalent increase in serum phosphate concentration was observed when compared to the Voormolen study of patients with advanced CKD (0.11 ml/min/1.73m^2/month vs. 0.15 ml/min/1.73m^2/month decline per 1 mg/dl increase in serum phosphate; a 1 mg/dl change in serum phosphate is equivalent to a 0.32 mmol/l change). Similarly in a community study of African-

Americans with hypertensive renal disease and eGFR ranging from 20–65 ml/min/1.73m^2 serum phosphate was independently associated with an increased risk of decline in eGFR (by 50% or 25 ml/min/1.73m^2) or progression to ESKD (Norris *et al.*, 2006). Serum phosphate was also an independent predictor of CKD progression in a cohort study of 985 males with stages 1–5 CKD (Schwarz *et al.*, 2006). A 1 mg/dl increase in serum phosphate was associated with an adjusted hazard ratio of 1.29 of the composite endpoint of ESKD or doubling of serum creatinine, although only the highest quartile of serum phosphate showed a significant hazard ratio for the composite endpoint; this quartile comprised subjects with predominantly stage 4 CKD. Our results confirm that phosphate predicts rate of decline in renal function across a broad range of patients with widely varying severity and aetiology of CKD. Furthermore, the higher phosphate concentration observed amongst patients reaching the combined renal end-point of dialysis or ≥25% decline in eGFR is consistent with the findings of the CRIB longitudinal cohort study of 382 patients with CKD, in which serum phosphate was an independent predictor of progression to ESKD in Cox regression analyses (Landray *et al.*, 2010). In a *post hoc* analysis of 331 participants of the Ramipril Efficacy In Nephropathy (REIN) study, the cumulative incidence of ESKD or doubling of serum creatinine increased across increasing phosphate quartiles, and each 1 mg/dl (0.32 mmol/l) increase in serum phosphate was associated with an 84% increase in the risk of ESKD (Zoccali *et al.*, 2011). Another recent study has shown similar results, with phosphate levels >4 mg/dl (>1.28 mmol/l) increasing the risk of ESKD by a factor of 1.9 compared to levels <4 mg/dl in 13372 participants of NHANES III (O'Seaghdha *et al.*, 2011). The same group reported a 2.1-fold increased risk of incident CKD amongst 2269

subjects from the Framingham Heart Study in those with serum phosphate >4 mg/dl (>1.28 mmol/l) compared to those with a serum phosphate of 2.5–3.5 mg/dl (0.8–1.12 mmol/l).

The mechanism underlying the association between serum phosphate and progression of renal dysfunction is unknown. The Western diet is typically rich in bio-available phosphate, resulting in high renal phosphate load (Ferro *et al.*, 2009). A study of variable dietary phosphate content in uraemic rat models in the 1980s provided interesting insights into potential mechanisms of kidney damage (Haut *et al.*, 1980). Higher dietary phosphate was associated with greater urinary phosphate excretion and greater calcium and phosphate deposition in the renal tubules and interstitium of subtotally nephrectomised rats. Interstitial oedema and fibrosis, together with tubular atrophy and dilatation, were present histologically. The nephrotoxicity of phosphate appeared to increase as renal functional mass decreased. It has been hypothesised that high intracellular phosphate promotes precipitation of calcium-phosphate product within renal tubules, or that phosphate itself acts as a direct tubular toxin (Loghman-Adham, 1993). Dietary restriction of phosphate in animal models has been shown to reduce progression of kidney disease (Ibels *et al.*, 1978), and use of the non-calcium-based phosphate binder sevelamer hydrochloride reduces renal calcium content and appears to protect against further deterioration in renal function (Cozzolino *et al.*, 2003; Nagano *et al.*, 2003). In the *post hoc* analysis of participants from the Ramipril Efficacy in Nephropathy (REIN) study, in which subjects were randomly allocated to ACEI therapy or placebo, higher serum phosphate levels appeared to blunt the reno-

protective effects of ACEI, which may represent an alternative mechanism of acceleration of kidney dysfunction (Zoccali *et al.*, 2011).

There are limited data linking higher FGF-23 levels with a greater decline in renal function. In a longitudinal study that followed up 177 patients with non-diabetic CKD over a median of 53 months, FGF-23 independently predicted decline in renal function after adjustment for age, gender, proteinuria and serum phosphate, calcium and PTH (Fliser *et al.*, 2007). In a *post hoc* analysis of 1099 participants of the Homocysteine in Kidney and End Stage Renal Disease study, higher FGF-23 levels were independently associated with initiation of dialysis therapy (Kendrick *et al.*, 2011). It is possible that the decline in renal function observed with higher serum phosphate levels is mediated by FGF-23, which is elevated in CKD as a compensatory response to hyperphosphataemia. Further studies are required to assess this relationship to determine whether this is causal or merely an association (Larsson *et al.*, 2003).

Serum phosphate has been established as a predictor of cardiovascular mortality in the general population and in CKD, including renal transplant and dialysis patients (Block *et al.*, 2004; Connolly *et al.*, 2009; Kestenbaum *et al.*, 2005; Tonelli *et al.*, 2005). We have speculated that much of this cardiovascular risk might be attributed to direct effects of phosphate on the vasculature and myocardium (Ferro *et al.*, 2009), but considering the strong relationships between renal function and rate of decline in renal function and cardiovascular risk, it is also possible that phosphate might exert its effects on cardiovascular risk indirectly via the kidney.

3.4.2 Markers of Arterial Stiffness and Renal Function Decline

Brachial and aortic systolic BP both independently predicted progression of kidney disease in our cohort, despite brachial BP being controlled to national targets (mean 138/80 mmHg). This has been well established in previous observational studies (K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification, 2002). We were unable to demonstrate a relationship between markers of arterial stiffness and decline in renal function. These findings are in keeping with a prospective longitudinal analysis of the Framingham Offspring Study which did not find an association between baseline aortic PWV and incident CKD or microalbuminuria (Upadhyay *et al.*, 2009). Three small studies have found an association. Taal *et al* measured radial-dorsalis pedis PWV in 35 patients with stage 4 and 5 CKD and found PWV and AIx predicted progression to ESKD (Taal *et al.*, 2007). Studying a cohort of patients with advanced CKD and the unorthodox use of radial-dorsalis pedis PWV rather than the gold standard carotid-femoral PWV may explain this difference. In a Japanese study of only 41 subjects with non-diabetic CKD AIx predicted a greater decline in renal function (Takenaka *et al.*, 2005), although a subsequent study by this group of 42 patients failed to replicate this finding (Takenaka *et al.*, 2009). A third study of 133 patients with stage III-IV CKD showed PWV predicted decline in renal function (Ford *et al.*, 2010). Although this study examined a similar patient cohort, a number of differences may collectively account for their contrasting findings: firstly, they used the Complior device, which generates higher aortic PWV measurements compared to the SphygmoCor (Rajzer *et al.*, 2008); secondly, this group had poorer renal function

with a mean eGFR of 32 compared to 43 ml/min/1.73m^2 in our cohort; thirdly, although their subjects were treated to a target BP of 130/80 mmHg their actual achieved mean BP was 155/83 mmHg compared with 138/80 mmHg in our study, with a considerably higher pulse pressure potentially accounting for their higher reported PWV; fourthly, despite similar usage in the number of antihypertensives, our cohort had much greater use of agents targeting the renin-angiotensin-aldosterone axis which potentially have a greater influence on reducing arterial stiffness, particularly in CKD (Chue *et al.*, 2010); fifthly, their patients were on average 11 years older than those in our study, and age is known to be a significant determinant of arterial stiffness (McEniery *et al.*, 2005); finally, they had a higher proportion of diabetics compared to our study (23% vs. 12%). Our study is larger than these three studies combined and has a much longer follow-up period. The differences between all of these studies including our own, however, highlight the fact that very little is known about the complex and presumably two-way interactions between arterial stiffness and kidney disease. Research is hindered by the multiplicity of factors that influence this relationship in CKD patients including age, BP and medication (Chue *et al.*, 2010; Moody *et al.*, 2011). The recent formation of collaborative networks such as EURECAM (Covic *et al.*, 2009a) and UREKA (Tomlinson *et al.*, 2010) will hopefully facilitate the recruitment of patients into much larger studies in order to address these important questions.

Our results raise the possibility that therapeutic lowering of phosphate levels might be an effective method of reducing the rate of decline of renal function. Similarly, our data support the control of systolic BP as a means of reducing progression of kidney

dysfunction, although we have found no indication that lower arterial stiffness is associated with improved renal outcomes in this population.

3.4.3 Study Limitations

There are limitations to this study. Although prospective, it is observational and cross-sectional in design and therefore subject to potential residual confounding from missing variables. Only baseline measurements of arterial stiffness were acquired and thus the relationship between changes in arterial stiffness and kidney disease was not examined longitudinally. All subjects recruited into this study were under regular nephrology review and most had primary renal disease, potentially limiting applicability to patients with CKD treated solely in the community. Phosphate, however, is associated with cardiovascular morbidity and mortality in the general population as well as in CKD. Data on serum vitamin D or FGF-23 concentrations were not collected, which may have provided greater insight into potential aetiological mechanisms.

3.4.4 Conclusion

Serum phosphate is an independent predictor of decline in renal function in early stage chronic kidney disease. Further work is required to determine the underlying mechanisms through which phosphate influences kidney function decline, and randomised controlled trials are warranted to investigate the benefits of phosphate lowering in the preservation of kidney function.

4 RELATIONSHIP BETWEEN LEFT VENTRICULAR MASS AND PHOSPHATE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

4.1 Introduction

4.1.1 Background

The mechanism through which phosphate influences cardiovascular risk is currently unknown. It has been postulated that phosphate might affect cardiac structure and function indirectly through promotion of vascular calcification and an increase in arterial stiffness, but direct effects on the myocardium, such as cardiac fibrosis and ventricular hypertrophy, have also been demonstrated. In a rat model of CKD, rats fed a high phosphate diet developed more cardiac fibrosis compared to those fed a low phosphate diet (Amann *et al.*, 2003). Increased left ventricular mass has also been demonstrated in a similar experimental rat model of CKD (Neves *et al.*, 2004). Left ventricular hypertrophy is an established predictor of cardiovascular and all-cause mortality in several patient populations including CKD (Shlipak *et al.*, 2005). There is also emerging evidence that LV mass assessed as a continuous variable, rather than using the dichotomy of LVH being present or absent, has prognostic value in predicting cardiovascular disease (Schillaci *et al.*, 2000). Furthermore, reductions in LV mass have been associated with reduced mortality in a variety of groups at high cardiovascular risk (Devereux *et al.*, 2004).

4.1.2 Objective

The majority of cardiovascular deaths in late stage CKD are attributable to structural heart disease, such as LVH, which acts as a substrate for the development of CHF and SCD. The relationship between serum phosphate, arterial stiffness and LV mass in patients with CKD is unclear. The aim of this study was to investigate these relationships to determine whether serum phosphate was associated with increased LV mass and arterial stiffness in early CKD.

4.2 Methods

4.2.1 Study Design, Setting and Participants

The patients studied in this cross-sectional cohort study were recruited prospectively from renal clinics at the Queen Elizabeth Hospital Birmingham, United Kingdom for the previously published trial of spironolactone in CKD (Edwards *et al.*, 2009) and for the randomised controlled trial of sevelamer described in section 5. Patients were included if aged 18–80 years, with stage 2 (GFR 60–89 ml/min/1.73m^2 and evidence of kidney damage for ≥3 months), stage 3 (GFR 30–59 ml/min/1.73m^2) or stage 4 CKD (GFR 15–29 ml/min/1.73m^2) (K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification, 2002) and no change in medication in the preceding three months. Patients with diabetes mellitus, uncontrolled BP (mean daytime ambulatory BP >130/85 mmHg), atrial fibrillation, known LV dysfunction, valvular disease and signs and symptoms of congestive heart failure were excluded. Patients receiving treatment with a phosphate binder or vitamin D analogue were also excluded.

All subjects underwent baseline investigations including measurement of height and weight and collection of serum and plasma. Glomerular filtration rate was estimated by the 4-variable MDRD formula with serum creatinine recalibrated to be traceable to an isotope derived mass spectroscopy method (Levey *et al.*, 1999). Serum calcium levels were corrected for serum albumin. Albuminuria was measured using spot albumin: creatinine ratio (ACR). Office BP and 24-hour ambulatory BP were measured in accordance with the methods described in section 2.3. Arterial stiffness was assessed through measurement of PWV as described in section 2.2.1. Cardiovascular magnetic resonance imaging was used to determine LV volumes, function and mass according to the methods described in section 2.1.1.

4.2.2 Statistical Analysis

Statistical analysis was performed as described in section 2.8. Reproducibility of LV mass index was determined as described in section 2.1.3.

4.3 Results

4.3.1 Demographics

Of 3349 patients screened, a total of 736 patients met the study entry criteria. Of these, 528 patients declined the study and therefore 208 subjects were recruited. Renal diagnoses and prescribed medication of the entire cohort are shown in Table 4-1. Patients were on a mean of 1.8 ± 1.2 antihypertensive agents with 89% of patients either on an ACEI or ARB. No patients were taking erythropoietin-

stimulating agents. Fifty-seven per cent were male, 86% Caucasian, 10% South Asian and 4% Afro-Caribbean with a mean age of 54 ± 13 years. Seventeen per cent were current smokers. The biochemical and haematological characteristics of the cohort are shown in Table 4-2. The mean eGFR was 50 ± 15 ml/min/1.73m^2 with a mean phosphate of 1.11 ± 0.21 mmol/l. Frequency of CKD stage was as follows: stage 2 22%, stage 3a 39%, stage 3b 34% and stage 4 6%. Fifty-nine per cent of patients had renal disease confirmed by biopsy, with a further 23% diagnosed using ultrasonography.

Primary kidney disease (%)	
Glomerulonephritis	29
Renal vascular disease/nephrosclerosis	17
Multi-system disease	13
Reflux nephropathy/nephrolithiasis/obstruction	10
Polycystic kidney disease	9
Other	18
Unknown	4
Medication (%)	
ACEI or ARB	89
β-blocker	18
Calcium channel blocker	28
Diuretic	31
α-blocker	12
Statin	40
Aspirin	17
Warfarin	0.5

Table 4-1. Renal diagnoses and medication of study population.

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

		Phosphate quartiles				
	All	**1**	**2**	**3**	**4**	**P value**
	(n=208)	**(n=53)**	**(n=55)**	**(n=48)**	**(n=52)**	
Phosphate (mmol/l)	1.11 ± 0.21	0.86 ± 0.08	1.04 ± 0.03	1.16 ± 0.04	1.39 ± 0.13	<0.001
Age (years)	54 ± 13	56 ± 13	54 ± 15	51 ± 12	55 ± 12	0.4
Male (%)	57	70	60	58	41	0.03*
Number of antihypertensives	1.8 ± 1.2	1.4 ± 1.2	2.0 ± 1.0	1.9 ± 1.2	2.0 ± 1.0	0.03
eGFR (ml/min/1.73m^2)	50 ± 15	50 ± 11	50 ± 17	46 ± 16	53 ± 17	0.2
ACR (mg/mmol)	8 (1–47)	8 (1–40)	3 (1–53)	7 (2–43)	34 (28–44)	0.3
Body mass index (kg/m^2)	28 ± 5	28 ± 4	28 ± 5	28 ± 6	28 ± 4	1.0
Haemoglobin (g/dl)	13.3 ± 1.6	13.6 ± 1.4	13.4 ± 1.7	13.4 ± 1.4	12.9 ± 1.6	0.09
Cholesterol (mmol/l)	4.89 ± 1.17	4.93 ± 1.15	4.79 ± 0.99	4.78 ± 0.94	5.07 ± 1.49	0.5
HDL (mmol/l)	1.53 ± 0.52	1.49 ± 0.45	1.50 ± 0.59	1.45 ± 0.47	1.68 ± 0.55	0.1
Calcium (mmol/l)	2.25 ± 0.14	2.22 ± 0.11	2.25 ± 0.11	2.24 ± 0.12	2.28 ± 0.20	0.3
PTH (pg/ml)	46 (32–66)	57 (31–74)	52 (37–66)	45 (34–69)	34 (28–44)	0.08

Table 4-2. Demographic, haematological and biochemical characteristics of the entire cohort and according to quartiles of increasing serum phosphate.

*Analysed using χ^2. Data are mean ± standard deviation, percentages or median (interquartile range). Analysed with one-way analysis of variance. eGFR, estimated glomerular filtration rate; ACR, urinary albumin: creatinine ratio; HDL, high-density lipoprotein cholesterol; PTH, parathyroid hormone.

Imaging, BP and PWV parameters are shown in Table 4-3 and Table 4-4. Left ventricular mass index was higher in men than women (62.5 vs. 49.9 g/m^2; P<0.0001). Five patients (2%) had LVH, of which three were male. Mean PWV was 8.6 ± 2.1 m/s with no differences between males and females.

		Phosphate quartiles				
	All	**1**	**2**	**3**	**4**	**P value**
	(n=208)	**(n=53)**	**(n=55)**	**(n=48)**	**(n=52)**	
Phosphate (mmol/l)	1.11 ± 0.12	0.86 ± 0.08	1.04 ± 0.03	1.16 ± 0.04	1.39 ± 0.13	<0.001
LV mass index (g/m^2)	57 ± 15	54 ± 13	55 ± 13	58 ± 17	61 ± 16	0.04
Absolute LV mass (g)	110 ± 35	102 ± 27	107 ± 31	114 ± 42	118 ± 36	0.1
LVH (%)	2	0	0	2	8	0.03*
LVEF (%)	72 ± 8	73 ± 7	73 ± 9	72 ± 8	70 ± 9	0.3
LVEDVI (ml/m^2)	59 ± 12	59 ± 11	59 ± 12	59 ± 12	60 ± 14	0.9
LVSVI (ml/m^2)	43 ± 10	44 ± 10	44 ± 10	42 ± 7	43 ± 12	0.9

Table 4-3. Cardiac magnetic resonance parameters for the entire cohort and according to quartiles of increasing serum phosphate.

*Analysed using χ^2. Data are mean ± standard deviation or percentages. Analysed with one-way analysis of variance. LV, left ventricular; LVH, left ventricular hypertrophy; LVEF, left ventricular ejection fraction; LVEDVI, left ventricular end diastolic volume index; LVSVI, left ventricular stroke volume index.

		Phosphate quartiles				
	All (n=208)	**1 (n=53)**	**2 (n=55)**	**3 (n=48)**	**4 (n=52)**	**P value**
Phosphate (mmol/l)	1.11 ± 0.12	0.86 ± 0.08	1.04 ± 0.03	1.16 ± 0.04	1.39 ± 0.13	<0.001
Office blood pressure (mmHg)						
Systolic	129 ± 17	128 ± 19	128 ± 18	131 ± 17	129 ± 19	0.9
Diastolic	75 ± 11	76 ± 10	75 ± 11	76 ± 11	73 ± 12	0.6
24-hour blood pressure (mmHg)						
Systolic	122 ± 11	121 ± 13	123 ± 9	122 ± 14	121 ± 11	0.8
Diastolic	73 ± 8	71 ± 8	73 ± 9	73 ± 7	73 ± 8	0.7
Day-time average blood pressure (mmHg)						
Systolic	125 ± 11	125 ± 13	125 ± 10	126 ± 13	126 ± 9	0.9
Diastolic	76 ± 9	75 ± 9	76 ± 11	77 ± 8	76 ± 8	0.9
Night-time average blood pressure (mmHg)						
Systolic	114 ± 14	11 ± 14	117 ± 15	116 ± 16	112 ± 13	0.4
Diastolic	66 ± 10	65 ± 10	67 ± 12	67 ± 12	66 ± 9	0.7
PWV (m/s)	8.6 ± 2.1	8.6 ± 2.2	9.2 ± 2.2	8.4 ± 2.0	8.2 ± 1.8	0.1

Table 4-4. Blood pressure and pulse wave velocity for the entire cohort and according to quartiles of increasing serum phosphate.

Data are mean ± standard deviation. Analysed with one-way analysis of variance. PWV, pulse wave velocity.

4.3.2 Relationships With Serum Phosphate

Sixty-two (30%) patients had a serum phosphate higher than the recommended treatment threshold of 1.2 mmol/l as defined in the KDIGO guidelines (KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic

Kidney Disease-Mineral and Bone Disorder (CKD-MBD), 2009). Patients with serum phosphate >1.2 mmol/l had a greater LV mass index than those with serum phosphate <1.2 mmol/l (61 vs. 55 g/m^2; P=0.03) with no differences in eGFR (52 ± 16 vs. 49 ± 15 ml/min/1.73m^2; P=0.3), age (54 ± 12 vs. 54 ± 13 years; P=0.8), or office BP (systolic 130 ± 18 vs. 129 ± 17 mmHg; P=0.6) and 24-hour ambulatory BP (systolic day-time average 122 ± 12 vs. 121 ± 11 mmHg; P=0.4). This association was also present when gender-specific LV mass index was assessed with serum phosphate (men 70 vs. 60 g/m^2; P=0.001, women 53 vs. 48 g/m^2; P=0.03) with no differences in eGFR, body mass index, age, PWV or office and 24-hour ambulatory BP between phosphate groups.

When the cohort was divided into quartiles according to serum phosphate concentrations, LV mass index increased across phosphate quartiles (P=0.04; Table 4-3). There were no differences in age, office or 24-hour ambulatory BP, PWV, eGFR, calcium, PTH, haemoglobin, albuminuria or cholesterol across the quartiles (Table 4-2). The relationship between serum phosphate quartiles and LV mass index persisted when analysed separately for men (P=0.002, Figure 4-1) and women (P=0.04, Figure 4-2). There was no observed difference in absolute LV mass across quartiles (P=0.1), but more women were in the higher serum phosphate quartiles than in the lower quartiles (Table 4-2; P=0.03). When men and women were analysed separately, absolute LV mass increased across phosphate quartiles (P=0.002 and P=0.04 respectively).

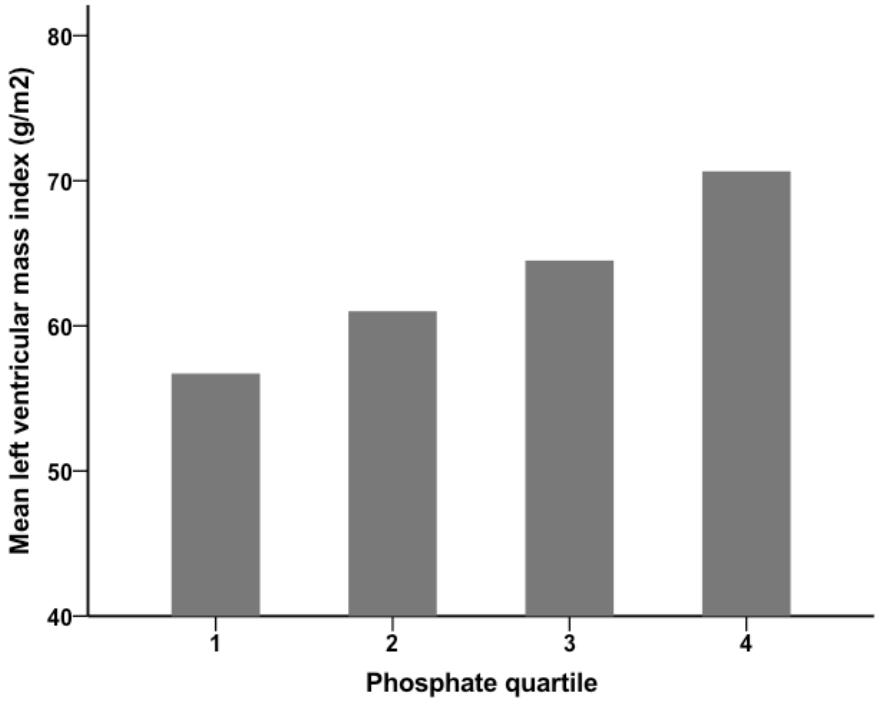

Figure 4-1. Left ventricular mass index in men (n=119) according to serum phosphate quartile.

One-way analysis of variance, P=0.002.

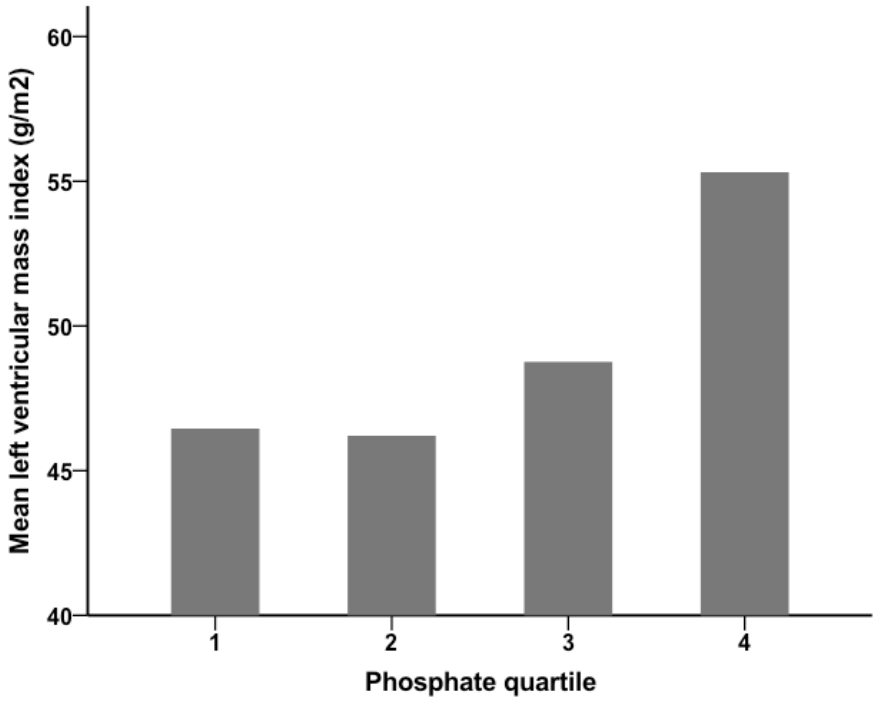

Figure 4-2. Left ventricular mass index in women (n=89) according to serum phosphate quartile. One-way analysis of variance, P=0.04.

4.3.3 Correlations with Left Ventricular Mass

Log-ACR (r=0.184; P=0.009), haemoglobin (r=0.162; P=0.02), serum phosphate, (r=0.173; P=0.01; Figure 4-3) and office systolic BP (r=0.323; P<0.0001), diastolic BP (r=0.260; P<0.0001) and pulse pressures (r=0.191; P=0.008) all correlated with LV mass index. There was no correlation between PWV and LV mass index (P=0.2). Age, eGFR, calcium, log-PTH, total cholesterol and HDL-cholesterol did not correlate with LV mass index.

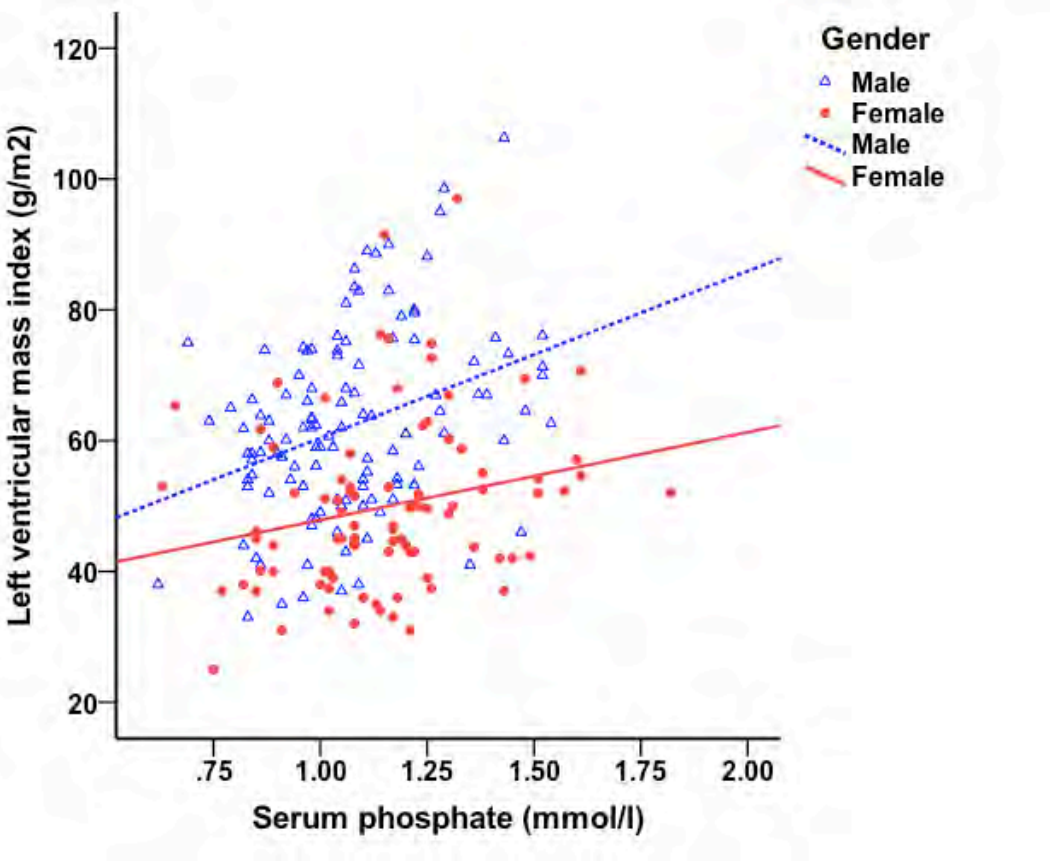

Figure 4-3. Scatterplot of serum phosphate versus left ventricular mass index labelled by gender. Pearson's correlation: whole cohort r=0.17, P=0.01, n=208; men r=0.34, P<0.0001, n=119; women r=0.23, P=0.03, n=89.

4.3.4 Determinants of Left Ventricular Mass

All variables that significantly correlated with LV mass index (including gender) were entered into an "enter" linear regression model using LV mass index as a continuous variable and office systolic BP used as the most highly correlated measure for BP (Table 4-5). Log-ACR and haemoglobin were not significantly associated with LV mass index in this model. The model explained 30% of the variation in LV mass index (P<0.0005). The importance of serum phosphate and systolic BP in explaining the variance in LV mass index was confirmed, with serum phosphate accounting for 5% and systolic BP 6% of the variance. Substitution of central BP for peripheral BP

in the model made no appreciable difference to the results. When analysis was repeated with absolute LV mass as the outcome variable and BMI as an additional covariate, the model remained significant with 37% of the variation in absolute LV mass being explained ($P<0.0005$). Serum phosphate, systolic BP and gender were the only significant independent predictors of absolute LV mass, with serum phosphate and systolic BP accounting for 5% and 4% of the variance respectively.

	B	95% CI	β	P
Male gender	-12.45	-16.49–-8.41	-0.41	<0.0005
Phosphate (mmol/l)	16.66	7.51–25.81	0.23	<0.0005
Office systolic blood pressure (mmHg)	0.21	0.11–0.32	0.25	<0.0005
Log albumin: creatinine ratio	1.41	-0.37–3.18	0.10	0.12
Haemoglobin (g/dl)	0.41	-0.86–1.68	0.04	0.53

Table 4-5. Enter linear regression. Predictors of left ventricular mass index. Predicted variable: left ventricular mass index in g/m^2 (r^2 for model 0.303). Independent variables used in model: gender (male 0 female 1), serum phosphate, office systolic blood pressure, log albumin: creatinine ratio and haemoglobin. B, unstandardised coefficient; β, standardised coefficient; 95% CI, 95% confidence interval.

4.4 Discussion

In this study it was shown that serum phosphate is associated with LV mass in both men and women with early CKD. The association is maintained after adjusting for multiple confounders and is independent of BP, age and renal function. A relationship between serum phosphate and arterial stiffness was not demonstrated. In this cohort of patients with well-controlled BP, the variance in LV mass explained by serum phosphate is of a very similar magnitude to that explained by systolic BP. These findings raise the possibility that lowering serum phosphate might be a novel way of reducing LV mass and thus provide alternative means of improving cardiovascular outcome in this group of patients.

Although the higher cardiovascular risk conferred by the presence of LVH as a categorical variable is well accepted, it is increasingly recognised that there is no biological dichotomy; LV mass is a continuous variable with a graded relationship with cardiovascular risk (Devereux *et al.*, 2004; Schillaci *et al.*, 2000). The reasons for this association are unclear; LV mass may merely reflect the cumulative burden of exposure to high BP, or may directly influence cardiovascular risk through factors such as increased myocardial oxygen consumption, reduced coronary flow reserve and ventricular scarring and fibrosis causing an increased risk of arrhythmia (Schillaci *et al.*, 2000). In patients with CKD, increased LV mass is just one manifestation of a "uraemic cardiomyopathy". In favour of a causative relationship with cardiovascular risk is the finding that in patients with hypertension and LVH, a reduction in LV mass during antihypertensive therapy is associated with improved cardiovascular outcome in addition to the benefit gained from lower BP (Devereux *et al.*, 2004). Furthermore, reduction of BP within the normal range is associated with further reductions in LV mass (Edwards *et al.*, 2009; Simpson *et al.*, 2010). There may be limits to how low BP can be reduced without increasing cardiovascular events and mortality, however, and although chronic pressure overload is generally accepted as a major determinant of LVH, the contribution of other biological variables is now acknowledged (Cuspidi, 2010).

Two studies have demonstrated a similar association between serum phosphate and LV mass in ESKD patients (Patel *et al.*, 2009; Strozecki *et al.*, 2001). Strozecki et al used echocardiography to demonstrate a correlation between serum phosphate and LV mass in 22 normotensive haemodialysis patients (Strozecki *et al.*, 2001). These

results were verified in a more recent study of 246 haemodialysis patients in which serum calcium-phosphate product was a major determinant of LV mass measured using CMR (Patel *et al.*, 2009). Our study extends these findings to the early CKD population, indicating that phosphate may have an influence on cardiovascular structure in the early stages of CKD when the majority of patients have a serum phosphate level that lies below the recommended treatment threshold according to KDIGO guidelines (KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD), 2009). Improved control of hyperphosphataemia through use of a daily dialysis regimen has been shown to be independently associated with a reduction in LV mass over 12 months compared to conventional haemodialysis in ESKD patients (Ayus *et al.*, 2005). Whether similar reductions in serum phosphate are associated with decreased LV mass in pre-dialysis CKD patients requires further investigation.

Associations have been demonstrated between serum phosphate and LVH in the non-CKD population. In a cohort study of 4055 subjects with normal renal function (mean eGFR 119ml/min/1.73m^2) and normal serum phosphate (mean 3.7 mg/dl; 1.2 mmol/l), serum phosphate levels were associated with the presence of LVH (Foley *et al.*, 2009a). This finding was confirmed in a later study evaluating the association between serum phosphate and incident heart failure, which demonstrated an association between serum phosphate and LV mass at baseline in a cohort of 3300 subjects with normal cardiac and renal function (Dhingra *et al.*, 2010). In an observational study of 978 patients with stable coronary artery disease and normal renal function, serum phosphate was associated with LV mass in males but not in

females (Saab *et al.*, 2010). In our cohort the strength of association between phosphate and LV mass was weaker in females compared to males. It is possible that hormonal factors may explain this difference in strength of association, but this is an area that requires further study.

Phosphate may play a causative role in the development of LVH and myocardial fibrosis through a number of possible actions. The most widely accepted mechanism is by an increase in arterial stiffness and therefore LV afterload. *In vitro* experiments have shown that high levels of intracellular phosphate actively promote osteogenic transformation of VSMC, resulting in vascular calcification (Giachelli, 2003). Deposition of calcium-phosphate mineral in the vasculature is highly prevalent in dialysis patients, and the extent of calcification correlates with severity of arterial stiffness and is a predictor of all cause and cardiovascular mortality in ESKD (London *et al.*, 2003). An association between vascular calcification, arterial stiffness and LV mass has been demonstrated in small numbers of ESKD patients (Nitta *et al.*, 2004; Yildiz *et al.*, 2005), and in larger studies of non-CKD populations serum phosphate has been associated with surrogate measures of arterial stiffness (Ix *et al.*, 2009; Kendrick *et al.*, 2010). Our cohort of early CKD patients with a mean age of 54 ± 13 years had well-controlled BP and only marginally elevated PWV (mean 8.6 ± 2.1 m/s), which may account for the absence of any demonstrable relationship between PWV and serum phosphate or LV mass. Our study thus provides no support for this hypothesis but being observational in nature does not exclude such a mechanism. Alternatively, animal studies suggest that phosphate might exert direct actions on the myocardium, inducing fibrosis and LVH, although the mechanism by which this

occurs requires further study (Amann *et al.*, 2003; Neves *et al.*, 2004). Given concerns regarding the use of gadolinium-based contrast agents in patients with renal dysfunction, this was not studied in our population.

An alternative explanation for the relationship between serum phosphate and LV mass is the existence of a common factor present in CKD that influences both of these variables. Hyperphosphataemia is associated with increased levels of FGF-23, increased PTH and decreased vitamin D levels. The phosphaturic hormone FGF-23 regulates calcium-phosphate metabolism and was associated with LV mass independently of serum phosphate levels in a study of 220 pre-dialysis CKD patients (Gutierrez *et al.*, 2009). The authors did not demonstrate a relationship between serum phosphate and LV mass in their cohort. Approximately half of their cohort was diabetic, however, and comprised a large proportion of Afro-Caribbean subjects. They also included patients with coronary artery disease and congestive heart failure. Furthermore, 15% of subjects with the lowest eGFR were receiving phosphate binders and 25% were on activated vitamin D. All of these factors could have significantly affected the relationship between serum phosphate and LV mass in their cohort. Levels of FGF-23 and serum phosphate are intricately linked (Gutierrez *et al.*, 2005), and activation of FGF receptors has been shown to induce myocyte hypertrophy *in vitro* (Corda *et al.*, 1997). There is evidence from *in vitro* and animal models that FGF-23 directly induces LVH (Faul *et al.*, 2011), but it remains unclear whether FGF-23 increases arterial stiffness in CKD. The effects of FGF-23 are mediated by the renal co-receptor klotho, expression of which is reduced in early CKD. It has been suggested that klotho is the initiator of renal mineral bone disorder

and abnormal phosphate handling in CKD, although its relationships with arterial stiffness and LV mass are currently unknown (Kuro-o, 2011). Parathyroid hormone has been implicated in the development of cardiac fibrosis in animal models (Amann *et al.*, 1994), and a small study of normotensive haemodialysis patients has demonstrated an association between PTH and echocardiographically-derived LV mass (Strozecki *et al.*, 2001). A larger study of haemodialysis patients using CMR-derived LV mass was unable to replicate this finding (Patel *et al.*, 2009). We were also unable to demonstrate an association. Vitamin D receptor gene polymorphisms have been linked to LVH in ESKD patients (Testa *et al.*, 2010) and treatment with vitamin D analogues is associated with reductions in LV mass in animal models (Bodyak *et al.*, 2007) and regression of LVH in haemodialysis patients (Park *et al.*, 1999). It remains unclear whether these observations are due to direct cardiovascular effects of vitamin D or whether other mediators are involved.

Phosphate plays a key role in multiple aspects of myocyte metabolism including regulation of cardiac energetics (Beadle and Frenneaux, 2010). There is some evidence that reduced phosphate is associated with impaired myocardial performance (O'Connor *et al.*, 1977), and abnormal cardiac energetics have been demonstrated in patients with increased LV mass and may play a role in the progression of structural heart disease and development of heart failure (Smith *et al.*, 2006). Impaired cardiac energetics have also been demonstrated in animal models of CKD (Raine *et al.*, 1993). The relationship between cardiac energetics and a high phosphate state is unknown but represents an area worthy of future study.

There are limitations to this study. All subjects recruited were under regular review by nephrologists and most had primary renal disease, potentially limiting the applicability of our results to patients with CKD treated solely in the community. Data was not collected on serum vitamin D or FGF-23 concentrations, which would have provided greater insight into potential aetiological mechanisms. Unlike these tests, however, serum phosphate is an easily measured variable that is widely available and regularly used in routine clinical practice. The use of gadolinium-enhanced CMR may have provided useful insights into the effects of phosphate on myocardial fibrosis. This study was observational and cross-sectional in design and thus subject to potential confounding from missing variables, therefore we are able only to describe associations and not infer causality. Nevertheless, this is the first study to report a clear association between serum phosphate and LV mass, a prognostically important cardiovascular variable, in patients with early stage CKD. Further longitudinal and interventional studies are required to determine whether the association between phosphate and LV mass is causative and whether lowering phosphate exposure is effective at reducing LV mass and therefore cardiovascular risk in this group of patients.

5 EFFECTS OF PHOSPHATE BINDING WITH SEVELAMER CARBONATE IN PATIENTS WITH STAGE 3 CHRONIC KIDNEY DISEASE

5.1 Introduction

5.1.1 Background

The association between serum phosphate and increased arterial stiffness (Ix *et al.*, 2009) as well as increased LV mass (Chue *et al.*, 2012; Dhingra *et al.*, 2010; Foley *et al.*, 2009a) suggests a potential mechanism by which phosphate might cause cardiovascular disease, although the cellular processes remain unclear. The ability of the kidneys to excrete phosphate is reduced at GFRs below 60 ml/min/1.73m^2. Serum phosphate, however, remains within the normal range until the GFR falls below 30 ml/min/1.73m^2 due to increased production of the phosphatonins PTH and FGF-23, which promote urinary phosphate excretion (Craver *et al.*, 2007; Isakova *et al.*, 2011b). Recent data have linked FGF-23 to increases in LV mass and development of LVH (Faul *et al.*, 2011).

It is likely that non-atheromatous pathogenic processes such as increased arterial stiffness and structural cardiac abnormalities predominate and are responsible for the excess cardiovascular morbidity and mortality in CKD (Chue *et al.*, 2010). Heart failure and arrhythmias are the commonest cause of cardiovascular death, and the current paradigm is that these events are a consequence of LV disease, predominantly LVH and fibrosis (Tonelli *et al.*, 2006). It is increasingly recognised

that LV mass, assessed as a continuous variable, has a graded relationship with cardiovascular risk (Schillaci *et al.*, 2000). Given that over 70% of incident dialysis patients have established LVH (Foley *et al.*, 1995), an understanding of the early pathophysiology of increased LV mass and LVH is essential for the targeting of therapeutic strategies to attenuate cardiovascular disease in CKD.

5.1.2 Hypothesis

We hypothesised that lowering gastrointestinal phosphate absorption through use of the non-calcium based phosphate binder, sevelamer carbonate, would reduce serum levels of phosphatonins such as FGF-23, thereby reducing LV mass and arterial stiffness as well as improving LV systolic and diastolic function in patients with early stage CKD.

5.2 Methods

5.2.1 Study Design

The study was a single-centre, prospective, randomised, double-blind, placebo-controlled trial (Chue *et al.*, 2011b). The non-calcium-based phosphate binder sevelamer carbonate was chosen due to its lack of calcium content and proven efficacy in the treatment of hyperphosphataemia in advanced CKD (Bleyer *et al.*, 1999). The study was in two phases: all subjects were administered 1600 mg of sevelamer carbonate with meals during a four-week open-label run-in phase; subjects that tolerated sevelamer then underwent 1:1 randomisation to continue a

further 36 weeks of treatment with either sevelamer or placebo with meals (Figure 5-1) during the second part of the study, the blinded treatment phase.

5.2.2 Setting and Participants

Subjects were recruited from renal outpatient clinics at the Queen Elizabeth Hospital Birmingham, United Kingdom from 2009 to 2011 with the following inclusion criteria: age 18–80 years with stage 3 CKD (eGFR between 30–59 ml/min/1.73m^2), total cholesterol <5.5 mmol/l and office BP controlled to <140/90 mmHg for ≥12 months prior to enrolment. Inclusion and exclusion criteria for the study are detailed in Table 5-1. Treatment with vitamin D analogues and other phosphate binders was not permitted during study enrolment. Regular medications unrelated to mineral metabolism that were taken at study entry were continued; no changes were made to regular medications during the study period.

Inclusion Criteria
Age 18–80 years
Chronic kidney disease stage 3 (estimated glomerular filtration rate 30–59 ml/min/1.73m^2)
Office blood pressure controlled to <140/90 mmHg for ≥12 months before entry
Total cholesterol <5.5 mmol/l
Exclusion Criteria
Existing or previous treatment within the past year with a phosphate binder or vitamin D analogue
Hyperphosphataemia (serum phosphate >1.8 mmol/l)
Hypophosphataemia (serum phosphate <0.8 mmol/l)
Uncontrolled secondary hyperparathyroidism (parathyroid hormone >80 pg/ml)
Diabetes mellitus
Pregnancy
Women of child-bearing age not on contraception
Bowel obstruction
Dysphagia or other swallowing disorder
Severe gastrointestinal motility disorders including severe constipation
Previous major gastrointestinal tract surgery

Table 5-1. Inclusion and exclusion criteria.

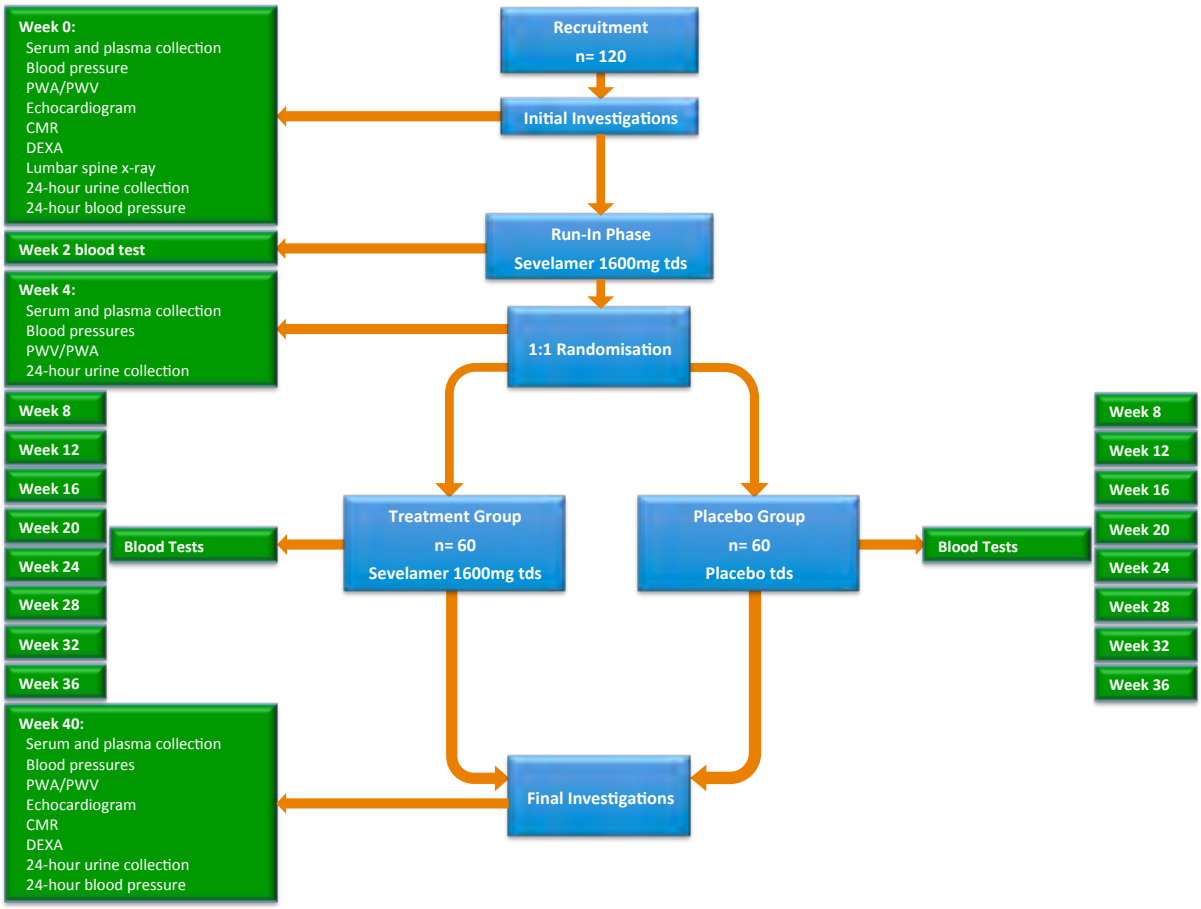

Figure 5-1. Study flow diagram.

PWA, pulse wave analysis; PWV, pulse wave velocity; CMR, cardiovascular magnetic resonance; DEXA, dual energy x-ray absorptiometry.

5.2.3 Baseline Visit

Subjects were assessed at baseline (week 0), at the end of the four-week open-label run-in phase (week 4) and at the end of the study (week 40). The following were performed at the baseline visit following an overnight fast: i) questionnaire detailing past medical history, drug history and tobacco and alcohol consumption; ii) clinical examination of all systems; iii) measurement of height (in centimetres), weight (in kilograms), hips and waist (both in centimetres); iv) 12-lead ECG; v) office brachial

BP and heart rate measurement as described in section 2.3; vi) applanation tonometry for assessment of PWA and aortic pulse wave velocity PWV as in section 2.2; vii) dual energy x-ray absorptiometry scanning for assessment of lumbar spine and femoral bone density as described in section 2.5; viii), collection of serum and plasma for biochemical and haematological analysis; ix) spot urine collection for ACR; x) 24-hour urine collection for phosphate excretion; xi) lateral lumbar spine radiography to assess presence and extent of aortic calcification at baseline as previously described (Kauppila *et al.*, 1997); xii) transthoracic echocardiography to assess ventricular dimensions, systolic function and diastolic function together with myocardial deformation (strain), ventricular rotation and twist as detailed in section 2.4; xiii) CMR to determine LV mass, volumes and function together with aortic distensibility as described previously in section 2.1; and xiv) 24-hour ambulatory BP monitoring as detailed in section 2.3. Eligible female subjects of childbearing age underwent urine pregnancy testing prior to entry into the study. Reproducibility for the primary endpoint of LV mass was assessed using the methods described in section 2.1.3. No dietary changes were enforced during the study and no dietary counselling was provided.

5.2.4 Open-Label Run-In Phase

Following baseline studies all subjects were prescribed 1600mg (two 800mg tablets) of sevelamer carbonate with meals for 4 weeks during an open-label run-in phase to assess tolerability, short-term efficacy, compliance and side effect profile. This was reduced to 800mg with meals only if persistent adverse effects or hypophosphataemia occurred. Subjects were seen at week 2 to monitor for adverse

effects that may be related to treatment, to assess compliance and for monitoring of serum phosphate and renal function. In the event that serum phosphate fell below 0.8 mmol/l, the dose of sevelamer was halved to 800 mg with meals (Table 5-2) and levels rechecked two weeks later to ensure serum phosphate was ≥0.8 mmol/l. If serum phosphate remained below this level after 6 weeks and/or a dose reduction subjects were withdrawn from the study after a final set of measurements.

	Serum Phosphate (mmol/l)	Action
Week 2	<0.8	Reduce dose to 800 mg tds and continue to week 4
	≥0.8	Continue to week 4
Week 4	<0.8	Reduce dose to 800 mg tds and continue to week 6
		If already on 800 mg tds withdraw from study after week 4 visit
	≥0.8	Randomise
Week 6	<0.8	Final measurements and withdraw from study
	≥0.8	Randomise

Table 5-2. Management of serum phosphate levels during run-in phase.

5.2.5 Randomisation and Treatment Phase

At the end of the open-label run-in phase subjects attended for a second study visit and the following were repeated following an overnight fast: i) office brachial BP and heart rate; ii) applanation tonometry to determine aortic pulse wave velocity; iii) applanation tonometry to derive central pressure waveforms from pulse wave analysis; iv) collection of serum and plasma for haematological and biochemical analysis; v) collection of a spot urine sample to determine ACR; and vi) 24-hour urine

collection for determination of phosphate excretion. Samples were collected at the same time of day as on the baseline visit.

Participants were then randomised by computer assignment to continue 1600 mg (or half-dose) sevelamer with meals or receive an identical placebo for the remaining 36 weeks. During this blinded treatment phase subjects underwent monitoring of renal function and serum calcium, phosphate and PTH every four weeks (Figure 5-1). Serum phosphate levels were managed according to a pre-defined protocol during the treatment period (Table 5-3). Adherence to study medication was also assessed at every patient contact (every four weeks) using pill counts of returned medication, taking into consideration the typical number of meals per day. The number of pills taken was determined by subtracting the number of pills returned from the number of pills dispensed; compliance was then calculated as a percentage of prescribed pills taken. The occurrence of any adverse effects was assessed and documented at each patient visit.

Participants were regularly encouraged to take tablets with meals as prescribed; recommendations to improve compliance were given when deemed appropriate. These suggestions included: i) use of a small container of study medication in the event of meals taken outside the home; ii) a drug diary; iii) involvement of relatives to assist with medication reminders; iv) use of pill counts; and v) patient education to ensure understanding of the study and its aims. All measurements performed at the initial main study visit (except for the plain lateral abdominal radiograph) were

repeated at a final visit 40 weeks after baseline studies, marking the end of subject participation in the study.

	Serum Phosphate (mmol/l)	Action
At visit	<0.8	Recheck serum phosphate within 1 week
	≥0.8	Continue routine 4-weekly checks
Within 1 week of visit	<0.8	Reduce treatment/placebo dose to 800 mg tds and recheck in 2 weeks
	≥0.8	Continue routine 4-weekly checks
2 weeks after visit	<0.8	Final measurements and withdraw from study
	≥0.8	Continue routine 4-weekly checks

Table 5-3. Management of serum phosphate levels during treatment phase.

5.2.6 Subject Withdrawal

Withdrawal criteria are listed in Table 5-4. The average serum phosphate level of patients with stage 3 CKD enrolled in a previous study by the investigators was 1.2 ± 0.2 mmol/l (Edwards *et al.*, 2009). It was therefore felt unlikely that significant numbers of participants would need to be withdrawn because of hypophosphataemia. The majority of patients with stage 3 CKD do not require phosphate-binding medication for overt hyperphosphataemia and the prevention of metabolic bone disease. If any subjects in the placebo group developed serum phosphate >1.8 mmol/l, they would have been withdrawn from the study and non-trial therapy with appropriate phosphate binders would have been administered. Subjects would also have been withdrawn if they developed persistent hypophosphataemia,

defined as serum phosphate <0.8 mmol/l on two sequential blood tests, or persistent adverse effects related to treatment, despite halving of therapy to 800 mg three times daily. Subjects withdrawn after randomisation were included in final intention-to-treat analyses where data were available.

Pregnancy
Hypophosphataemia (serum phosphate <0.8 mmol/l despite dose reduction of treatment/placebo)
Hyperphosphataemia (serum phosphate >1.8 mmol/l in subjects receiving placebo)
Occurrence of any serious adverse event, intercurrent illness or laboratory abnormality which, in the opinion of the investigators, warrants the subject's permanent withdrawal from the study
Poor compliance with study medication
Poor attendance at study visits
Subject inability to tolerate study medication due to side effect profile
Subject decision to withdraw
Deteriorating renal function
Subject lost to follow-up (loss of contact before final study visit)

Table 5-4. Withdrawal criteria.

5.2.7 Biochemical Analyses

Serum and plasma were stored at -80^{o}C for later analysis for FGF-23, soluble klotho and vitamin D. Methods for these assays are detailed in section 2.7. Twenty-four-hour urine collections were used to determine urinary phosphate excretion (mmol/24 hours), urinary phosphate concentration (mmol/l) and fractional excretion of phosphate, calculated by the equation:

$$Fractional\ Excretion\ Phosphate = \frac{Urine\ Phosphate \times Serum\ Creatinine}{Urine\ Creatinine \times Serum\ Phosphate} \times 100$$

Fractional excretion of phosphate allows correction for incomplete urine collections over 24 hours and standardises urinary phosphate excretion according to degree of renal impairment (Isakova *et al.*, 2011b).

5.2.8 Outcomes and Follow-up

The primary endpoint for the study was change in LV mass from baseline assessed using CMR after 40 weeks of treatment. Left ventricular mass is a powerful predictor of cardiovascular and all-cause mortality in the general population (Levy *et al.*, 1990; Schillaci *et al.*, 2000) and in CKD (Silberberg *et al.*, 1989). A therapeutic reduction in LV mass has been shown to effectively reduce cardiovascular morbidity and mortality (Devereux *et al.*, 2004; Wachtell *et al.*, 2007). Secondary endpoints included change in aortic distensibility measured by CMR, change in arterial stiffness as assessed using PWV and PWA, change in LV function as assessed by tissue Doppler imaging, strain and strain rate analysis and torsion, change in serum FGF-23 and change in bone density on DEXA scanning.

5.2.9 Monitoring and Safety Assessments

All adverse events, including serious adverse events (SAEs), were recorded and followed up for the duration of the study or until resolution. Assessment of adverse events was performed by the study investigators. All SAEs were graded and reported to the sponsor. Any suspected unexpected serious adverse reactions were

reported to the sponsor, ethics committee and Medicines and Healthcare products Regulatory Agency.

5.2.10 Power Calculation

Sample size calculations were based on the primary endpoint of change in LV mass after 40 weeks of treatment. A previous study by our group evaluating the cardiovascular effects of spironolactone in patients with early chronic kidney disease revealed a 14 ± 12 gram drop in LV mass from baseline following 40 weeks of therapy (Edwards *et al.*, 2009). Using data from this previous study and a more conservative estimate of effect size, it was calculated that 55 subjects in each arm would provide >90% power of detecting an 8 gram difference in the change in LV mass from baseline to 40 weeks using a two-tailed t-test at the 5% significance level, assuming a standard deviation of the change in LV mass from baseline of 12 grams. Recruiting 60 subjects to each group would allow a 10% withdrawal or dropout rate.

5.2.11 Statistical Analyses

Statistical analyses were performed as described in the methods section 2.8. Differences between groups after 40 weeks of treatment were assessed using repeated measures analysis of variance. Analysis was by intention-to-treat.

5.3 Results

5.3.1 Recruitment

Recruitment for the study began in February 2009 and was completed in January 2011. During this period a total of 1297 patients with non-diabetic CKD were screened in the outpatient renal clinics at University Hospital Birmingham NHS Foundation Trust (Figure 5-2). 695 were not eligible for the study, leaving 602 potential subjects. 494 of these were approached and formally invited to take part in the study; the remaining 108 failed to attend their clinic appointments or were missed. Of those approached, 120 agreed to participate, 197 declined and 177 did not return the reply slip included with the study information.

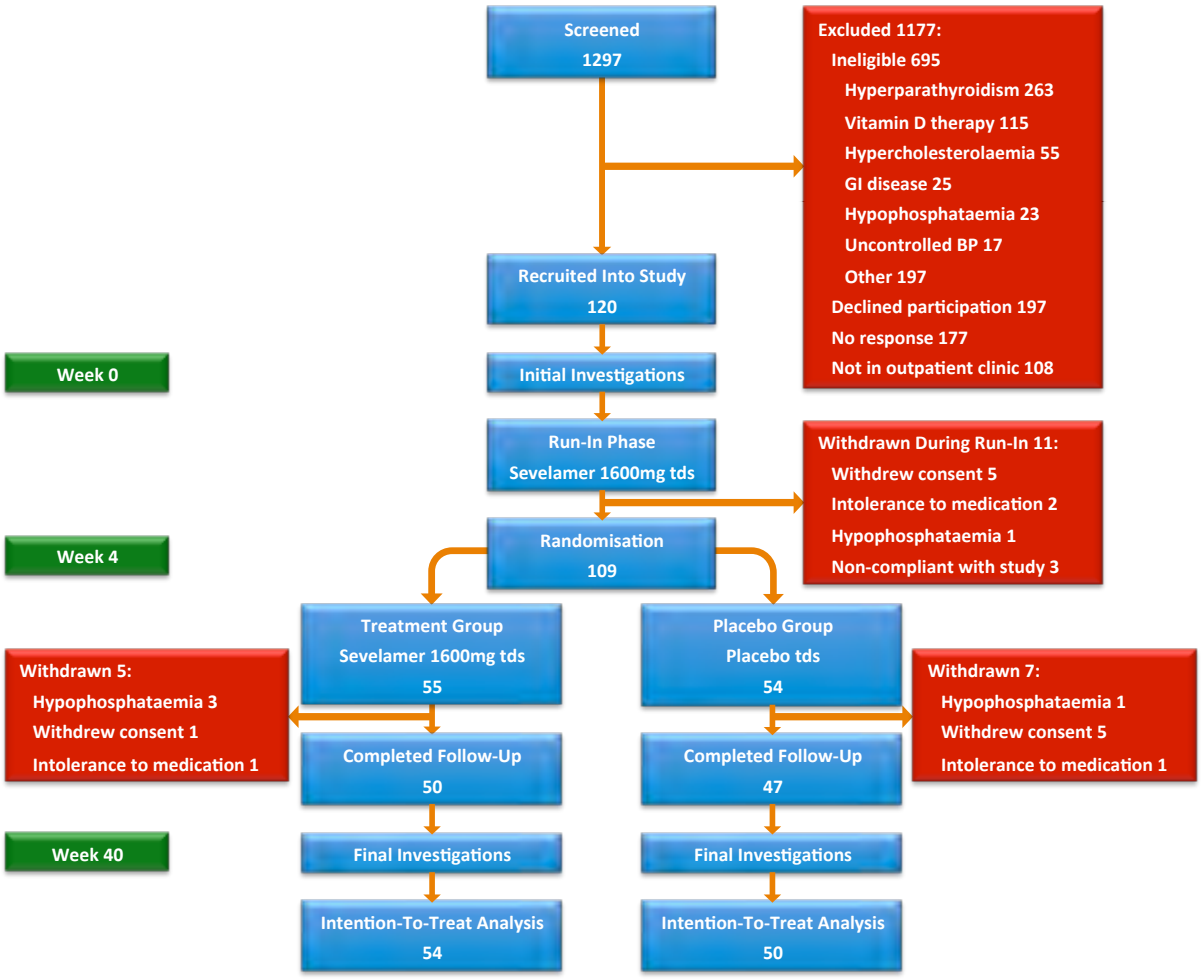

Figure 5-2. Study recruitment, follow-up and withdrawal.

5.3.2 Baseline Results

Demographic data for the 120 subjects recruited into the study are detailed in Table 5-5. The mean age was 55 ± 14 years with 54% male. The prevalence of hypertension was high at 73% and 11% of subjects had a history of established cardiovascular disease. Aortic calcification was present in 48% of subjects at baseline with calcification scores ranging from 1 to 18 (median score of 4 for those with calcification). Compared to baseline data of participants of the Calcification Outcome in Renal Disease (CORD) study, an epidemiological study that aimed to

identify risk factors for vascular calcification and arterial stiffness (Honkanen *et al.*, 2008), our cohort comprised subjects who were younger (mean age 55 vs. 61 years), had fewer males (54 vs. 61%) and had a lower prevalence of previous cardiovascular disease (11 vs. 44%).

Demographic	
Age (years)	55 ± 14
Males	65 (54)
Hypertension	88 (73)
Ethnicity:	
Caucasian	105 (88)
Afro-Caribbean	4 (3)
Asian Indian	7 (6)
Asian Pakistani	4 (3)
Previous cardiovascular disease:	13 (11)
Transient ischaemic attack	6 (5)
Stroke	3 (3)
Coronary artery disease	6 (5)
Peripheral vascular disease	2 (2)
Current smoker	21 (18)
Previously smoked	44 (37)
Pack years*	15 (4–25)
Height (cm)	169 ± 10
Weight (kg)	83 ± 16
Body mass index (kg/m^2)	29.0 ± 5.5
Body surface area (m^2)	1.9 ± 0.2
Hips (cm)	105 ± 12
Waist (cm)	98 ± 13
Hip: waist ratio	1.1 ± 0.1

Table 5-5. Baseline demographic data.

*n= 65 for those with a history of smoking. Data are mean ± standard deviation, frequency (percentage) or median (interquartile range).

Causes of kidney disease are detailed in Table 5-6. The cause of renal disease was diagnosed by biopsy in 53% and ultrasound in 34% (predominantly adult polycystic kidney disease and reflux nephropathy). Medications are detailed in Table 5-7. Most patients were receiving either an ACEI or ARB as per national guidelines.

Diagnosis	
Hypertensive nephropathy	16 (13)
IgA nephropathy	13 (11)
Reflux nephropathy	13 (11)
Adult polycystic kidney disease	12 (10)
Systemic vasculitis	9 (8)
Focal segmental glomerulosclerosis	8 (7)
Nephrectomy for neoplasm	5 (4)
Other glomerulonephritis	11 (9)
Renovascular disease	4 (3)
Nephrolithiasis	3 (3)
Obstructive nephropathy	3 (3)
Cystic renal disease	2 (2)
Other	18 (15)
Unknown	3 (3)

Table 5-6. Renal diagnoses.

Data are frequency (percentage).

Medication	
ACEI	64 (53)
ARB	32 (27)
ACEI or ARB	92 (77)
Aspirin	23 (19)
Warfarin	1 (1)
Statin	53 (44)
Fibrate	3 (3)
Ezetimibe	3 (3)
β-blocker	20 (17)
Calcium-channel blocker	33 (28)
Diuretic	32 (27)
α-blocker	17 (14)
Aldosterone antagonist	2 (2)
Nitrate	1 (1)
Calcium supplement	8 (7)
Bisphosphonate	9 (8)
Immunosuppressant	23 (19)
Erythropoietin	1 (1)
Number of antihypertensives	1.7 ± 1.2

Table 5-7. Medication at study entry.

Data are frequency (percentage) or mean ± standard deviation. ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Biochemical and haematological data are summarized in Table 5-8 to Table 5-10. Mean GFR was 50 ± 13 ml/min/1.73m^2. Mean serum phosphate, calcium, PTH,

klotho and vitamin D levels were within the normal laboratory reference range. Serum FGF-23 levels and urinary fractional excretion of phosphate were both above the upper limit of normal from published reference ranges (Isakova *et al.*, 2011b; Yamazaki *et al.*, 2002).

Biochemical and haematological parameter	
Haemoglobin (g/dl)	13.3 ± 1.5
Sodium (mmol/l)	142 ± 3
Potassium (mmol/l)	4.4 ± 0.4
Urea (mmol/l)	8.9 ± 3.2
Creatinine (µmol/l)	123 ± 33
Estimated glomerular filtration rate (ml/min/1.73m^2)	50 ± 13
Albumin (g/l)	44 ± 4
Bilirubin (µmol/l)	9 ± 4
Total protein (g/l)	70 ± 6
Aspartate aminotransferase (U/l)	23 ± 9
Total cholesterol (mmol/l)	4.92 ± 1.22
High density lipoprotein cholesterol (mmol/l)	1.45 ± 0.51
Low density lipoprotein cholesterol (mmol/l)	2.74 ± 0.92
Triglycerides (mmol/l)	1.2 (0.8–2.0)
Glucose (mmol/l)	5.1 ± 1.0
Bicarbonate (mmol/l)	26.7 ± 2.8
Uric acid (µmol/l)	414 ± 111
hsCRP (µg/ml)	2.17 (1.09–6.35)

Table 5-8. Baseline biochemical and haematological data: serum and plasma.

Data are mean ± standard deviation or median (interquartile range). hsCRP, high sensitive C-reactive protein.

Phosphate and calcium metabolism	
Alkaline phosphatase (U/l)	182 ± 56
Phosphate (mmol/l)	1.03 ± 0.16
Corrected calcium (mmol/l)	2.21 ± 0.09
Calcium phosphate product	2.3 ± 0.4
Parathyroid hormone (ng/l)	52 (38–71)
Fibroblast growth factor 23 (pg/ml)	68 (50–85)
Soluble klotho (pg/ml)	943 ± 396
1,25-dihydroxyvitamin D (pmol/l)	76 ± 31
25-hydroxyvitamin D (pmol/l)	56 ± 30

Table 5-9. Baseline markers of calcium and phosphate metabolism.

Data are mean ± standard deviation or median (interquartile range).

Urine	
Albumin: creatinine ratio (mg/mmol)	6.3 (0.8–48.3)
24-hour urine volume (ml)	2015 ± 805
24-hour phosphate excretion (mmol)	22.3 ± 8.2
24-hour urinary phosphate concentration (mmol/l)	11.7 ± 5.0
Fractional excretion of phosphate (%)	20.8 (12.7–32.3)

Table 5-10. Baseline biochemical data: urine.

Data are mean ± standard deviation or median (interquartile range).

Blood pressures and arterial stiffness indices are shown in Table 5-11 and Table 5-12. Despite a high prevalence of hypertension (73%), BP was well controlled with an office mean of 128/71 mmHg and 24-hour ambulatory mean of 124/71 mmHg.

Office brachial blood pressure	
Systolic (mmHg)	128 ± 17
Diastolic (mmHg)	71 ± 11
Pulse pressure (mmHg)	57 ± 17
Mean arterial pressure (mmHg)	91 ± 11
Heart rate (beats/minute)	63 ± 10
Central aortic blood pressure	
Systolic (mmHg)	117 ± 17
Diastolic (mmHg)	72 ± 11
Pulse pressure (mmHg)	45 ± 16
Mean arterial pressure (mmHg)	91 ± 12
Arterial stiffness indices	
AIx	26.1 ± 11.9
AIx_{75}	19.9 ± 11.8
Pulse wave velocity (m/s)	8.9 (7.3–11.2)
Pulse wave $velocity_{adj}$ (m/s)*	9.1 (7.7–10.6)

Table 5-11. Baseline haemodynamic and arterial stiffness data.

*Adjusted for mean arterial pressure. Data are mean ± standard deviation or median (interquartile range). AIx, augmentation index; AIx_{75}, augmentation index adjusted to heart rate of 75bpm.

24 hour blood pressure data	
Day-time:	
Systolic (mmHg)	128 ± 12
Diastolic (mmHg)	75 ± 10
Pulse pressure (mmHg)	53 ± 11
Mean arterial (mmHg)	93 ± 9
Heart rate (beats/minute)	75 ± 11
Night-time*:	
Systolic (mmHg)	116 ± 16
Diastolic (mmHg)	64 ± 10
Pulse pressure (mmHg)	51 ± 12
Mean arterial (mmHg)	82 ± 11
Heart rate (beats/minute)	66 ± 11
24-hour average:	
Systolic (mmHg)	124 ± 12
Diastolic (mmHg)	71 ± 9
Pulse pressure (mmHg)	53 ± 11
Mean arterial (mmHg)	89 ± 9
Heart rate (beats/minute)	72 ± 10
Nocturnal dipper*	58 (50)

Table 5-12. Baseline 24-hour ambulatory blood pressure data.

*Data available for 116 subjects. Data are mean ± standard deviation or frequency (percentage).

Baseline 2-dimensional echocardiographic parameters are shown in Table 5-13 and Table 5-14. Mean LV dimensions and ejection fraction were within normal limits.

Strain, strain rate, rotation and twist data obtained with speckle tracking analysis are displayed in Table 5-15.

Left ventricular chamber measurement	
Interventricular septum diastole (cm)	0.9 ± 0.2
Left ventricular internal dimension diastole (cm)	4.9 ± 0.7
Left ventricular internal dimension systole (cm)	3.0 ± 0.6
Left ventricular posterior wall diastole (cm)	0.9 ± 0.2
Fractional shortening (%)	39 ± 10
Left ventricular ejection fraction (%)	62 ± 6
Left ventricular end diastolic volume index (ml/m^2)	49 ± 11
Left atrial volume index (ml/m^2)	28 ± 8
Left ventricular mass index (g/m^2)	92 ± 34

Table 5-13. Baseline echocardiographic data: left ventricular chamber quantification. Data are mean ± standard deviation.

Left ventricular functional parameter	
Transmitral E (m/s)	0.68 ± 0.13
Transmitral A (m/s)	0.71 ± 0.18
Transmitral E/A	0.9 (0.8–1.1)
E wave deceleration time (ms)	223 ± 54
Velocity propagation (cm/s)	57 (46–75)
Transmitral E/velocity propagation	1.13 ± 0.48
E/mean e’	7.6 (6.5–9.4)
Isovolumic relaxation time (ms)	110 ± 27
Pulmonary vein S:D	1.21 ± 0.34
a duration – A duration (ms)	-39 ± 24
Left ventricular Tei index	0.32 ± 0.07
Anterolateral s’ (m/s)	8.9 ± 2.4
Anterolateral e’ (m/s)	10.3 ± 3.8
Inferoseptal s’ (m/s)	9.1 ± 2.1
Inferoseptal e’ (m/s)	7.8 ± 2.7
Mean s’ (m/s)	9.0 ± 1.9
Mean e’ (m/s)	9.1 ± 3.0
Calibrated integrated backscatter (dB)	-15.7 ± 6.4
Right ventricular measurements	
Right ventricular internal dimension (cm)	3.1 ± 0.7
Right ventricular Tei index	0.48 ± 0.27
TAPSE (mm)	26 ± 5

Table 5-14. Baseline echocardiographic data: left ventricular function and right ventricular measurements.

Data are mean ± standard deviation or median (interquartile range). TAPSE, tricuspid annular plane systolic excursion.

Left ventricular rotation	
Basal peak rotation (^{o})*	-9.8 ± 2.7
Time to peak basal rotation (ms)*	342 ± 44
Apical peak rotation (^{o})†	12.0 ± 4.1
Time to peak apical rotation (ms)†	359 ± 57
Left ventricular twist‡	
Peak twist (^{o})	19.7 ± 4.9
Time to peak twist (ms)	350 ± 47
Peak systolic twist rate (^{o})	124 ± 28
Time to peak systolic twist rate (ms)	182 ± 52
Peak early diastolic untwist rate (^{o})	-134 ± 41
Time to peak early diastolic untwist (ms)	447 ± 50
Longitudinal strain§	
Global longitudinal peak systolic strain (%)	-19.3 ± 2.6
Global longitudinal end systolic strain (%)	-19.1 ± 2.6
Global longitudinal peak systolic strain rate (s^{-1})	-1.35 ± 0.22
Global longitudinal early diastolic strain rate (s^{-1})	1.58 ± 0.32

Table 5-15. Baseline echocardiographic data: strain, rotation and twist.

*Data available in 59 pts; †data available in 84 patients; ‡data available in 53 patients; §data available in 112 patients. Data are mean ± standard deviation.

Cardiovascular magnetic resonance imaging was unable to be performed in 13 subjects (11%) due to claustrophobia. Baseline ventricular measurements are shown for the 107 subjects that underwent CMR in Table 5-16. Mean LV mass, LV systolic and diastolic function and LV volumes were within normal limits; only one

patient had LVH. Aortic distensibility data derived from CMR are shown in Table 5-17.

Left ventricle	
Ejection fraction (%)	74 ± 7
End diastolic volume index (ml/m^2)	60 ± 16
End systolic volume index (ml/m^2)	16 ± 7
Stroke volume index (ml/m^2)	44 ± 9
Cardiac index (l/min/m^2)	3.0 ± 0.8
Mass (g)	101 ± 30
Mass index (g/m^2)	52 ± 13
Left ventricular hypertrophy	1 (1)
Right ventricle	
Ejection fraction (%)	73 ± 7
End diastolic volume index (ml/m^2)	59 ± 14
End systolic volume index (ml/m^2)	16 ± 7
Stroke volume index (ml/m^2)	42 ± 9
Cardiac index (l/min/m^2)	2.9 ± 0.8

Table 5-16. Baseline cardiac magnetic resonance imaging data: left and right ventricle. Data available for 107 subjects. Data are mean ± standard deviation or frequency (percentage).

Aortic distensibility	
Ascending aortic distensibility ($x10^{-3}$ $mmHg^{-1}$)	2.7 ± 2.0
Ascending aortic $distensibility_{adj}$ ($x10^{-3}$ $mmHg^{-1}$)*	2.6 ± 2.0
Proximal descending aortic distensibility ($x10^{-3}$ $mmHg^{-1}$)	3.3 ± 1.6
Proximal descending aortic $distensibility_{adj}$ ($x10^{-3}$ $mmHg^{-1}$)*	3.2 ± 1.5
Distal descending aortic distensibility ($x10^{-3}$ $mmHg^{-1}$)	4.2 ± 2.1
Distal descending aortic $distensibility_{adj}$ ($x10^{-3}$ $mmHg^{-1}$)*	4.1 ± 2.0
Ascending: proximal descending ratio	0.76 ± 0.30
Ascending: distal descending ratio	0.61 ± 0.31
Proximal descending: distal descending ratio	0.82 ± 0.28

Table 5-17. Baseline cardiac magnetic resonance imaging data: aortic distensibility. Data available for 107 subjects. *Adjusted for mean arterial pressure. Data are mean ± standard deviation.

Bone density data for the lumbar spine and both hips are shown in Table 5-18. 28% of subjects had evidence of osteopenia at one or more sites; one patient had overt osteoporosis.

Bone density	
Left hip:	
Bone mineral density (g/cm^2)	1.00 ± 0.14
T-score	0.03 ± 1.00
Z-score	0.61 ± 1.08
Right hip:	
Bone mineral density (g/cm^2)	0.99 ± 0.14
T-score	0.00 ± 0.95
Z-score	0.57 ± 1.05
Both hips:	
Bone mineral density (g/cm^2)	1.00 ± 0.14
T-score	0.01 ± 0.98
Z-score	0.59 ± 1.06
Lumbar spine:	
Bone mineral density (g/cm^2)	1.09 ± 0.17
T-score	0.16 ± 1.53
Z-score	0.94 ± 1.67
Osteopenia	34 (28)
Osteoporosis	1 (1)

Table 5-18. Baseline bone mineral density data.

Data are mean ± standard deviation or frequency (percentage).

5.3.3 Open-Label Run-In Phase

Data are available for 112 subjects at week 0 and week 4. Biochemical and haematological data are displayed in Table 5-19 and Table 5-20. Following four weeks of open-label treatment with sevelamer carbonate significant reductions in

serum cholesterol (4.91 ± 1.20 vs. 4.46 ± 1.13 mmol/l, P<0.01), low density lipoprotein cholesterol (2.74 ± 0.84 vs. 2.26 ± 0.85 mmol/l, P<0.01), serum urate (415 ± 111 vs. 396 ± 97 µmol/l, P<0.01), hsCRP (2.17 [1.09–6.35] vs. 1.74 [0.79–5.12] µg/ml, P<0.01) and urinary ACR (5.7 [0.8–42.7] vs. 4.2 [1.9–46.7] mg/mmol, P<0.01) were seen. There was also a small but statistically significant increase in serum phosphate (1.02 ± 0.17 vs. 1.05 ± 0.16 mmol/l, P<0.05). Levels of both 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D decreased significantly after 4 weeks of treatment, but this was not accompanied by any significant changes in FGF-23, klotho or measures of 24-hour urinary phosphate excretion (Table 5-20). Small but significant changes were also noted with haemoglobin, serum albumin, total protein, aspartate aminotransferase, alkaline phosphatase and triglycerides.

Haemodynamic data and indices of arterial stiffness are shown in Table 5-21. There was a small but significant decrease in brachial systolic BP (128 ± 16 vs. 125 ± 16 mmHg, P<0.05), brachial pulse pressure (57 ± 17 vs. 54 ± 16 mmHg, P<0.01) and central pulse pressure (45 ± 16 vs. 43 ± 15 mmHg, P<0.01), but no observed differences in central systolic BP or any indices of arterial stiffness.

Eleven subjects were withdrawn during the four-week open-label run-in phase (five withdrew consent, two were unable to tolerate the study drug, one had persistent hypophosphataemia and three subjects did not comply with study instructions) leaving 109 subjects to be randomised to receive sevelamer (n=55) or placebo (n=54; Figure 5-2).

	Week 0	Week 4
Haematological:		
Haemoglobin (g/dl)	13.3 ± 1.5	13.0 ± 1.6*
White cell count ($x10^9$/l)	6.7 ± 1.9	6.5 ± 1.8
Platelets ($x10^9$/l)	240 ± 59	236 ± 59
Biochemical:		
Urea (mmol/l)	8.9 ± 3.2	9.0 ± 3.5
Creatinine (µmol/l)	124 ± 33	126 ± 34
Estimated glomerular filtration rate (ml/min/1.73m^2)	50 ± 13	49 ± 13
Albumin (g/l)	44 ± 4	43 ± 4†
Total protein (g/l)	70 ± 6	68 ± 6*
Bilirubin (µmol/l)	9.2 ± 4.2	9.4 ± 5.3
Aspartate aminotransferase (U/l)	23 ± 10	26 ± 13‡
Bicarbonate (mmol/l)	26.7 ± 2.9	27.0 ± 2.8
Total cholesterol (mmol/l)	4.91 ± 1.20	4.46 ± 1.13*
High density lipoprotein cholesterol (mmol/l)	1.45 ± 0.53	1.44 ± 0.52
Low density lipoprotein cholesterol (mmol/l)	2.74 ± 0.84	2.26 ± 0.85*
Triglycerides (mmol/l)	1.1 (0.8–1.9)	1.3 (0.9–1.9)‡
Glucose (mmol/l)	5.1 ± 1.0	5.0 ± 1.1
Urate (µmol/l)	415 ± 111	396 ± 97*
hsCRP (µg/ml)	2.17 (1.09–6.35)	1.74 (0.79–5.12)†

Table 5-19. Biochemical and haematological data from the 4-week open-label run-in phase. *P<0.001; †P<0.005; ‡P<0.05. Data are mean ± standard deviation or median (interquartile range). Analysed with paired t-tests, n=112. hsCRP, high sensitive C-reactive protein.

	Week 0	Week 4
Serum and plasma markers of phosphate metabolism:		
Alkaline phosphatase (U/l)	181 ± 56	194 ± 69*
Phosphate (mmol/l)	1.02 ± 0.17	1.05 ± 0.16†
Corrected calcium (mmol/l)	2.21 ± 0.10	2.21 ± 0.08
Parathyroid hormone (ng/l)	53 (38–71)	55 (44–71)
Fibroblast growth factor 23 (pg/ml)	69 (51–85)	68 (53–86)
Soluble klotho (pg/ml)	936 ± 403	945 ± 359
1,25-dihydroxyvitamin D (pmol/l)	76 ± 31	68 ± 25*
25-hydroxyvitamin D (nmol/l)	55 ± 29	52 ± 28‡
Urine:		
Albumin: creatinine ratio (mg/mmol)	5.7 (0.8–42.7)	4.2 (1.9–46.7)‡
24-hour urine volume (ml)§	2021 ± 778	2073 ± 903
24-hour phosphate excretion (mmol)§	22.7 ± 7.8	21.4 ± 10.2
Phosphate concentration (mmol/l)§	11.7 ± 4.7	11.0 ± 5.9
Fractional excretion of phosphate§	21.2 (13.1–32.8)	20.6 (13.8–33.2)

Table 5-20. Markers of phosphate metabolism and urinary data from the 4-week open-label run-in phase.

*P<0.001; †P<0.05; ‡P<0.005; §data available for 101 subjects. Data are mean ± standard deviation or median (interquartile range). Analysed with paired t-tests, n=112.

	Week 0	Week 4
Haemodynamics:		
Brachial systolic BP (mmHg)	128 ± 16	125 ± 16*
Brachial diastolic BP (mmHg)	71 ± 11	71 ± 10
Brachial pulse pressure (mmHg)	57 ± 17	54 ± 16†
Brachial mean arterial pressure (mmHg)	90 ± 11	90 ± 11
Central systolic BP (mmHg)	117 ± 16	115 ± 16
Central diastolic BP (mmHg)	72 ± 11	72 ± 10
Central pulse pressure (mmHg)	45 ± 16	43 ± 15†
Central mean arterial pressure (mmHg)	91 ± 11	90 ± 11
Heart rate (beats/minute)	63 ± 10	64 ± 11*
Arterial stiffness indices:		
AIx	25.8 ± 12.0	25.1 ± 11.6
AIx_{75}	19.7 ± 11.9	19.8 ± 10.8
Pulse wave velocity (m/s)	9.0 (7.4–11.2)	8.7 (7.4–10.6)
Pulse wave $velocity_{adj}$ (m/s)‡	9.1 (7.7–10.6)	8.6 (7.4–10.3)

Table 5-21. Haemodynamics and indices of arterial stiffness at baseline and week 4. *P<0.05; †P<0.01; ‡adjusted for mean arterial pressure. Data are mean ± standard deviation or median (interquartile range). Analysed with paired t-tests, n=112. BP, blood pressure; AIx, augmentation index; AIx_{75}, augmentation index adjusted to heart rate of 75bpm.

5.3.4 Follow-up

Participants were well matched at baseline (Table 5-22 to Table 5-24). There were significantly more individuals with hypertension in the placebo group (83% vs. 60%, P<0.05) and consequently a greater mean number of antihypertensive agents (1.9 ± 1.2 vs. 1.4 ± 1.2, P<0.05) prescribed per patient. Aortic calcification was present in

35% of subjects in the placebo group and 55% in the sevelamer group (P=0.06). Of those randomised, twelve patients (11%) did not complete follow-up to 40 weeks (seven in placebo group, five in sevelamer group, P=0.6; Figure 5-2). Six patients declined continued participation in the study due to difficulties with tablet frequency and a wish to discontinue study visits, four experienced persistent hypophosphataemia despite dose reduction of study drug and two became intolerant of medication. No patients died.

	Placebo n=54	Sevelamer n=55
Age (years)	55 ± 14	55 ± 13
Male gender	28 (52)	32 (58)
Hypertension	45 (83)	33 (60)*
Hypercholesterolaemia	28 (52)	25 (46)
Ethnicity:		
Caucasian	48 (89)	47 (86)
Afro-Caribbean	0 (0)	4 (7)
Asian Indian	3 (6)	3 (6)
Asian Pakistani	3 (6)	1 (2)
Previous cardiovascular disease	5 (9)	6 (11)
Smoking status:		
Current smoker	8 (15)	8 (15)
Previous smoker	17 (32)	24 (44)
Alcohol drinker	29 (54)	36 (66)
Body mass index (kg/m^2)	29.7 ± 6.7	28.6 ± 4.3
Hip: waist ratio	1.08 ± 0.10	1.08 ± 0.09
Aortic calcification present	19 (35)	30 (55)

Table 5-22. Baseline demographics of placebo and treatment group.

*P<0.05. Data are mean ± SD or frequency (%). Analysed using independent samples t-test or χ^2.

	Placebo n=54	Sevelamer n=55
Hypertensive nephropathy	10 (19)	3 (6)*
IgA nephropathy	3 (6)	7 (13)
Reflux nephropathy	4 (7)	7 (13)
Adult polycystic kidney disease	8 (15)	4 (7)
Systemic vasculitis	5 (9)	4 (7)
Lupus nephritis	0 (0)	3 (6)
Focal segmental glomerulosclerosis	4 (7)	3 (6)
Nephrectomy for neoplasm	2 (4)	3 (6)
Other glomerulonephritis	2 (4)	3 (6)
Other	15 (28)	16 (29)
Unknown	1 (2)	2 (4)

Table 5-23. Renal diagnoses of placebo and treatment group.

*P<0.05. Data are frequency (percentage) and analysed using χ^2.

	Placebo n=54	Sevelamer n=55
Angiotensin converting enzyme inhibitor	32 (59)	25 (46)
Angiotensin receptor blocker	16 (30)	13 (24)
Aspirin	9 (17)	10 (18)
Aldosterone antagonist	1 (2)	1 (2)
Beta blocker	12 (22)	6 (11)
Calcium channel blocker	15 (28)	14 (26)
Diuretic	19 (35)	10 (18)
Statin	26 (48)	24 (44)
Other lipid lowering therapy	3 (6)	3 (6)
Prednisolone	8 (15)	9 (16)
Immunosuppressant	11 (20)	11 (20)
Number of antihypertensives	1.9 ± 1.2	1.4 ± 1.2*

Table 5-24. Baseline medication use in placebo and treatment group.

*P<0.05. Data are frequency (percentage) or mean ± standard deviation. Analysed using independent samples t-test or χ^2.

5.3.5 Treatment Effects

Data for effects of treatment compared to placebo over 40 weeks are shown in Table 5-25 to Table 5-34. After 40 weeks of treatment with sevelamer carbonate there was a significant decrease in total cholesterol when compared to placebo (P<0.05) but LDL and HDL did not change significantly (Table 5-25). There were no significant changes in any other biochemical parameters measured, in particular phosphate, calcium, PTH, FGF-23, klotho, vitamin D or urinary phosphate excretion (Table 5-26).

There were no significant changes in the primary endpoint of LV mass (Figure 5-3 and Table 5-27) or LV mass indexed to body surface area. There were no changes in any indices of LV systolic or diastolic function (Table 5-27 and Table 5-30), strain or twist (Table 5-31). There were no observed differences in peripheral office, central or ambulatory BP, or in any indices of arterial stiffness (Table 5-32, Table 5-28 and Table 5-33). Bone density remained unaltered in both groups (Table 5-34). When patients with aortic calcification were excluded from analyses no significant differences were observed in LV mass (P=0.2), AIx (P=0.6) or PWV (P=0.6) at 40 weeks. Further subgroup analyses that only included patients with the greatest falls in LDL and FGF-23 also showed no significant difference in the above endpoints.

	Placebo n=50		Sevelamer n=54	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Haemoglobin (g/dl)	13.0 ± 1.5	13.1 ± 1.7	13.5 ± 1.4	13.4 ± 1.4
Creatinine (µmol/l)	124 ± 33	125 ± 33	126 ± 35	130 ± 41
eGFR (ml/min/1.73m^2)	49 ± 13	50 ± 14	49 ± 13	48 ± 14
Albumin (g/l)	43 ± 3	43 ± 3	44 ± 4	43 ± 4
Alkaline phosphatase (U/l)	190 ± 55	186 ± 53	173 ± 58	189 ± 68*
Total cholesterol (mmol/l)	4.69 ± 1.06	4.54 ± 1.00	5.03 ± 1.34	4.38 ± 1.23†
HDL cholesterol (mmol/l)	1.40 ± 0.49	1.41 ± 0.58	1.45 ± 0.43	1.39 ± 0.56
LDL cholesterol (mmol/l)	2.71 ± 0.94	2.58 ± 1.10	2.75 ± 0.91	2.37 ± 1.00
Triglycerides (mmol/l)	1.1 (0.8–1.7)	1.1 (0.8–1.5)	1.3 (0.9–2.2)	1.4 (1.0–1.9)
Glucose (mmol/l)	5.2 ± 1.1	5.1 ± 1.8	5.1 ± 0.9	4.9 ± 0.8
Bicarbonate (mmol/l)	26.4 ± 2.8	27.2 ± 3.4	27.0 ± 2.7	27.2 ± 6.2
Urate (µmol/l)	410 ± 105	414 ± 102	414 ± 106	411 ± 103
hsCRP (µg/ml)	3.37 (1.09–6.42)	2.64 (1.06–8.35)	1.96 (0.96–1.89)	1.42 (0.77–2.94)

Table 5-25. Biochemical data at week 0 and week 40 for placebo and treatment groups. *P<0.005; †P<0.05. Data are mean ± standard deviation or median (interquartile range). Data analysed with repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups. eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high sensitive C-reactive protein.

	Placebo n=50		Sevelamer n=54	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Markers of phosphate metabolism:				
Phosphate (mmol/l)	1.05 ± 0.17	1.07 ± 0.17	1.02 ± 0.16	1.02 ± 0.23
Corrected calcium (mmol/l)	2.20 ± 0.10	2.19 ± 0.08	2.22 ± 0.09	2.21 ± 0.08
Calcium phosphate product	2.38 ± 0.43	2.35 ± 0.40	2.34 ± 0.38	2.25 ± 0.52
Parathyroid hormone (ng/l)	54 (37–73)	51 (39–72)	52 (39–70)	52 (35–75)
FGF-23 (pg/ml)	68 (51–88)	64 (52–84)	71 (53–83)	66 (49–91)
Klotho (pg/ml)	869 ± 279	873 ± 320	1001 ± 500	980 ± 533
1,25-dihydroxyvitamin D (pmol/l)	75 ± 33	68 ± 28	74 ± 27	71 ± 27
25-hydroxyvitamin D (nmol/l)	56 ± 31	55 ± 31	57 ± 28	59 ± 33
Urine:				
ACR (mg/mmol)	3.0 (0.7–31.4)	2.6 (0.7–21.1)	8.9 (0.9–63.7)	7.5 (0.8–63.7)
24-hour phosphate excretion (mmol)	21.1 ± 7.2	21.9 ± 8.6	24.2 ± 7.6	21.9 ± 7.4
UPi (mmol/l)	11.7 ± 4.2	11.3 ± 4.8	12.1 ± 5.2	10.8 ± 4.4
FEPi (%)	21 (13–28)	20 (10–24)	22 (13–34)	18 (13–30)

Table 5-26. Biochemical data for markers of phosphate metabolism and urinary data at week 0 and week 40 for placebo and treatment groups.

Data are mean ± standard deviation or median (interquartile range). Data analysed with repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups. FGF-23, fibroblast growth factor 23; ACR, albumin: creatinine ratio; UPi, urinary phosphate concentration; FEPi, urinary fractional excretion of phosphate.

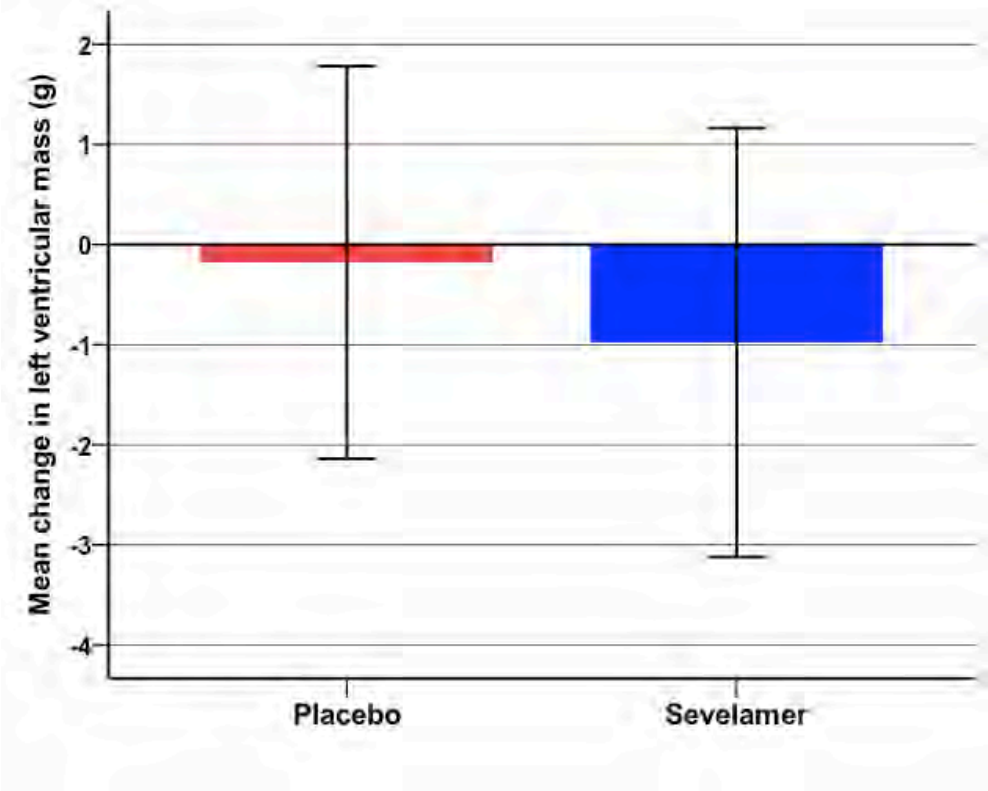

Figure 5-3. Mean change in left ventricular mass between placebo group and treatment group.

P=0.58, independent samples t-test of difference in change between groups over 40 weeks.

	Placebo n=45		Sevelamer n=45	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Ejection fraction (%)	76 ± 7	76 ± 6	73 ± 6	72 ± 13
End diastolic volume index (ml/m^2)	59 ± 11	57 ± 11	62 ± 20	61 ± 16
End systolic volume index (ml/m^2)	15 ± 6	14 ± 6	17 ± 7	16 ± 7
Stroke volume index (ml/m^2)	44 ± 8	43 ± 8	44 ± 10	44 ± 13
Cardiac index (l/min/m^2)	3.0 ± 0.7	2.9 ± 0.7	3.1 ± 0.9	2.9 ± 0.9
Mass (g)	99 ± 31	99 ± 30	102 ± 29	101 ± 27
Mass index (g/m^2)	51 ± 13	51 ± 12	52 ± 13	52 ± 13

Table 5-27. Left ventricular cardiovascular magnetic resonance imaging data at week 0 and week 40 for placebo and sevelamer groups.

Data are mean ± standard deviation. Data analysed using repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups.

	Placebo n=45		Sevelamer n=45	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Ascending	2.34 ± 1.79	2.40 ± 1.49	2.54 ± 1.64	2.70 ± 1.88
$Ascending_{adj}$†	2.27 ± 1.73	2.29 ± 1.25	2.52 ± 1.62	2.81 ± 1.72
Proximal descending	3.07 ± 1.51	3.13 ± 1.43	3.22 ± 1.45	3.42 ± 1.55
$Proximal\ descending_{adj}$†	2.96 ± 1.39	3.01 ± 1.05	3.22 ± 1.42	3.55 ± 1.36
Distal descending	3.94 ± 2.00	3.94 ± 1.79	4.44 ± 2.27	4.37 ± 2.08
$Distal\ descending_{adj}$†	3.82 ± 1.82	3.77 ± 1.42	4.41 ± 2.29	4.50 ± 1.79
Ascending: proximal descending	0.71 ± 0.29	0.73 ± 0.27	0.76 ± 0.31	0.76 ± 0.29
Ascending: distal descending	0.54 ± 0.24	0.58 ± 0.23	0.60 ± 0.27	0.60 ± 0.23
Proximal descending: distal descending	0.82 ± 0.27	0.83 ± 0.23	0.82 ± 0.29	0.82 ± 0.21

Table 5-28. Aortic distensibility cardiovascular magnetic resonance imaging data at week 0 and week 40.

*Adjusted for mean arterial pressure. Data are mean ± standard deviation and analysed using repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups.

	Placebo n=50		Sevelamer n=54	
	Week 0	**Week 40**	**Week 0**	**Week 40**
LVIDd (cm)	5.0 ± 0.6	4.9 ± 0.6	4.8 ± 0.6	4.8 ± 0.5
LVIDs (cm)	3.1 ± 0.6	2.9 ± 0.6	2.9 ± 0.6	2.9 ± 0.5
Fractional shortening (%)	39.1 ± 7.6	40.0 ± 7.2	39.0 ± 7.3	39.0 ± 6.7
LVEF (%)	62 ± 5	62 ± 4	62 ± 6	61 ± 4
LVEDVI (ml/m^2)	48.6 ± 9.6	45.6 ± 9.9	48.6 ± 12.1	45.3 ± 10.7
LAVI (ml/m^2)	28.9 ± 8.2	26.4 ± 7.3	27.3 ± 8.1	25.7 ± 8.1
RVID (cm)	3.2 ± 0.5	3.2 ± 0.5	3.1 ± 0.7	3.1 ± 0.5

Table 5-29. Echocardiographic chamber dimension data at week 0 and week 40 for placebo and treatment groups.

Data are mean ± standard deviation and analysed using repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups. LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole; LVEF, left ventricular ejection fraction; LVEDVI, left ventricular end diastolic volume index; LAVI, left atrial volume index; RVID, right ventricular internal dimension in diastole.

	Placebo n=50		Sevelamer n=54	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Transmitral E/A	0.96 (0.81–1.13)	0.99 (0.81–1.17)	0.93 (0.77–1.06)	0.97 (0.73–1.16)
Transmitral DT (ms)	227 ± 57	219 ± 44	216 ± 50	202 ± 40
VP (cm/s)	62 (45–74)	57 (48–69)	57 (47–79)	53 (46–64)
Transmitral E/VP	1.19 ± 0.44	1.23 ± 0.37	1.10 ± 0.45	1.17 ± 0.38
Mean e' (m/s)	9.3 ± 3.4	9.1 ± 2.8	8.6 ± 2.7	8.8 ± 2.6
E/mean e'	7.3 (6.6–9.3)	7.4 (6.5–9.3)	7.8 (6.5–9.4)	7.2 (6.2–9.4)
IVRT (ms)	108 ± 23	113 ± 26	108 ± 31	119 ± 19
Pulmonary vein S:D	1.2 ± 0.4	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3
a duration – A duration (ms)	-35.8 ± 22.7	-30.0 ± 24.1	-39.3 ± 21.3	-31.9 ± 27.4
Left ventricular Tei index	0.32 ± 0.07	0.35 ± 0.06	0.32 ± 0.08	0.37 ± 0.07
Anterolateral s' (m/s)	9.3 ± 2.5	9.5 ± 2.3	8.4 ± 2.3	9.2 ± 2.3
Inferoseptal s' (m/s)	9.1 ± 1.7	9.0 ± 1.8	8.9 ± 1.9	9.0 ± 1.7
cIB (dB)	-15.1 ± 6.5	-15.6 ± 4.7	-16.2 ± 6.1	-17.8 ± 7.5
RV Tei index	0.47 ± 0.25	0.61 ± 0.26	0.52 ± 0.31	0.59 ± 0.26
TAPSE (mm)	26 ± 5	25 ± 5	27 ± 5	26 ± 5

Table 5-30. Echocardiographic functional data at week 0 and week 40 for placebo and treatment groups.

Data are median (interquartile range) or mean ± standard deviation. Analysed using repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups. DT, deceleration time; VP, velocity propagation; IVRT, isovolumic relaxation time; cIB, calibrated integrated backscatter; RV, right ventricular; TAPSE, tricuspid annular plane systolic excursion.

	Placebo n=50		Sevelamer n=54	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Global peak longitudinal systolic strain (%)	-19.9 ± 2.6	-20.8 ± 2.0	-19.2 ± 2.6	-19.5 ± 2.8
Global peak strain rate (s^{-1})	-1.37 ± 0.22	-1.36 ± 0.18	-1.36 ± 0.23	-1.32 ± 0.23
Global early diastolic strain rate (s^{-1})	1.58 ± 0.30	1.60 ± 0.32	1.57 ± 0.32	1.53 ± 0.31
Peak systolic twist (º)	19.4 ± 3.6	17.0 ± 5.8	18.9 ± 5.1	19.6 ± 5.3
Peak systolic torsion (º/cm)	2.72 ± 0.61	2.57 ± 0.84	2.75 ± 0.76	2.47 ± 0.65
Peak apical rotation (º)	12.2 ± 4.5	11.6 ± 4.6	11.8 ± 3.7	12.0 ± 4.6
Peak twist rate (º/s)	124 ± 23	115 ± 40	120 ± 30	118 ± 40
Peak early diastolic untwist rate (º/s)	-129 ± 28	-115 ± 43	-129 ± 46	-135 ± 37

Table 5-31. Echocardiographic strain and twist data at week 0 and week 40 for placebo and treatment groups.

Data are mean ± standard deviation. Analysed using repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups.

	Placebo n=50		Sevelamer n=54	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Brachial office blood pressure (mmHg):				
Systolic	126 ± 17	124 ± 21	129 ± 15	124 ± 15
Diastolic	70 ± 11	67 ± 10	73 ± 10	71 ± 10
Pulse pressure	56 ± 17	57 ± 19	56 ± 15	53 ± 13
Mean arterial pressure	89 ± 12	87 ± 13	92 ± 10	89 ± 11
Central aortic blood pressure (mmHg):				
Systolic	115 ± 18	113 ± 21	119 ± 15	114 ± 16
Diastolic	71 ± 11	68 ± 11	74 ± 10	72 ± 10
Pulse pressure	44 ± 17	46 ± 19	45 ± 14	42 ± 13*
Mean arterial pressure	90 ± 12	87 ± 13	92 ± 10	89 ± 11
Arterial stiffness indices:				
AIx	24.6 ± 11.5	26.7 ± 13.7	27.8 ± 11.6	27.4 ± 12.1
AIx_{75}	18.9 ± 12.0	20.1 ± 13.2	21.4 ± 10.9	20.8 ± 12.2
Pulse wave velocity (m/s)	9.3 (7.6–11.2)	8.8 (7.9–10.6)	8.5 (7.3–10.7)	8.6 (7.5–10.7)
Pulse wave $velocity_{adj}$ (m/s)†	9.2 (8.1–10.6)	8.9 (8.1–10.6)	8.6 (7.1–10.7)	8.7 (7.7–10.7)

Table 5-32. Haemodynamic data and arterial stiffness indices at week 0 and week 40. *P<0.05; †adjusted for mean arterial pressure. Data are mean ± standard deviation or median (interquartile range). Data analysed with repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups. AIx, augmentation index; AIx_{75}, augmentation index adjusted to heart rate of 75 bpm.

	Placebo n=50		Sevelamer n=54	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Day-time:				
Systolic (mmHg)	128 ± 15	126 ± 15	128 ± 10	127 ± 13
Diastolic (mmHg)	74 ± 10	72 ± 11	76 ± 9	75 ± 10
Pulse pressure (mmHg)	54 ± 11	54 ± 11	52 ± 11	52 ± 11
Mean arterial pressure (mmHg)	92 ± 11	90 ± 11	94 ± 8	92 ± 9
Night-time:				
Systolic (mmHg)	119 ± 19	115 ± 17	114 ± 12	113 ± 14
Diastolic (mmHg)	65 ± 11	63 ± 9	65 ± 10	64 ± 9
Pulse pressure (mmHg)	54 ± 14	53 ± 13	49 ± 10	49 ± 10
Mean arterial pressure (mmHg)	83 ± 12	80 ± 11	81 ± 10	80 ± 10
24-hour average:				
Systolic (mmHg)	125 ± 15	123 ± 16	124 ± 10	122 ± 13
Diastolic (mmHg)	71 ± 9	69 ± 10	73 ± 9	71 ± 9
Pulse pressure (mmHg)	54 ± 12	54 ± 11	51 ± 10	51 ± 10
Mean arterial pressure (mmHg)	89 ± 10	87 ± 11	90 ± 8	88 ± 9
Heart rate (beats/minute)	71 ± 11	69 ± 10	71 ± 9	71 ± 10
Nocturnal dipper (%)	19 (42)	25 (53)	29 (56)	32 (62)

Table 5-33. 24-hour ambulatory blood pressure data at week 0 and week 40.

Data are mean ± standard deviation or frequency (percentage). Analysed using repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups.

	Placebo n=50		Sevelamer n=54	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Lumbar spine:				
Bone mineral density (g/cm^2)	1.14 ± 0.20	1.14 ± 0.20	1.06 ± 0.16	1.06 ± 0.16
T-score	0.61 ± 1.77	0.62 ± 1.75	-0.16 ± 1.34	-0.19 ± 1.36
Z-score	1.51 ± 1.89	1.55 ± 1.87	0.60 ± 1.40	0.63 ± 1.40
Both hips:				
Bone mineral density (g/cm^2)	1.02 ± 0.13	1.03 ± 0.18	1.00 ± 0.14	1.00 ± 0.15
T-score	0.24 ± 0.91	0.20 ± 0.93	-0.08 ± 0.94	-0.14 ± 0.96
Z-score	0.89 ± 0.99	0.87 ± 1.01	0.50 ± 1.01	0.47 ± 1.01

Table 5-34. Bone density data at week 0 and week 40 for placebo and treatment groups. Data are mean ± standard deviation. Analysed using repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups.

A mean of 79 ± 21% of tablets was taken during the open-label run-in phase. There was no significant difference in compliance between groups during the blinded treatment phase (sevelamer group 80 ± 20% vs. placebo group 81 ± 30%, P=0.9). Overall, 61 patients (56%) returned <20% of prescribed medication (Figure 5-4). A subgroup analysis that included only these highly compliant subjects revealed significant decreases in FGF-23 (-8.9 ± 19.8 vs. 1.0 ± 17.7 pg/ml, P<0.05) and urinary phosphate concentration (-2.4 ± 4.3 vs. 0.6 ± 3.6 mmol/l, P<0.01) over 40 weeks in those receiving sevelamer compared to placebo (Table 5-35) but no differences in serum phosphate, PTH, klotho or vitamin D. There were also no

differences in BP, indices of arterial stiffness, LV mass or measures of LV systolic or diastolic function (Table 5-36).

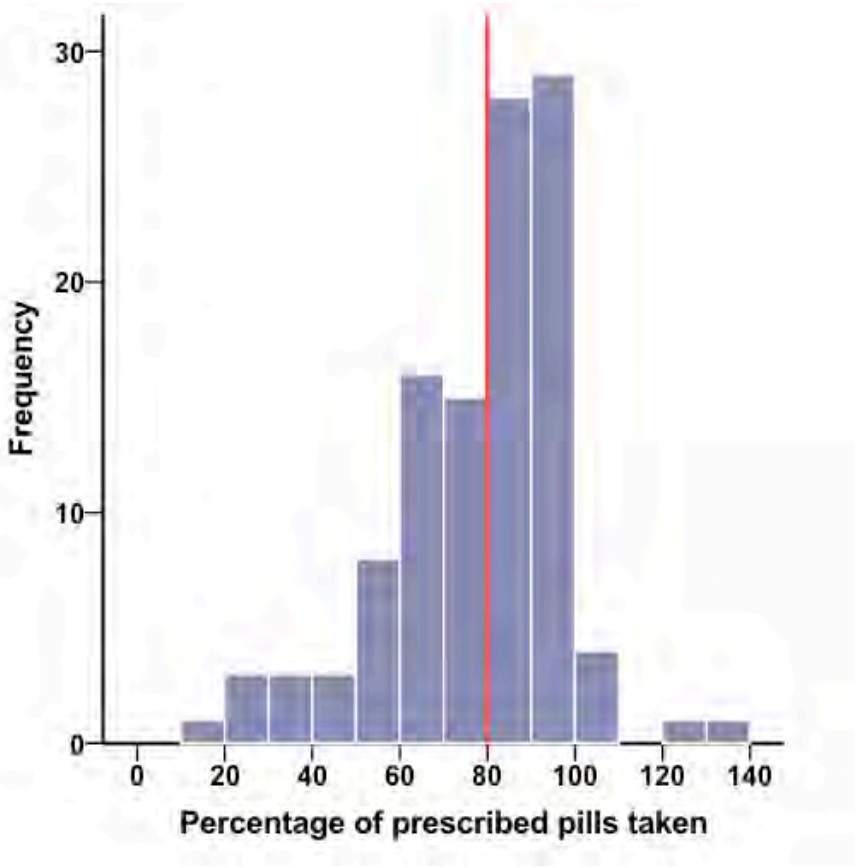

Figure 5-4. Compliance displayed as percentage of prescribed pills taken.

	Placebo n=31		Sevelamer n=30	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Biochemical:				
Phosphate (mmol/l)	1.04 ± 0.20	1.06 ± 0.17	1.03 ± 0.15	0.98 ± 0.24
FGF-23 (pg/ml)	75 ± 35	76 ± 29	76 ± 31	67 ± 28*
Klotho (pg/ml)	888 ± 278	921 ± 355	1060 ± 617	1044 ± 667
1,25-dihydroxyvitamin D (pmol/l)	67 ± 27	65 ± 27	73 ± 31	68 ± 28
25-hydroxyvitamin D (nmol/l)	60 ± 36	60 ± 34	65 ± 31	62 ± 32
UPi (mmol/l)	10.4 ± 3.4	11.0 ± 4.6	12.4 ± 4.6	10.0 ± 3.0†
FEPi (%)	21.4 (13.5–34.0)	16.3 (11.8–23.2)	23.8 (15.0–33.8)	17.1 (10.8–29.8)
Total cholesterol (mmol/l)	4.7 ± 1.1	4.3 ± 1.3	4.8 ± 1.1	4.2 ± 1.3
Haemodynamics:				
Brachial systolic BP (mmHg)	125 ± 17	121 ± 18	126 ± 15	122 ± 13
Brachial diastolic BP (mmHg)	69 ± 11	66 ± 11	70 ± 10	67 ± 8
Brachial MAP (mmHg)	89 ± 12	85 ± 12	89 ± 11	86 ± 9
Heart rate (beats/min)	64 ± 11	63 ± 10	62 ± 9	62 ± 9
Central systolic BP (mmHg)	114 ± 17	111 ± 18	116 ± 15	113 ± 14
Central diastolic BP (mmHg)	70 ± 11	66 ± 11	71 ± 10	68 ± 8
Central MAP (mmHg)	89 ± 12	85 ± 12	89 ± 11	86 ± 9

Table 5-35. Biochemical and haemodynamic data for patients with ≥80% compliance. *P<0.05, †P<0.01. Data are mean ± standard deviation or median (interquartile range). Analysed using repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups. FGF-23, fibroblast growth factor 23, UPi, urinary phosphate concentration; FEPi, urinary fractional excretion of phosphate; BP, blood pressure; MAP, mean arterial pressure.

	Placebo n=31		Sevelamer n=30	
	Week 0	**Week 40**	**Week 0**	**Week 40**
Cardiac structure and function:				
Left ventricular mass (g)	104 ± 33	102 ± 33	94 ± 21	94 ± 19
LVEF (%)	75 ± 7	76 ± 7	74 ± 7	75 ± 7
Mean TDi s' (m/s)	9.0 ± 1.2	8.9 ± 1.2	9.1 ± 1.8	9.2 ± 1.7
Transmitral E/A	0.98 (0.81–1.13)	0.99 (0.81–1.13)	0.93 (0.79–1.10)	0.94 (0.70–1.19)
Mean TDi e' (m/s)	9.5 ± 3.6	9.4 ± 3.1	8.9 ± 3.0	8.9 ± 3.0
E/mean e'	7.2 (6.2–9.0)	7.5 (6.4–9.4)	8.4 (6.4–9.6)	8.3 (6.1–9.5)
Arterial stiffness indices:				
Pulse wave velocity (m/s)	9.0 (7.5–11.7)	8.9 (7.9–10.6)	8.4 (7.2–10.3)	8.8 (7.2–10.8)
AIx	24.7 ± 10.5	25.8 ± 12.6	28.0 ± 11.4	28.8 ± 10.5
AIx_{75}	19.1 ± 11.4	19.2 ± 12.4	21.8 ± 11.1	22.3 ± 10.1
Aortic distensibility ($x10^{-3}$ $mmHg^{-1}$):				
Ascending	2.38 ± 1.98	2.38 ± 1.63	2.58 ± 1.83	2.81 ± 2.18
Proximal descending	3.10 ± 1.74	3.08 ± 1.44	3.27 ± 1.65	3.57 ± 1.62
Distal descending	3.99 ± 2.24	3.95 ± 1.73	4.62 ± 2.68	4.49 ± 2.30

Table 5-36. Cardiac structure and function data and arterial stiffness data for patients with ≥80% compliance.

Data are mean ± standard deviation or median (interquartile range). Analysed using repeated measures analysis of variance with time point (week 0, week 40) as the within-subjects factor and group (placebo, sevelamer) as the between-subjects factor, testing the difference in change over time between groups. LVEF, left ventricular ejection fraction; TDi, tissue Doppler; AIx, augmentation index; AIx_{75}, augmentation index adjusted to heart rate of 75 bpm.

5.3.6 Comparison with Spironolactone Cohort

Table 5-37 compares the baseline demographic and biochemical characteristics of the patients from the current sevelamer study with those of participants from the spironolactone study described previously (Edwards *et al.*, 2009). There were significantly fewer patients with a smoking history in the spironolactone study. Furthermore, the spironolactone cohort had lower body mass index and hsCRP at baseline, although the mean serum phosphate level was higher. Table 5-38 shows a comparison of baseline haemodynamic data, arterial stiffness indices and left ventricular mass data between the study cohorts. Brachial diastolic BP, brachial mean arterial pressure and central diastolic BP were higher in the spironolactone cohort, although 24-hour systolic BP was slightly lower. Patients in the spironolactone study had stiffer arteries at baseline, with a higher median PWV (8.9 (7.3–11.2) vs. 7.9 (7.2–9.3) m/s, $P<0.01$). Left ventricular mass was also greater at baseline (114 ± 30 vs. 101 ± 30 g, $P<0.01$).

	Sevelamer study n=120	Spironolactone study n=117
Age (years)	55 ± 14	54 ± 12
Male gender	65 (54)	71 (61)
Body mass index (kg/m^2)	29.0 ± 5.5	27.2 ± 4.2*
Smoking history	64 (54)	17 (15)†
Mean eGFR (ml/min/1.73m^2)	50 ± 13	50 ± 17
Phosphate (mmol/l)	1.03 ± 0.16	1.19 ± 0.22†
Total cholesterol (mmol/l)	4.92 ± 1.22	4.82 ± 1.05
ACR (mg/mmol)	6.3 (0.8–48.3)	8.2 (1.5–47.9)
Haemoglobin (g/dl)	13.3 ± 1.5	13.4 ± 1.6
hsCRP (µg/ml)	2.17 (1.09–6.35)	1.89 (0.57–4.31)*
Number of antihypertensives	1.7 ± 1.2	2.0 ± 1.0*

Table 5-37. Comparison of baseline demographic and biochemical characteristics of sevelamer and spironolactone study populations.

*P<0.05; †P<0.0005. Data are mean ± standard deviation, median (interquartile range) or frequency (percentage). eGFR, estimated glomerular filtration rate; ACR, albumin: creatinine ratio; hsCRP, high sensitive C-reactive protein. Analysed using independent samples t-test or χ^2.

	Sevelamer study n=120	Spironolactone study n=117
Haemodynamics:		
Brachial systolic BP (mmHg)	128 ± 17	125 ± 12
Brachial diastolic BP (mmHg)	71 ± 11	77 ± 10*
Brachial pulse pressure (mmHg)	57 ± 17	54 ± 12
Brachial mean arterial pressure (mmHg)	91 ± 12	95 ± 11†
Central systolic BP (mmHg)	117 ± 17	121 ± 17
Central diastolic BP (mmHg)	72 ± 11	78 ± 10*
Central pulse pressure (mmHg)	45 ± 16	43 ± 12
Central mean arterial pressure (mmHg)	91 ± 12	92 ± 11
24-hour average systolic BP (mmHg)	124 ± 12	121 ± 11‡
24-hour average diastolic BP (mmHg)	71 ± 9	73 ± 8
24-hour average pulse pressure (mmHg)	53 ± 11	48 ± 9*
24-hour mean arterial pressure (mmHg)	89 ± 9	89 ± 8
Nocturnal dipper	58 (50)	54 (47)
Arterial stiffness and left ventricular mass indices:		
AIx_{75}	20 ± 12	25 ± 10*
PWV (m/s)	7.9 (7.2–9.3)	8.9 (7.3–11.2)†
LV mass (g)	101 ± 30	114 ± 30†
LV mass index (g/m^2)	52 ± 13	60 ± 12*

Table 5-38. Comparison of baseline haemodynamic indices, arterial stiffness indices and left ventricular mass between sevelamer and spironolactone study populations.

*P<0.0005; †P<0.01; ‡P<0.05. Data are mean ± standard deviation, median (interquartile range) or frequency (percentage). BP, blood pressure; AIx_{75}, augmentation index adjusted to heart rate of 75bpm; PWV, pulse wave velocity; LV, left ventricular. Analysed using independent samples t-test or χ^2.

	Sevelamer study n=53	Spironolactone study n=55
Change in LV mass (g)*	-1.0 ± 7.2	-14.0 ± 12.7†
Change in LV mass index (g/m^2)	-0.1 ± 4.1	-6.5 ± 5.6†
Change in PWV (m/s)	0.0 ± 1.1	-0.8 ± 1.0†
Change in AIx_{75}	-0.7 ± 8.4	-4.9 ± 6.1‡
Change in brachial systolic BP (mmHg)	-5 ± 15	-5 ± 8

Table 5-39. Comparison of changes observed in treatment groups between sevelamer and spironolactone study populations.

*Data available for 45 subjects from sevelamer study and 47 subjects from spironolactone study; †P<0.0005; ‡P<0.01. LV, left ventricular; BP, blood pressure; PWV, pulse wave velocity, AIx_{75}, augmentation index adjusted to heart rate of 75bpm. Analysed using independent samples t-test.

Table 5-39 compares the changes observed in the main endpoints after 40 weeks of active treatment in each study. Treatment with spironolactone resulted in significant decreases in LV mass, PWV and AIx_{75} when compared to treatment with sevelamer.

5.3.7 Adverse Effects

During the open-label run-in phase nine subjects (8%) developed hypophosphataemia (phosphate <0.8 mmol/l) necessitating a halving of the dose of sevelamer to 800 mg with meals. One subject was withdrawn during run-in due to persistent hypophosphataemia despite dose reduction. Nine patients (8%) experienced dyspepsia, six developed constipation (5%) and two (2%) complained of nausea and vomiting. In eight patients a dose-reduction was required to alleviate symptoms. Two patients were withdrawn due to persistence of adverse effects

despite dose reduction (Figure 5-2). Following randomisation a further seven subjects developed hypophosphataemia (three [6%] in the placebo group, four [7%] in the treatment group, P=0.7) requiring a dose reduction to 800 mg three times daily or equivalent. Three subjects from the treatment group and one from the placebo group were withdrawn from the treatment phase due to persistent hypophosphataemia. Twelve patients were hospitalised during their time in the study for unrelated medical conditions (two during run-in, six from the treatment group and four from the placebo group).

5.3.8 Subject Withdrawal

The commonest reason for withdrawal from the study was withdrawal of consent (Table 5-40). Four participants were intolerant to medication, either due to persistent adverse effects despite a dose-reduction (three subjects) or finding the tablets too large and unpalatable (one participant). Only 5 subjects were withdrawn due to persistent hypophosphataemia.

Run-in phase	
Hypophosphataemia	1
Poor compliance with study	3
Intolerance to medication	2
Other	5
Treatment phase	
Hypophosphataemia	4
Poor compliance with study	0
Intolerance to medication	2
Other	4

Table 5-40. Subject withdrawal.

5.4 Discussion

In patients with stage 3 non-diabetic CKD, 40 weeks of treatment with sevelamer carbonate resulted in no change in LV mass compared with placebo. Furthermore, there were no significant effects on measures of arterial stiffness and wave reflection or LV systolic and diastolic function. Although a small reduction in brachial systolic BP was observed after 4 weeks of treatment with sevelamer in the open-label uncontrolled phase of the study, this did not persist in the double blind, placebo-controlled phase. Despite repeated reminders and suggested methods to maximise compliance, adherence to medication was low in both phases of the study with only 56% of subjects taking ≥80% of their study medication. Furthermore, several

patients withdrew from the study because of difficulty with the frequency and tolerability of the study agents.

Chronic kidney disease is the commonest condition associated with deranged phosphate homeostasis. Abnormal phosphate handling, coupled with phosphate-rich Western diets, may contribute to the elevated cardiovascular risk observed in this patient group (Ferro *et al.*, 2009). Observational studies have also linked phosphate to an increased risk of cardiovascular events (Tonelli *et al.*, 2005) and development of symptomatic heart failure in the general population (Dhingra *et al.*, 2010; Dhingra *et al.*, 2007), together with a higher prevalence of vascular calcification (Adeney *et al.*, 2009; Foley *et al.*, 2009b; Tuttle and Short, 2009). Cellular mechanisms of phosphate cardiovascular toxicity are unclear, but post-prandial increases in serum phosphate seen following acute phosphate loading are associated with transient endothelial dysfunction (Shuto *et al.*, 2009). Studies *in vitro* have demonstrated that vascular smooth muscle cells undergo osteogenic transformation in the presence of hyperphosphataemia, with upregulation of genes that promote matrix mineralization and vascular calcium deposition (Jono *et al.*, 2000). The presence of vascular medial calcification is associated with increased arterial stiffness (Guerin *et al.*, 2000; McEniery *et al.*, 2009; Raggi *et al.*, 2007), which appears to be a principal driver of abnormalities in cardiac structure and function (Chue *et al.*, 2010). Relationships have been demonstrated between serum phosphate and increased arterial stiffness (Ix *et al.*, 2009) as well as increased LV mass (Chue *et al.*, 2012; Dhingra *et al.*, 2010) and LVH (Foley *et al.*, 2009a), which are all independent predictors of adverse cardiovascular outcome and may be responsible for the excess cardiovascular

morbidity and mortality observed in CKD (Chue *et al.*, 2010). Furthermore, evidence from haemodialysis patients suggests lowering of serum phosphate may reduce LV mass (Achinger and Ayus, 2006). These observations led to the hypothesis that in early stage CKD, when serum phosphate levels are normal, reducing phosphate exposure using a non-calcium-based phosphate binder would have beneficial effects on both arterial stiffness and LV mass (Ferro *et al.*, 2009).

The absence of improvement in cardiac and vascular parameters after 40 weeks of treatment with sevelamer would suggest our primary hypothesis has been refuted. Before this concept is discarded, however, a number of issues must be considered. Firstly, we chose subjects with stage 3 CKD because at this level of kidney function, cardiovascular risk is increased despite normal serum phosphate levels, and serum FGF-23 and urinary phosphate excretion are elevated (Isakova *et al.*, 2011b). This was indeed the case, although mean levels of klotho, 1,25-dihydroxyvitamin D and PTH levels were normal indicating that most subjects had only mildly impaired renal function and therefore only a minimally impaired capacity to excrete a phosphate load. Furthermore, at this level of kidney function it has recently been suggested that use of phosphate binders may cause upregulation of the gastrointestinal NPT-2b sodium-phosphate co-transporters or enhancement of passive paracellular diffusion of phosphate ions (Block *et al.*, 2012); this compensatory response, combined with observed reductions in urinary phosphate excretion, may mean that overall "phosphate flux" remains unchanged (Berndt *et al.*, 2007). Given the inaccuracy of the MDRD equation in near-normal renal function it is possible that a significant number of participants had true GFRs >60 ml/min/1.73m^2 (Levey *et al.*, 1999).

Despite this, 86% of patients enrolled had histological or radiological evidence of kidney disease.

In this study patients with well-controlled BP were selected and almost all had baseline LV mass within normal limits. This was to determine whether intervention with a phosphate binder would have beneficial effects on cardiovascular structure and function over and above optimal conventional treatment, a concept validated in previous research with spironolactone (Edwards *et al.*, 2009). This could have potentially masked any benefits of reducing phosphate absorption. There is increasing evidence highlighting the prognostic value of LV mass as a continuous variable, however, even when values lie within normal limits (Schillaci *et al.*, 2000). Furthermore, over 70% of incident dialysis patients have established LVH, indicating that structural heart disease develops in the earlier stages of CKD (Foley *et al.*, 1995). Multiple indices of cardiovascular structure and function were examined, which remained unchanged during the controlled phase of the study. Cardiovascular magnetic resonance imaging was also utilised, which is a highly sensitive technique for detecting changes in LV mass and which permits a smaller sample size when compared to echocardiography (Myerson *et al.*, 2002). The possibility that the cardiovascular effects of sevelamer were too small to detect with a sample size of 60 in each group cannot be excluded. The power calculations used in this study were based on changes in LV mass observed in a previous trial of the same size, duration and design with similar inclusion and exclusion criteria but utilising spironolactone as the treatment agent (Edwards *et al.*, 2009). The calculation of required sample size used in the current study was conservative, aiming for >90% power. Although LV

mass data were only available for 45 subjects in each arm of the study, detection of a change in LV mass of 8 grams with a standard deviation of the change in LV mass of 12 grams was possible with 88% power. The rate of withdrawal from the study was higher than the 10% accounted for when calculating sample size but compares favourably to other studies of phosphate binders in CKD (Block *et al.*, 2012; Qunibi *et al.*, 2011). Similarly, 40 weeks was chosen for the study duration based on the previous spironolactone trial, which demonstrated notable changes in LV mass, arterial stiffness and LV function over this time period (Edwards *et al.*, 2009). Furthermore, treatment with allopurinol over a 40-week period was also sufficient to reduce LV mass and improve endothelial function in patients with CKD (Kao *et al.*, 2011). A longer treatment duration with sevelamer may be required to exert detectable effects on cardiovascular structure and function, particularly with normal BP and LV mass at baseline. Our findings parallel those of the recently published Paricalcitol Capsule Benefits in Renal Failure-Induced Cardiac Morbidity (PRIMO) study in which 227 patients with stage 3 and 4 CKD (eGFR 15–59 ml/min/1.73m^2) failed to show any reductions in LV mass after 48 weeks of treatment with paricalcitol, a once daily vitamin D analogue, compared to placebo (Thadhani *et al.*, 2012). This was despite a lower mean eGFR at baseline compared to our cohort and inclusion of patients with mild to moderate LVH at study entry.

Adherence to both active treatment and placebo was low with 56% of subjects taking ≥80% of prescribed study medication. This is likely to be an underestimate of the actual tablets taken given the known limitations of pill counts (Lee *et al.*, 2007). This low level of compliance occurred despite regular encouragement and monitoring

throughout the study period, and may have reduced the efficacy of treatment; this may also account for the modest effects on urinary phosphate excretion, cholesterol and urate levels. Several participants were unable to maintain a regimen of two tablets three times a day and this contributed significantly to reasons given by patients when withdrawing from the study. A subgroup analysis of the 61 subjects with ≥80% compliance indicated some biochemical changes in those taking sevelamer compared to placebo, with significant reductions in serum FGF-23 and urinary phosphate excretion. This is consistent with previous studies of CKD patients with normal serum phosphate levels, in which sevelamer reduced urinary phosphate excretion and serum FGF-23 without altering serum phosphate (Block *et al.*, 2012; Oliveira *et al.*, 2010). In our compliant patients, no changes in LV mass or arterial stiffness were observed between groups. Such sub-group analyses are likely underpowered to detect any differences in these parameters. Effective testing of our original hypothesis may thus require the introduction of a well-tolerated, once-daily agent that significantly lowers gastrointestinal phosphate absorption and is associated with improved compliance. An analogous situation may have occurred with cholesterol lowering; early studies with cholesterol lowering agents failed to show significant benefits (Muldoon *et al.*, 1990) and the concept that cholesterol lowering was beneficial was not supported until statins were introduced.

Despite excluding patients with diabetes mellitus and including a study population with early stage CKD and no history of treatment with calcium-based phosphate binders or vitamin D analogues, 48% of subjects had evidence of aortic calcification. This is higher than the reported prevalence in non-diabetic stage 3 CKD (Russo *et*

al., 2004). The use of more sensitive techniques such as computed tomography would have increased the proportion of patients with detectable calcification. It is possible that this high prevalence would have made it very difficult, if not impossible, to lower arterial stiffness and therefore LV mass in these patients over 40 weeks. Treatment with sevelamer over twelve months has been shown to reduce progression of arterial calcification in haemodialysis patients but not cause regression (Chertow *et al.*, 2002; Kakuta *et al.*, 2011). In a subgroup analysis that only included subjects without aortic calcification there were no differences in arterial stiffness or LV mass between groups at 40 weeks, although only 25 patients were included in each group.

It remains possible that the association between phosphate and adverse cardiovascular outcome is mediated by other pathophysiological processes unrelated to arterial stiffness and structural heart disease. Serum phosphate correlates with angiographic severity of coronary artery disease (Narang *et al.*, 1997), and may contribute to changes in plaque structure (Ellam and Chico, 2012). Such changes would not have been detected by any of the techniques used in our study. Finally, it is possible that the relationship between poor outcome and serum phosphate is not causative but reflects an association observed between worse outcomes and declining renal function. Although studies have attempted to mathematically correct for renal function, most have done so by adjusting for eGFR. The accuracy of such calculations is reduced at eGFR values close to 60 ml/min/1.73m^2 when compared to true GFR measurements.

5.4.1 Conclusion

In summary, in patients with stage 3 CKD the use of sevelamer carbonate for 40 weeks was not associated with any significant changes in LV mass, arterial stiffness, or any parameters of LV systolic and diastolic function. Adherence to the study medication was low, however, and more rigorous testing of this hypothesis may be achieved with the introduction of a well-tolerated once-daily drug regimen that more effectively reduces cardiovascular phosphate exposure.

6 SUMMARY AND FUTURE WORK

6.1 Summary of Principal Findings

In this volume of work it was shown that in 225 patients with early stage CKD (mean eGFR 43 ± 19 ml/min/1.73m^2), serum phosphate was an independent predictor of GFR decline, with a 1 mmol/l increase in serum phosphate associated with a 0.34 ml/min/1.73m^2/month steeper slope of renal function decline (P=0.01) (Chue *et al.*, 2011a). This finding was supported by the result that serum phosphate was significantly higher amongst the subset of patients reaching the combined endpoint of commencement of dialysis or ≥25% decline in eGFR, and was independently associated with this combined endpoint following adjustment for multiple variables known to influence decline in renal function. These results were in keeping with those of an earlier study of patients with more advanced CKD (mean eGFR 13 ± 5.4 ml/min/1.73m^2), and suggest an alternative mechanism through which phosphate may increase cardiovascular risk.

In the second cross-sectional study, serum phosphate was shown to be independently associated with LV mass in 119 males and 89 females with stage 2–4 non-diabetic CKD and normal LV mass and well-controlled BP at baseline (Chue *et al.*, 2012). The majority of subjects in this study had serum phosphate levels within the normal reference range. Similar associations between serum phosphate and increased LV mass have been demonstrated in ESKD (Strozecki *et al.*, 2001) but also in individuals with normal cardiac and renal function (Dhingra *et al.*, 2010; Foley

et al., 2009a). The results of this study add support to the original hypothesis that lowering serum phosphate in patients with early CKD could represent a novel way of reducing LV mass and thus cardiovascular risk. The notion that LV mass should be considered a continuous variable, rather than according to whether LVH is present or absent, was suggested following the demonstration that increasing LV mass has a graded relationship with cardiovascular risk (Schillaci *et al.*, 2000). The question raised by this study was whether a reduction in phosphate exposure in early CKD would be linked to a reduction in LV mass, which formed the premise for the third study described in section 5.

Although an association was not demonstrated between serum phosphate and indices of arterial stiffness, or between arterial stiffness and LV mass, the lack of such relationships in this observational study does not exclude the possibility that phosphate-induced changes in LV mass are mediated by increased arterial stiffness and increased afterload. The use of subjects with normal LV mass, normal serum phosphate and only mildly elevated PWV would increase the difficulty of detecting such associations. Previous studies have demonstrated an association between serum phosphate and vascular calcification (Foley *et al.*, 2009b; Goodman *et al.*, 2000) as well as serum phosphate and arterial stiffness (Ix *et al.*, 2009; Kendrick *et al.*, 2010; Toussaint *et al.*, 2008). Several techniques of arterial stiffness measurement were therefore adopted in the final study.

The double-blind, randomised, placebo-controlled trial of 120 patients with stage 3 non-diabetic CKD did not demonstrate a significant reduction in LV mass or arterial

stiffness after 40 weeks of treatment with the oral phosphate binder sevelamer carbonate when compared to placebo (Chue *et al.*, 2013). There were also no significant changes in indices of systolic or diastolic LV function, aortic distensibility or bone density over this period. Furthermore, there were no detectable changes in any biochemical indices of phosphate metabolism, including serum phosphate, serum FGF-23, klotho, vitamin D and 24-hour urinary phosphate excretion. Compliance with trial medication during the 40-week study period was low, with only 56% of subjects taking ≥80% of the prescribed study drug. This level of compliance occurred despite repeated reminders to take medication, pill counting, suggested methods to improve compliance and monitoring of adverse effects. A subgroup analysis of the 61 patients with ≥80% compliance revealed a significant reduction in serum FGF-23 and urinary phosphate excretion in those taking sevelamer compared to placebo, suggesting some degree of modification of phosphate handling, although no difference in LV mass or arterial stiffness was detected due to the small subgroup sample size.

6.2 Future Directions

6.2.1 Drug Efficacy and Acceptability

There is clear evidence that medication compliance decreases with increasing dose frequency (Claxton *et al.*, 2001). All currently available phosphate binders have a multiple daily dosing regimen; the absence of a phosphate binder with a low pill-burden undoubtedly has adverse effects upon compliance, and in turn efficacy. Following initial recruitment and randomisation of the first few patients into the study,

sachets providing 2.4 g of sevelamer carbonate in powder form (equivalent to 1600 mg in tablet form) were introduced. Although still requiring three-times-daily dosing with meals, the administration of a drug in suspension form over a tablet formulation has the potential benefit of improved acceptance amongst patients, particularly those that find large tablets unpalatable. An open-label randomised crossover study, however, demonstrated equivalent short-term rates of compliance between sevelamer hydrochloride tablets and sevelamer carbonate sachets (84% vs. 86%) over two 4-week periods; control of hyperphosphataemia was also equivalent (Fan *et al.*, 2009). The lack of a clear improvement in compliance over the traditional tablet form highlights the need for a treatment that effectively lowers phosphate, or the more sensitive FGF-23, whilst providing a more acceptable dosing regimen. Until such a treatment is introduced, compliance is likely to remain a shortcoming of phosphate-lowering therapy.

A novel salivary phosphate-binding chewing gum has been shown to be efficacious in reducing serum phosphate when used twice daily in conjunction with an oral phosphate binder taken with meals in a small number of patients with ESKD (Savica *et al.*, 2009). It is unknown whether this treatment is effective at lowering serum phosphate concentrations when used as sole therapy, and also whether there is efficacy in the early CKD population.

A number of studies have examined the phosphate-lowering efficacy of niacin (nicotinic acid) and its related compounds, including its metabolite nicotinamide (also known as niacinamide) (Cheng *et al.*, 2008; Maccubbin *et al.*, 2010; Muller *et al.*,

2007; Sampathkumar *et al.*, 2006; Takahashi *et al.*, 2004). Nicotinamide reduces gastrointestinal phosphate absorption by inhibiting the gastrointestinal sodium-phosphate co-transporter (Eto *et al.*, 2005). This alternative mechanism of action allows for an extended-release form of the drug, which has the advantage of once-daily dosing. Despite promising results of effective phosphate lowering, even in patients with early stage CKD (Maccubbin *et al.*, 2010), these compounds are limited by their side effect profile, which includes hepatotoxicity, thrombocytopenia and flushing, which may limit their regular use (Berns, 2008). Extended release niacin is often given alongside the selective prostaglandin D2 receptor subtype I inhibitor laropiprant, which effectively halves the incidence of niacin-induced flushing (Maccubbin *et al.*, 2008).

It is possible that novel compounds targeting the FGF-23 pathway, including the non-specific FGF-23 receptor antagonist PD173074, which has been shown to attenuate development of LVH in animal models of CKD (Faul *et al.*, 2011), may be effective in improving structural and functional cardiovascular parameters in early stage CKD. Although a monoclonal antibody against FGF-23 was shown to improve many biochemical parameters of CKD-MBD in a rat model and effectively treat secondary hyperparathyroidism, the resulting hyperphosphataemia and vascular calcification resulted in excess mortality (Shalhoub *et al.*, 2012). It is possible that a more targeted approach aiming to antagonise the specific FGF-23 receptor responsible for the induction of LVH, rather than blanket inhibition of all FGF-23 activity, may prove to be more effective in the CKD setting. Such compounds require further careful evaluation to determine whether FGF-23 antagonism is truly beneficial or whether

such treatments suppress an important compensatory mechanism designed to protect against the harmful effects of hyperphosphataemia.

6.2.2 Study Population

The population studied in this body of work were predominantly patients with stage 3 CKD (mean eGFR 50 ml/min/1.73m^2) and normal LV mass and BP at baseline. Furthermore, these individuals had relatively preserved phosphate handling, and despite elevated urinary phosphate excretion and FGF-23 levels at study entry, mean levels of klotho, 1,25-dihydroxyvitamin D and PTH were within normal limits. Despite the above, and exclusion of patients with diabetes mellitus, 48% of participants had calcification of the abdominal aortic wall, detectable using a relatively insensitive imaging modality.

It is possible that repeating a study of sevelamer versus placebo in patients with more advanced CKD (for example stage 3b [eGFR 30–44 ml/min/1.73m^2] or stage 4 CKD [eGFR 15–29 ml/min/1.73m^2]), and therefore a greater derangement in phosphate handling and potentially higher LV mass at baseline, would yield significant changes in the endpoints studied. However, a number of issues relating to the study of patients with more advanced kidney disease should be considered. Firstly, a greater proportion of these patients will have secondary hyperparathyroidism and also hyperphosphataemia, necessitating treatment with vitamin D analogues or phosphate binders according to the KDIGO guidelines (KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD), 2009).

Use of these therapies introduces a source of confounding, potentially limits the number of patients suitable for such a study, and may require cessation of recommended therapy. Secondly, more advanced CKD is likely to be associated with a higher prevalence of vascular calcification (Kramer *et al.*, 2005) and established aortopathy, which may limit the ability of any treatment to reduce aortic stiffness. Thirdly, a high pill burden and low compliance will still remain as limiting factors. An alternative approach would be to replicate the inclusion criterion of the PRIMO study and only include subjects with LVH at baseline (Thadhani *et al.*, 2012). Given the low prevalence of LVH in the population currently studied, it is possible that many potential subjects will undergo screening transthoracic echocardiography or CMR but ultimately be excluded on the basis of normal LV mass. It should also be noted that the PRIMO study did not demonstrate any reduction in LV mass or improvement in LV diastolic function after 48 weeks of treatment with the vitamin D analogue paricalcitol.

6.2.3 Future Analyses

In addition to the analyses performed in this body of work, specialised tagging sequences have also been acquired during CMR in order to assess LV systolic and diastolic function with greater detail and sensitivity. Dynamic tissue-tagging MRI magnetic resonance imaging allows direct non-invasive assessment of regional systolic myocardial shortening and has been previously validated (Yeon *et al.*, 2001). Spatial modulation of magnetization was used to generate a uniform grid pattern with 8mm tag separation on the left ventricular myocardium at three short axis sections (basal, equatorial and apex) and the horizontal long axis image using a fast field

echo multi-shot sequence (temporal resolution 40–50 ms, repetition time 3.9 ms, echo time 4.4 ms, voxel size 1.8/1.3/6.0 mm^3, flip angle 14º, tag grid angle 45º with slice thickness 6 mm and a minimum number of 15 phases per cardiac cycle) with prospective ECG gating as previously described (Young *et al.*, 1994). The myocardial grid was followed through systole for deformation caused by ventricular contraction. Analysis will be performed offline (Argus Software, Siemens, Erlangen, Germany) by blinded observers for left ventricular longitudinal shortening and apical and basal rotation as previously described (Young *et al.*, 1994). Comparisons between healthy controls and CKD patients are planned, along with further analyses to determine any changes relating to sevelamer treatment compared to placebo over 40 weeks.

Further cross-sectional analyses of this patient cohort are also planned, in particular examination of serum levels of soluble klotho and their associations with LV mass, arterial stiffness and LV systolic and diastolic functional indices for future hypothesis generation.

6.2.4 The Next Study

Taking the above into consideration, the proposed study to further test our hypothesis would be a prospective, randomised, double-blind placebo-controlled trial to determine the effects of modification of phosphate homeostasis on the primary endpoint of LV mass in patients with stage 3b and 4 CKD. A minimum of 120 patients with stage 3b or stage 4 CKD with established LVH at baseline would be required. These patients may be recruited from secondary care as in the previously

reported study, or alternatively from a primary care setting. The presence of increased LV mass at study initiation, which could be determined through screening echocardiography, would improve the likelihood of detecting an improvement in structural heart disease related to therapy. The presence of significantly abnormal phosphate homeostasis could be confirmed through measurement of serum FGF-23, which is the most sensitive marker of impaired renal phosphate excretion. An FGF-23 level of 100 pg/ml or greater (twice the upper limit of the normal range) would suggest abnormal phosphate handling. Proposed inclusion and exclusion criteria are listed in Table 6-1. Cardiac magnetic resonance imaging would again be employed to determine the primary endpoint of LV mass, as well as LV volumes and function. This would be complemented with transthoracic echocardiography for the determination of LV systolic and diastolic function as secondary endpoints. The non-invasive assessment of arterial stiffness, namely measurement of carotid-femoral PWV and AIx using applanation tonometry and the SphygmoCor system, would again be used. As previous studies of alternative therapies have demonstrated changes in LV mass over a 40-week period in patients with CKD, this time frame would be considered to be appropriate. The obvious phosphate-modifying treatment of choice, however, is unclear at present. The use of alternative, non-calcium based phosphate binders such as lanthanum would be associated with a similar pill burden to sevelamer (and therefore similar issues with compliance) with a comparable adverse effect profile. Agents that modify the FGF-23 axis, such as antibodies and antagonists against FGF-23 and its receptor, require further testing in animal models to identify an appropriate target for treatment that is not associated with hyperphosphataemia and excess mortality. Such agents will also require testing in

humans before trial use and are therefore currently beyond our reach. This leaves the use of an extended-release, once daily niacin-based compound in combination with laropiprant to minimise the adverse effect of flushing. Regular monitoring (4-weekly at initiation) of liver function, creatine kinase, serum glucose level and platelets would be required. Despite the once-daily dosing regimen, a randomised trial assessing the lipid-lowering effect of extended-release niacin compared to placebo over a 24-week treatment period was still associated with a 30% withdrawal rate before trial completion (Maccubbin *et al.*, 2008). This would need to be considered when performing a power calculation, and such a study may require a larger sample size than has been used previously.

Inclusion Criteria
Age 18–80 years
Chronic kidney disease stage 3b or 4 (estimated glomerular filtration rate 15–44 ml/min/1.73m^2)
Total cholesterol <5.5 mmol/l
LVH on echocardiography
Serum FGF-23 ≥100pg/ml
Exclusion Criteria
Existing or previous treatment within the past year with a phosphate binder or vitamin D analogue
Hyperphosphataemia (serum phosphate >1.8 mmol/l)
Hypophosphataemia (serum phosphate <0.8 mmol/l)
Existing or previous treatment within the past 6 months with a niacin-based compound
Aspartate transaminase or alanine transaminase >1.5x upper limit of normal
Creatine kinase >2x upper limit of normal
Thrombocytopenia (platelets <150 x10^9/l)
Diabetes mellitus
Pregnancy
Women of child-bearing age not on contraception

Table 6-1. Proposed inclusion and exclusion criteria.

6.2.5 Other Studies in Progress

The IMPROVE-CKD trial (IMpact of Phosphate Reduction On Vascular Endpoints in Chronic Kidney Disease) is a randomised, double-blind, placebo-controlled trial of 488 patients with stage 3b–4 CKD designed to assess the effect of phosphate lowering using lanthanum carbonate on arterial stiffness and vascular calcification over 24 months (Australian New Zealand Clinical Trials Registry Number

ACTRN12610000650099). The primary endpoint of this study is arterial stiffness measured using PWV, with secondary endpoints including change in aortic calcification and bone density assessed using computed tomography scanning, change in renal function measured by eGFR, and change in biochemical markers of phosphate metabolism. Although yet to begin recruiting, the results of this study would be most compelling: firstly, the changes in arterial stiffness and vascular calcification observed as a consequence of phosphate lowering in more advanced CKD, and secondly compliance with therapy in subjects required to adhere to a three-times-daily dosing regimen over a 24 month period.

An open-label randomised study is comparing the effects of sevelamer carbonate against calcium acetate on markers of “vascular health” in 50 patients with stage 3–4 CKD over a 12 week period (clinicaltrials.gov identifier: NCT 01277497). In addition to the primary endpoint of change in serum FGF-23 levels, the investigators have included a number of biochemical markers as secondary endpoints, including markers of vascular calcification, endothelial dysfunction and inflammation. As demonstrated in the subset of 61 patients studied in section 5 with ≥80% compliance, it is likely that a decrease in FGF-23 levels will be observed in this study, particularly the sevelamer arm.

Our group is currently recruiting potential kidney donors to examine the short and long-term effects of an acute decrease in GFR (uninephrectomy) on arterial stiffness and LV mass. Kidney donors represent a healthy subset of the general population and are carefully screened for co-morbidities including hypertension and

cardiovascular disease. It is hoped that this study will provide insights into the cardiovascular effects of decreased renal function in the absence of potential confounders, such as the underlying cause of renal disease itself, and the sequelae, such as anaemia, chronic inflammation and hypertension (Moody *et al.*, 2011).

Although this body of work did not demonstrate any significant difference in arterial stiffness, LV mass or LV systolic or diastolic function in humans with CKD, a similar study using a mouse model of CKD is currently underway to investigate the effects of sevelamer hydrochloride on arterial stiffness, LV mass and LV diastolic function compared to placebo (Maizel *et al.*, 2013). The authors are planning invasive haemodynamic measurements to assess aortic stiffness and are using transthoracic echocardiography to measure LV mass and function. The outcome of such a study, which will be performed under controlled conditions and will not be disadvantaged by poor adherence to study medication, may lend support to or refute our hypothesis of phosphate-lowering being a novel mechanism of improving cardiovascular structure and function in CKD.

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8 APPENDIX I: LIST OF ABSTRACTS RELATED TO THIS WORK

Chue CD, Townend JN, Moody WE, Edwards NC, Steeds RP, Ferro CJ. Effects of sevelamer carbonate on arterial stiffness and left ventricular mass in patients with stage 3 chronic kidney disease: results of a randomized controlled trial. *Oral presentation as a finalist for the Elizabeth Barrett-Connor Research Award for Young Investigators in Training at the American Heart Association Scientific Sessions, Los Angeles 2012.*

Chue CD, Townend JN, Moody WE, Movchan N, Steeds RP, Ferro CJ. Increased arterial stiffness and left ventricular mass are associated with abdominal aortic calcification in patients with stage 3 chronic kidney disease. *Poster presentation at the British Renal Society/Renal Association Conference, Birmingham 2011.*

Chue CD, Townend JN, Edwards NC, Steeds RP, Ferro CJ. Left ventricular mass inversely correlates with femoral bone mineral density in stage 3 chronic kidney disease. *Poster presentation at the British Renal Society/Renal Association Conference, Birmingham 2011.*

Chue CD, Edwards NC, Townend JN, Steeds RP, Ferro CJ. Regional aortic distensibility in stage 3 chronic kidney disease: a cardiac magnetic resonance study. *Poster presentation at the British Renal Society/Renal Association Conference, Birmingham 2011.*

Chue CD, Edwards NC, Ferro CJ, Jones CM, Townend JN, Steeds RP. Insights into differential regional aortic distensibility in early chronic kidney disease: a cardiac magnetic resonance study. *Poster presentation at the European Society of Cardiology Congress, Stockholm 2010.*

9 APPENDIX II: LIST OF PUBLICATIONS ARISING FROM THIS WORK

Chue CD, Townend JN, Moody WE, Zehnder D, Wall NA, Harper L, Edwards NC, Steeds RP, Ferro CJ. Cardiovascular effects of sevelamer in stage 3 chronic kidney disease. *J Am Soc Nephrol* 2013; **24:** 842–52.

Chue CD, Edwards NC, Moody WE, Steeds RP, Townend JN, Ferro CJ. Serum phosphate is associated with left ventricular mass in patients with chronic kidney disease: a cardiac magnetic resonance study. *Heart* 2012; **98:** 219–24.

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10 APPENDIX III: LIST OF OTHER PUBLICATIONS RELATED TO THIS WORK

Wall NA, **Chue CD**, Edwards NC, Pankhurst T, Harper L, Steeds R, Lauder S, Townend JN, Moss P, Ferro CJ. Cytomegalovirus seropositivity is associated with increased arterial stiffness in patients with chronic kidney disease. *PLoS One* 2013; **8:** e55686.

Moody WE, Edwards NC, **Chue CD**, Ferro CJ, Townend JN. Arterial disease in chronic kidney disease. *Heart* 2012; **99:** 365–72.

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Chue CD, Wall NA, Crabtree NJ, Zehnder D, Moody WE, Edwards NC, Steeds RP, Townend JN, Ferro CJ. Aortic calcification and femoral bone density are independently associated with left ventricular mass in patients with chronic kidney disease. *PLoS One* 2012; **7:** e39241.

Moody WE, Ferro CJ, **Chue CD**, Edwards NC, Steeds RP, Townend JT. Invite all donors to participate in follow-up studies. *BMJ* 2012; **344:** e2724.

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11 APPENDIX IV: LIST OF AWARDS PRESENTED FOR THIS WORK

Finalist for Elizabeth Barrett-Connor Research Award in Epidemiology and Prevention for Investigators in Training. Oral presentation at American Heart Association Scientific Sessions, Los Angeles, 2012.