Improving the Outcomes of Patients with Chronic Kidney Disease – Mineral Bone Disorder

A thesis submitted to the University of Manchester for the degree of PhD in the Faculty of Medical and Human Sciences

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Chronic Kidney Disease – Mineral Bone Disorder (CKD-MBD) is a systemic disorder which includes abnormal bone chemistry, vascular or soft tissue calcification, and abnormal bone formation. Many of the parameters of CKD-MBD have been associated with an increased mortality risk in renal patients.

There were three main facets to this research project. The first aim of this research was to perform two different studies using the Chronic Renal Insufficiency Standards Implementation Study (CRISIS) data. This prospective epidemiological study is designed to identify factors associated with renal progression and survival in the pre-dialysis CKD population. We have shown that for each 0.323mmol/L (1mg/dL) increase in serum phosphate there was a significant stepwise increased risk of death. (HR1.3 (1.1, 1.5) P=0.01). The association of baseline phenotypic data against vascular stiffness measurements was also investigated. Augmentation index measured at the radial artery was associated with a raised systolic blood pressure but no association with biochemical abnormalities was found.

We hypothesised that the phosphate effect on survival was related to the effects within the CKD-MBD spectrum and therefore control of secondary hyperparathyroidism would improve bone and cardiovascular parameters. Therefore for the second part of this research we performed a randomised controlled trial to examine the effects of cinacalcet with standard therapy compared to standard therapy alone on bone and cardiovascular parameters in haemodialysis patients with uncontrolled hyperparathyroidism. The change of biochemical parameters and cardiovascular markers were also further explored in secondary analyses alongside survival data. The primary end point of change in vascular calcification at 52 weeks showed no significant difference between arms. As equivalent control of phosphate and iPTH was achieved in both arms secondary analyses were performed. This showed a significant regression of left ventricular hypertrophy and carotid intima-media thickness associated with phosphate but not iPTH reduction. Patients whose phosphate reduced during the study had a survival advantage when followed for 5 years (HR=10.2 (1.1, 104.5) P=0.049).

The third part of this research was to investigate iPTH assay variability. We explored the variation in iPTH assays across the North West and paired this with regional audit data. This study showed that despite there being significant variation among iPTH assays across the region the variation in clinical management was still accounting for some variation in achieving PTH targets.

In conclusion, serum phosphate, within the normal laboratory range, is associated with an increased mortality in CKD patients. Haemodialysis patients may have improvement of cardiovascular outcomes with tight control of secondary hyperparathyroidism, by whichever therapeutic means. Intact PTH assays variation may alter our clinical management but variation in practice still affects guideline achievement.
DECLARATION

No portion of this work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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This research would not have been possible without the patients who have taken part in all this research for which I am very grateful. I would especially like to acknowledge the patients who took part in the randomised trial. They gave up substantial personal time for which I am eternally thankful; they truly enlightened my research experience and will always be remembered.

I would also like to express gratitude to the following:

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DEDICATION

I could not have completed this research without my wonderful husband who has been so accommodating throughout my research journey. He has been wonderfully understanding and patient. During the final stages of this research his support has been above and beyond and he has tirelessly entertained our children to allow me to finish my thesis.

My whole family, including my mum and brother, have supported me throughout my life. They have carried me to this point in my career which I never thought I would reach and I cannot thank them enough for their constant belief in me.

I would like to dedicate my thesis to my husband and children.
PREFACE

All the experimental work included in this thesis was performed by me except for the following:

- Experimental chapters 1&2: recruitment and data input for the CRISIS study has been performed by a trained nurse in renal out-patients
- Experimental chapter 2: This paper was co-written with Dr Smeeta Sinha.
  - I collated all the data and performed all the statistical analysis alone. Dr Sinha and myself jointly performed the literature review and co-wrote the article included in this thesis
- Experimental chapters 3&4: Reporting of the CT, Bone and CMR scans were performed by people blinded to randomisation and therefore I could not perform these in the remit of the randomised trial
- Experimental chapters 3&4: All biomarkers were analysed at the Royal Liverpool Hospital Biochemistry Department at a GCP regulated laboratory as required for an MHRA approved study
- Experimental chapter 5: Analysis of all iPTH samples were performed by the respective biochemistry departments by trained staff as per normal clinical practice for the PTH assay variability study

I obtained the data presented in this thesis by the following methods:

- Experimental chapters 1&2: I collated the relevant phenotypic data from the CRISIS database for both studies. I assessed the AIX data for quality and paired with other phenotypic data.
- Experimental chapters 3&4: Application for approval to all relevant local ethics boards, R&D departments and the MHRA. Performed all screening, approached patients to participate in the study and obtained informed consent. Arranged all scans, blood tests, changes of clinical management and cinacalcet prescriptions and follow-up.
- Experimental Chapters 3&4: I performed all pulse wave velocity, augmentation index at the radial artery and carotid intima-media thickness measurements throughout the study.
- Experimental Chapter 5: discussed with the ethics committee and R&D departments. Logistical organisation on samples being processed frozen and shipped. All negotiations with the biochemistry departments. Approached patients.
- Data analysis: I analysed all the statistical data included in this thesis; this incorporated data interpretation and production of tables and graphs.
- LVMI – corrected for body surface area
PUBLISHED AND PRESENTED WORK

The following publications are included as chapters in the alternative thesis format. As a result I wish to acknowledge the contribution of the co-authors to each paper.

1. Serum Phosphate and mortality in patients with chronic Kidney Disease

   *Clinical Journal of the American Society of Nephrology* 2010 Dec 5; 12: 2251-2257

   Eddington H: Study design, main author
   Hoefield R: Study design, data collection, proof reading
   Sinha S: Study design, proof reading
   Chrysochou C: Proof reading
   Lane B: Data collection
   Foley RN: Study design, statistical advice, proof reading
   Hegarty J: Proof reading
   New J: Data collection
   O’Donoghue DJ: Proof reading
   Middleton RJ: Study design, proof reading
   Kalra PA: Study design, main editor

2. Factors associated with vascular stiffness: Cross-sectional analysis from the Chronic Renal Insufficiency Standards Implementation Study.

   *Nephron Clinical Practice* 2009; 112(3):c190-8

   H Eddington: Joint main author, study design, data analyses,
   S Sinha: Joint main author, study design
   J Hegarty: Proof reading
   J Ting: Data collection,
   B Lane: Data collection
   C Chrysochou: Proof reading
   R Hoefield: Proof reading
   R Foley: Study design, statistical advice, proof reading
   D O’Donoghue: Proof reading
   P A Kalra: Study design, main editor
   R Middleton: Study design, main editor
3. **The association of chronic kidney disease-mineral bone disorder and cardiovascular risk.**  
   *Journal of Renal Care 2010 May; Suppl 1:61-7*

   Eddington H: Main author  
   Kalra PA: Main editor

   *Journal of Renal Care 2009 Mar;35 Suppl 1:45-50*

   Eddington H: Main author  
   Sinha S: Proof reading  
   Kalra PA: Main editor

5. **Clinical management of disturbances of calcium and phosphate metabolism in dialysis patients.**  
   *Nephrology Dialysis Transplantation Plus 2009; 2(4): 267-272*

   Eddington H: Main author  
   Heaf JG Main editor

The following publications are included as thesis chapters and are due for submission.

1. **A Randomised controlled trial to examine the effects of calcimimetic on bone and cardiovascular health**

   Eddington H: Study design, Main author  
   Chrysochou C: CT reporting, proof reading  
   Green D: Data collection, proof reading  
   Erikosima I: Data collection  
   Espinosa O: Data collection  
   Hutchison A: Proof reading  
   Bubtana A: Data collection  
   Vail A: Statistical advice, proof reading  
   Fraser WD: Biomarker analysis  
   Heatlie G: CMR reporting  
   Taylor P: CT reporting  
   Adams J: Study design, Bone mineral density reporting  
   Kalra PA: Study design, Main editor
2. **Does tight control of secondary hyperparathyroidism lead to cardiovascular or survival benefits in haemodialysis patients? A Post hoc analysis.**

Authors as above

3. **Variation in PTH concentration measured in laboratories across the North West region of the UK; An assay variability study linked to clinical audit data**

   Eddington H: Study design, Data collection, Main author
   Hudson J: Study design, sample processing, proof reading
   Oliver R: Study design, sample processing, proof reading
   Fraser WD: Study design, sample processing, proof reading
   Hutchison AJ: Study design, proof reading
   Kalra PA: Study design, main editor

The following presentations to learned societies have resulted from work related to this thesis

1. **Poster presentation: ASN Renal Week November 2010 Denver**

   Difference in age-related patterns of arterial stiffness and wave reflections among patients with kidney disease: results of the UK research alliance into kidney disease and arterial stiffness (UREKA) collaboration

2. **Poster presentation: ASN Renal Week October 2009 San Diego**

   Left ventricular mass reduction in haemodialysis patients with Cinacalcet therapy: results from an open-label randomised pilot study

3. **Poster presentation: ASN Renal Week November 2008 Philadelphia**

   Serum phosphate is associated with mortality in non-dialysis CKD: A prospective study

4. **Oral Presentation: Renal Association May 2008 Glasgow**

   Serum phosphate: prognostic association in patients with CKD not on dialysis
5. **Oral Presentation:** *ERA-EDTA May 2008*  
*Stockholm*  
Prognostic association of serum phosphate in patients with CKD not on dialysis

6. **Oral Presentation:** *ASN Renal Week November 2007*  
*San Francisco*  
Factors influencing vascular stiffness: Cross-sectional study from the crisis cohort

7. **Oral Presentation:** *North West kidney club October 2007*  
*Manchester*  
Factors influencing vascular stiffness: Cross-sectional study from the crisis cohort
ABBREVIATIONS

1,25(OH)₂ vitamin D  1,25 dihydroxy-vitamin D
25(OH) vitamin D  25 hydroxy-vitamin D
Ab  Antibody
ABD  Adynamic bone disorder
ACEI  Angiotensin converting enzyme inhibitors
ADVANCE  A randomised vascular calcification study to evaluate the effects of cinacalcet
Alx  Augmentation Index
Alk Phos  Alkaline phosphatise
ALT  Alanine Transferase
ALTM  All laboratory trimmed mean
ANK  Major pyrophosphate plasma membrane transporter
ARB  Angiotensin receptor blockers
BD  Becton Dickinson
BMD  Bone mineral density
BMI  Body mass index
BMP-  Bone morphogenetic protein
BMU  Bone metabolic unit
BP  Blood pressure
Ca  Calcium
[Ca] x [P]  Calcium x phosphate product
CARDIA  Coronary artery Risk Development in Young Adults Trial
CARE-2  Calcium acetate Renagel evaluation -2
CaSR  Calcium sensing receptor
CCS  Canadian Cardiovascular Society
cfPWV  Carotid-femoral pulse wave velocity
CIMT  Carotid intima-media thickness
CKD  Chronic Kidney Disease
CKD-MBD  Chronic Kidney Disease – Mineral Bone Disorder
CMR  Cardiac magnetic resonance scan
CRISIS  Chronic Renal Insufficiency Standards Implementation Study
CRP  C- reactive protein
CT  Computed tomography
C-Term  Carboxy-terminal
CV  Coefficient of variance
CVD  Cardiovascular disease
DBP  Diastolic blood pressure
DCOR  Dialysis Clinical Outcomes Revisited Trial
DEQAS  Vitamin D External Quality Assessment Scheme
DPC  Diagnostic product corporation
DTI  Diastolic time index
DXA  Dual X-ray Absorptiometry
EBCT  Electron beam computerised tomography
ECG  Electro-cardiograph
ECLIA  Electrochemiluminescent immunoassay
EDTA  Ethylenediaminetetraacetic acid
eGFR  Estimated glomerular filtration rate
ELISA  Enzyme linked immunosorbent assay
ESR  Erythrocyte sedimentation rate
EVOLVE  Evaluation of Cinacalcet HCl therapy to Lower cardiovascular events
FGF23  Fibroblast growth factor 23
GI  Gastrointestinal
Hb  Haemoglobin
HDL  High density lipoprotein
HPLC  High performance liquid chromatography
HR  Hazard ratio
HRP  Horseradish peroxidase
HU  Hounsfield Units
IDS  Immuno diagnostic systems
IGF-  Insulin-like Growth Factor
IGFBP  Insulin-like growth factor binding protein
IL-  Interleukin
IMP  Investigational medicinal product
iPTH  Intact parathyroid hormone
IQR  Interquartile range
KDIGO  Kidney Disease: Improving Global Outcomes
KDOQI  National Kidney Foundation: Kidney Disease Outcomes Quality Initiative
LDL  Low density lipoprotein
LLOD  Lower limit of detection
LREC  Local research ethics committee
LVH  Left ventricular hypertrophy
LVMI  Left ventricular mass index
MCV  Mean corpuscular volume
MDRD  Modification of Diet in Renal Disease Study
MG  Matrix Gla Protein
MHRA  Medicines and Health Regulatory Authority
MIA  Malnutrition-inflammation-atherosclerosis
MRM  Multiple reaction mode
MS  Mass spectrometry
NEQAS  UK National External Quality Assessment Service
NF-κB  Nuclear Factor – KappaB
NIST  National Institute of Standards and Technology
NT-proBNP  N terminal pro-brain naturetic peptide
NYHA  New York Heart Association
OPG  Osteoprotegerin
OR  Odds ratio
PC-1/eNPP-1  Ectonucleotide pyrophosphatase phosphodiesterase
PCR  Protein: creatinine ratio
Pit-1  Sodium dependant phosphate co-transporter
PO₄  phosphate
PP  Pulse pressure
pQCT  Peripheral quantitative computerised tomography
PTH  Parathyroid hormone
PWV  Pulse wave velocity
QCT  Quantitative computerised tomography
RAAS Renin-angiotensin-aldosterone system
RANK Receptor activator of NF-κB
RANK-L RANK ligand
RCT Randomised controlled trial
RIA Radioimmunoassay
RRA Radio receptor assay
SBP Systolic blood pressure
SEVR Subendocardial viability ratio or Buckberg Index
SHARP Study of Heart and Renal Protection
SHPT Secondary hyperparathyroidism
SST Serum separation tube
TMB Tetramethylbenzidine
TNAP Tissue non-specific alkaline phosphatase
TNF Tumour necrosis factor
T<sub>f</sub> Time to wavefoot to foot of reflected wave
TTI Time tension index
UKRA UK renal association
ULN Upper limit of normal
URR Urea reduction ratio
UVB Ultra-violet B
VC Vascular calcification
VDR Vitamin D receptor
VSMC Vascular smooth muscle cells
WCC White cell count
1. INTRODUCTION AND OVERVIEW
Chronic Kidney Disease (CKD) is increasing in prevalence across our population mainly due to an increasing age of survival and incidence of diabetes. Unfortunately the CKD population suffers a high mortality; cardiovascular disease accounting for more than half and as a consequence of this both the American Heart Association and the National Kidney Foundation have stated that CKD stage 5 (estimated glomerular filtration rate <15ml/min) patients are at the highest risk of cardiovascular disease. Foley et al have shown that the expected mortality rate of a 25 year old with renal failure is similar to that of an 80yr old in the general population and this premature cardiovascular death in CKD is strongly associated with and predicted by increased vascular stiffness, vascular calcification and left ventricular mass beyond established conventional risk factors.

Disturbances in mineral and bone metabolism are common in patients with CKD, and a large body of evidence indicates that these derangements are also associated with increased mortality and morbidity. Phosphate has been shown to induce vascular calcification in vitro and even though calcification is associated with an increased mortality risk no study has shown that altering serum phosphate levels in humans can improve survival.

The mineral abnormalities and their correction may theoretically, improve survival in renal patients through changes in the vasculature and cardiac morphology. However achieving the targets has been difficult due to the limitations of our available therapies though with the introduction of cinacalcet, a calcimimetic, this could possibly be improved. The introduction of cinacalcet may have other benefits on the vasculature as its site of action is the calcium sensing receptor and this is found in many cells including cardiac myocytes and vascular smooth muscle cells and therefore may impact on the morbidity and mortality of these patients.

Unfortunately evidence is lacking regarding which biochemical ‘targets’ would improve patients’ morbidity and mortality; this may also be compounded by assay variation, especially within intact parathyroid hormone (iPTH) assays. In renal disease patients have increased levels of parathyroid hormone (PTH) peptides alongside the active 1,84 PTH molecule; these are sometimes measured by the available assays but may have antagonistic modes of action. Standardisation of the available intact PTH assays and further understanding of other PTH peptides in the circulation will be required.
In this introduction I aim to discuss the changes that occur with regards to mineral metabolism, bone and cardiovascular parameters in the setting of renal disease. The evidence supporting the benefits of the different medications used in this condition will also be discussed.
2. BACKGROUND AND MANAGEMENT OF CHRONIC KIDNEY DISEASE – MINERAL BONE DISORDER
2.1 Mineral metabolism: normal control

2.1.1 Calcium homeostasis

The calcium ion is of major importance for all biological systems of the body and is involved in multiple processes including hormone release, neurotransmission, muscle contraction and coagulation. Calcium is also necessary for enzymatic reactions and as a mediator for hormonal effects\textsuperscript{19, 20} and is a major ion in the structure of bone and teeth. Due to these crucial requirements for calcium it is necessary for the body to maintain tight regulation of its level in the plasma.

![Simplified diagram of calcium equilibrium in the normal state](image)

Figure 1: Simplified diagram of calcium equilibrium in the normal state

Figure 1 illustrates a simplified diagram of calcium turnover in the body and shows that the majority of calcium is absorbed and excreted in the digestive system though the kidney does play a role in finely adjusting the plasma calcium. The bones are responsible for the rapidly exchanging pool or buffering of calcium but in reality about 500mg of calcium are released from bone per day due to bone resorption and 500mg taken up for bone formation with 99\% of the body’s calcium found in the skeleton. Normal plasma levels of calcium
range from about 2.15 – 2.65 mmol/L and this varies little in the normal individual. Approximately 50% of plasma calcium is ionised and therefore active; the rest is either bound to proteins such as albumin or in non-ionic complexes. The percentage of calcium bound is affected by blood pH; as there is an increase in pH there is an increase in bound calcium and hence less active calcium.

The regulation of calcium is complex; intestinal absorption occurs mainly in the duodenum but can occur more distally in the colon. In the kidney passive reabsorption of calcium occurs in the proximal tubule and thick ascending limb of the loop of Henle. In the distal convoluted tubule and collecting duct further calcium is reabsorbed against an electrochemical gradient and involves an active process. This process is regulated via a number of factors including PTH, 1,25 dihydroxy vitamin D (1,25(OH)2 vitamin D), insulin, oestrogen, acidity, calcium ions, magnesium ions and klotho. Medications including diuretics and calcineurin inhibitors can affect cellular calcium transport within the kidney and therefore also have an impact on calcium regulation.

2.1.2 Phosphate homeostasis

For the purposes of this thesis the term phosphate will be used to describe phosphorus and phosphate. Phosphate is important to all biological systems playing a critical role in many cellular processes. It is integral to all glycolytic compounds and high energy transfer compounds such as Adenosine Triphosphate and it is present in serum or plasma as inorganic phosphorus or phosphate. Phosphate also modifies enzymatic activity and is a major anion in the structure of bone. Its normal concentration in plasma is 0.8-1.5 mmol/L and is mainly absorbed from the diet with the majority of phosphate excreted by the kidneys. Tubular reabsorption is regulated to maintain normal physiological levels though soft tissue stores are large and include the muscle mass. This equilibrium state is illustrated in figure 2.
Organs involved in phosphate regulation are the kidney, gastrointestinal tract, parathyroid glands and bone. Phosphate is absorbed from the gastrointestinal tract and this absorption is increased in the presence of 1,25(OH)$_2$ vitamin D. As phosphate levels rise phosphaturic hormones are released to increase removal and a recently identified phosphaturic hormone is fibroblast growth factor 23 (FGF23) which is now thought to be one of the most potent.\textsuperscript{23} Phosphate excretion and reabsorption is also regulated by a number of factors including PTH, phosphatoninns such as FGF23, acidosis, glucocorticoids, calcium levels, oestrogens and medications such as diuretics.\textsuperscript{22} This fine regulation of phosphate occurs in the proximal tubule of the kidney with 80-97\% of the phosphate filtered load reabsorbed through Sodium-Phosphate co-transporters.
2.1.3 Fibroblast Growth Factor 23 and Klotho

FGF23 is a 32-Kda protein secreted by osteocytes in bone and was originally identified as a causative factor for autosomal dominant hypophosphataemic rickets. It is now known to have a main physiological role in maintaining normal phosphate levels and is released from bone in response to increasing phosphate levels. The action of FGF23 is reliant on the presence of klotho as a cofactor to enable interaction with the FGF receptor. This therefore restricts the action of FGF23 as klotho is mainly present in the kidney and the brain but has also been identified in skeletal muscle, aorta, pancreas, testis ovary and colon, parathyroid gland and pituitary gland.

FGF23 mutations have been used to determine some of the functions of FGF23. Mice given excess FGF23 have suppressed phosphate reabsorption and inhibition of 1,25(OH)\(_2\) vitamin D synthesis whereas FGF23 deficient mice suffer hyperphosphataemia, excess 1,25(OH)\(_2\) vitamin D levels and soft tissue calcification and FGF23 is now thought to be a counter regulatory hormone to 1,25(OH)\(_2\) vitamin D.

In the kidney, the majority of FGF receptor-klotho complexes are found in the distal tubule whereas the majority of phosphate excretion is seen in the proximal tubules. The mechanism of this action is currently unknown though it has been hypothesised that there is an unknown paracrine pathway present, potentially with soluble klotho being released when activated at the distal tubule which then acts at the proximal tubule. FGF23 also inhibits the activity of 1α-hydroxylase reducing the amount of active 1,25(OH)\(_2\) vitamin D in circulation. This action will again lead to a reduced phosphate by reducing absorption at the gastro-intestinal tract.

Klotho can be found as membrane bound with intracellular, transmembrane and extracellular domains or in a soluble form where only the extracellular domain remains. Soluble klotho can act as a humoral factor through an, as yet, unknown receptor and as an enzyme that regulates glycoproteins. The soluble klotho is known to inhibit internalisation of a calcium channel within the kidney and is important in calcium regulation. Soluble klotho may also affect calcium by partly regulating PTH secretion as klotho deficient mice were found to have less PTH secretion with hypocalcaemic stimulation than wild type.
2.1.4 Normal parathyroid regulation

The parathyroid glands are situated posterior to the thyroid gland in the anterior of the neck. They release PTH which is the major regulator of calcium homeostasis and for the purposes of this thesis the parathyroid glands will be described as one gland.

The main cell in the parathyroid gland is the chief cell which is the normal source of PTH and depending on the activity of the cell there will be varying numbers of secretory granules which then undergo exocytosis at the time of PTH release. Active PTH is a single chain protein of 84 amino acids; the main biological activity of the molecule residing at the N-terminal within amino-acids 1 to 34.

Calcium acting at the calcium sensing receptor (CaSR) on the PTH gland is the main regulator of PTH release\textsuperscript{33}; the level to which the CaSR is activated by the serum calcium concentration determines how much PTH is released in a minute-to-minute response.\textsuperscript{34} The CaSR is a cell membrane G-protein coupled receptor which was cloned in 1993\textsuperscript{33} and is designed to recognise and respond to small changes in serum calcium levels leading to appropriate changes in cellular function. Activation of the CaSR is also known to engage mitogen-activating protein kinase C cascade through both G protein linked phospholipases and tyrosine kinases\textsuperscript{35} eventually leading to a decrease in PTH secretion. Hence decreased activation leads to an increase in PTH and increased activation leads to a reduction in PTH.\textsuperscript{34} The CaSR along with the Vitamin D receptor (VDR), directly mediate the changes in gene transcription and hormone synthesis within the parathyroid gland to facilitate calcium homeostasis. Active 1,25(OH)\textsubscript{2} vitamin D also leads to a reduction in PTH production and release.

FGF23 receptors and klotho can also be found on parathyroid cells however their suggested roles in the control of PTH release is still not certain. FGF23 has been shown to have a suppressive effect on PTH release.\textsuperscript{36, 37} Some studies have shown that 1,25(OH)\textsubscript{2} vitamin D and PTH stimulate FGF23 expression and potentially secretion\textsuperscript{38-40} therefore closing a feedback loop.

Other molecules that can affect PTH release are magnesium, adrenaline, and histamine. Magnesium is not effective in its regulation but longstanding hypomagnesaemia inhibits
PTH synthesis and impairs the PTH actions in target tissues. Adrenaline and histamine both stimulate PTH release via specific receptors.

The main actions of PTH are to increase plasma calcium and decrease plasma phosphate and this is accomplished via its effect on the bone, kidneys and the gastrointestinal tract, though the latter is an indirect effect. This is summarized in figure 3. The bone effects are described in more detail later. At the kidney PTH stimulates an increase in phosphate excretion by blocking the reabsorption of phosphate at the proximal tubule. PTH also increases reabsorption of calcium from the distal tubule. At the kidney PTH also stimulates 1α-hydroxylation of 25 hydroxy-vitamin D (25(OH) vitamin D) to produce the active 1,25(OH)₂ vitamin D. This then acts at the gastrointestinal tract to increase absorption of calcium and phosphate. As the calcium levels and active vitamin D levels rise, phosphate levels fall and these act as a negative feedback on the PTH gland and therefore homeostasis is maintained.

Figure 3: An overview of the actions of parathyroid hormone
2.1.5 Vitamin D

Vitamin D is either absorbed from food or produced in the skin when 7-dehydrocholesterol is converted to vitamin D3 when exposed to Ultraviolet-B radiation. Vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) are converted to 25 hydroxy vitamin D (25(OH) vitamin D, also known as calcidiol) in the liver and stored. This 25(OH) vitamin D is converted to 1,25(OH)₂ vitamin D by many cells throughout the body and is important for local autocrine and paracrine functions along with the more well-documented endocrine function.

25(OH) Vitamin D also undergoes 1α-hydroxylation in the kidney and the 1,25(OH)₂ Vitamin D formed circulates as an endocrine hormone. As previously discussed the enzyme activity of renal 1α hydroxylase is increased by PTH and inhibited by FGF23. The released 1,25(OH)₂ Vitamin D is known to act at the gastrointestinal tract to increase phosphate and calcium absorption and also at the bone to stimulate bone resorption. 1,25(OH)₂ Vitamin D leads to an increase in FGF23 and is required for normal bone mineralisation to occur. 1,25(OH)₂ Vitamin D is an inhibitor of PTH secretion and is inhibited by FGF23 and therefore two negative feedback loops are present to help maintain normal levels. This is shown in more detail in Figure 4.

The 25(OH) vitamin D is also converted to 1,25(OH)₂ vitamin D (calcitriol) within many cells and exerts its action locally before being broken down by 24α-hydroxylase. Low plasma levels of 25(OH) vitamin D have been associated with an increased risk of infection, diabetes, malignancy and hypertension, among others. These associations are thought to be modulated via the extra-renal 1α-hydroxylase and the autocrine/paracrine pathways. However there is little conclusive evidence that replacement will improve these disease outcomes. Low levels of 25(OH) vitamin D have been associated with an increased mortality, but there is currently no randomized trial to suggest replacement will improve survival in a general population. Many trials are underway to review whether 25(OH) vitamin D replacement can impart benefit but it is too early to recommend replacement in all deficient patients.
Figure 4: A diagram showing Vitamin D pathway and PTH and FGF23 feedback loops.
2.2 Chronic Kidney Disease – Mineral Bone Disorder (CKD-MBD)

In renal disease the regulatory mechanisms of bone and mineral factors become deranged. Bone abnormalities, calcification and deranged mineral metabolism are now thought to be part of the same disease spectrum in renal patients. The term CKD-MBD is used to describe the clinical systemic disorder encompassed by these irregularities. This syndrome is described as manifesting one or a combination of the following:

1) Abnormalities in bone turnover, mineralisation, volume, linear growth, or strength
2) Vascular or other soft tissue calcification
3) Abnormalities of calcium, phosphate, PTH or vitamin D metabolism

The abnormalities of mineral metabolism ultimately lead to the formation of secondary hyperparathyroidism (SHPT) which is a common complication of renal disease and its pathogenesis is multi-factorial. Figure 5 shows a simplified illustration of the changes associated with its formation. Traditional teaching suggests that as kidney function declines in CKD three main changes occur:

- Loss of renal function leads to less 25(OH) vitamin D converted by 1α-hydroxylase resulting in a decrease in 1,25(OH)\textsubscript{2} vitamin D levels. This active vitamin D has been shown to start a linear decline early in CKD; 13% of patients already having a low level with an estimated glomerular filtration rate (eGFR) >80ml/min.\textsuperscript{48} This decline continues and occurs before any rise in PTH is seen.
- Secondly an increase in phosphate burden develops due to the decreasing eGFR and reducing urinary phosphate excretion.

Finally, there is a decrease in serum calcium levels. This is due to low dietary calcium intake but mainly the low 1,25(OH)\textsubscript{2} vitamin D level leading to less intestinal calcium absorption and reduced renal clearance. Ultimately, this leads to a lower serum calcium level which stimulates the parathyroid glands to release more PTH to regulate the serum calcium concentration. As the parathyroid gland exerts most of its effect through action on the kidney, its effect is impaired in CKD. This is coupled with a down regulation of the PTH receptor in CKD leading to skeletal resistance to PTH.\textsuperscript{49}
Figure 5: Illustration of the derangement of parathyroid gland stimulation in the setting of renal disease

Figure 6: FGF23 role in secondary hyperparathyroidism
FGF23 and klotho are now known to have an important role in the development of secondary hyperparathyroidism in renal disease (see figure 6). FGF23 levels have been shown to rise early as glomerular filtration rate falls;\textsuperscript{50} this rise occurs even in the context of normal phosphate levels suggesting a compensatory rise to regulate phosphate. There is also a corresponding reduction in 1,25(OH)\textsubscript{2} vitamin D due to inhibition of 1\textalpha-hydroxylase in the kidney leading to less negative feedback at the parathyroid gland and increasing PTH levels. As previously discussed FGF23 does act at the parathyroid gland in a klotho-dependant manner and seems to have an inhibitory role.\textsuperscript{37}

As GFR falls there is less nephron mass and klotho deficiency occurs\textsuperscript{27} therefore restricting FGF23 activity in the kidney. This leads to a lower saturation of the active phosphate excretion pathways allowing phosphate accumulation despite very high FGF23 levels. The FGF23 levels have been shown to be the main predictor of 1,25(OH)\textsubscript{2} vitamin D levels even after further adjustment for phosphate and renal function\textsuperscript{51} and is closely correlated with PTH in patients with CKD.\textsuperscript{50, 52} FGF23 levels have also been associated with mortality in dialysis patients independent of serum phosphate and other risk factors.\textsuperscript{53} Klotho may have further roles independent of FGF23; klotho deficiency has now been shown in early in CKD and has been associated with complications such as calcification and left ventricular hypertrophy.\textsuperscript{27, 54} Mouse and rat studies suggest that replacement of soluble klotho protects the kidney from further damage and suppresses renal fibrosis\textsuperscript{27} and further investigation in this area is on-going.

SHPT is characterised by excess PTH synthesis and secretion, parathyroid gland hyperplasia and chronically elevated levels of PTH. The deleterious effects of SHPT are not isolated to the skeleton. Other complications include vascular calcification, soft tissue calcification, myocardial dysfunction, left ventricular hypertrophy, decreased erythropoiesis and endocrine and neurological consequences have also been noted.\textsuperscript{55, 56} It is not clear as to how much of the vascular calcification and its sequelae are due to the increased levels of PTH and phosphate or medications used to manage SHPT. The mineral metabolism parameters mentioned have all been associated with mortality. An increased serum phosphate has been shown to be associated with mortality in chronic kidney disease and in dialysis patients.\textsuperscript{10, 57, 58} An association of phosphate and mortality has also been demonstrated in the general population; the data suggesting that a phosphate at the upper
end of the normal range is associated with increased cardiovascular risk and death.\textsuperscript{59-61} The influence of FGF23 is also important and the phosphatonin has been associated with mortality independent of phosphate levels.\textsuperscript{53} Serum Calcium has been associated with mortality, though only in dialysis patients and in large observational studies of >40,000 patients.\textsuperscript{62} Laboratory measured serum calcium is not an accurate measure of the amount of active calcium present or the actual body calcium content and may explain this weaker association. Raised PTH has also been associated with mortality though this has been less consistent.\textsuperscript{10, 62-64} Low PTH levels have been associated with an increased risk of death leading to further controversy.\textsuperscript{63} This could be explained as low PTH is associated with low bone turnover and adynamic bone disorder. This in turn would lead to inadequate buffering capacity for calcium and phosphate at the bone exacerbating soft tissue calcification. Adynamic bone disorder has been associated with overuse of calcium based phosphate binders and active vitamin D therapy,\textsuperscript{65, 66} though an association with malnutrition-inflammation-atherosclerosis (MIA) syndrome has also been made.\textsuperscript{67} The type of bone disease found in patients with either high or low bone turnover, osteomalacia or osteopenia could not be correlated to PTH levels in all patients.\textsuperscript{68} The lack of consistent data may be related to the variations in assays used\textsuperscript{69} and the variation in iPTH levels seen in an individual on a daily basis.\textsuperscript{70}

The most common treatment used to lower PTH is calcitriol analogues and these have been associated with an improved survival in an observational study where haemodialysis patients receiving any form of vitamin D receptor activators have been shown to have a lower mortality risk compared to those not receiving replacement.\textsuperscript{71} As this was an observational study the difference in prescribing could be due to a number of unknown factors which could also be contributing to the difference in survival.
2.3 The Association of CKD-MBD and Cardiovascular Risk

Helen Eddington, Philip A. Kalra

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Summary:

Chronic Kidney Disease - Mineral Bone Disorder (CKD-MBD) is a multifaceted definition used to help describe the systemic derangement of mineral bone metabolism in renal disease. This was previously referred to, rather simplistically, as ‘renal osteodystrophy’ or ‘renal bone disease’. In this review we will try and show the evidence relating these factors to cardiovascular morbidity and mortality and give some evidence as to the mechanisms for this. The treatments used for this condition are also integral to the increased cardiovascular mortality seen in renal patients and a summary of these effects will also be covered.

Background:

As renal function worsens a deterioration of mineral metabolism occurs, involving calcium, phosphate, and hormones such as parathyroid hormone (PTH) and 25-hydroxyvitamin D and 1,25 dihydroxyvitamin D. The kidney becomes less able to excrete phosphate and to hydroxylate 25-hydroxyvitamin D as the glomerular filtration rate falls. This leads to higher levels of phosphate and lower levels of 1,25 dihydroxyvitamin D. As 1,25 dihydroxyvitamin D aids absorption of calcium from the gastrointestinal tract the lower levels also contribute to a fall in serum calcium. High serum phosphate, low serum calcium and low 1,25 dihydroxyvitamin D feedback to the parathyroid gland triggering release of PTH and drive the development of secondary hyperparathyroidism (see figure 7). This deranged metabolism is known to impact on bone re-modelling and is managed with a variety of medications that aim to correct these abnormalities. The medications along with the abnormal mineral metabolism are now potentially a causal factor of ectopic calcification.

The term Chronic Kidney Disease -Mineral Bone Disorder (CKD-MBD) was devised after a consensus conference in 2005 by Kidney Disease: Improving Global Outcomes (KDIGO). This has now been widely adopted to describe the clinical systemic disorder seen due to mineral and bone metabolism changes that occur in renal disease. This syndrome (see Table 1) is described as having evidence of one or more of the following:
1. Abnormalities of calcium, phosphate, PTH or vitamin D metabolism
2. Vascular or soft tissue calcification
3. Abnormalities in bone turnover, mineralisation, volume, linear growth, or strength

The prevalence of this condition in chronic kidney disease (CKD), including dialysis populations, is unknown as routine screening for the presence of calcification is not common practice and bone biopsies are rarely performed outside of the research setting; however it is likely to be almost universal based on above definition. There are many guidelines available suggesting when and how management of CKD-MBD should be instigated. The most recent guidelines by KDIGO\textsuperscript{17} highlight the lack of answers we have regarding this difficult complication of renal disease. In this review we will explore the evidence linking the above factors and their treatment with cardiovascular morbidity and mortality.

Figure 7: – Factors involved in the formation of secondary hyperparathyroidism (used with permission J of Ren Care 2009 35 (s1), 45-50)
CKD- Mineral Bone Disorder can be diagnosed if a renal patient has evidence of one or more of the following

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<td>Abnormalities in bone turnover, metabolism, volume, linear growth or strength</td>
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Table 1: Definition of Chronic Kidney Disease-Mineral Bone Disorder

*Mineral metabolism parameters*

As renal function deteriorates the kidney becomes less able to excrete phosphate. The resulting high serum phosphate levels have been associated with an increased carotid intima-media thickness\(^7\) which is a widely used marker for atherosclerosis. Hyperphosphataemia has also been associated with mortality in dialysis and non-dialysis dependant CKD populations.\(^10,57,58\) Block *et al*\(^10\) analysed data from over 6000 dialysis patients and demonstrated a U-shaped association with death suggesting both a low and high phosphate is associated with increased mortality in dialysis patients. The increased mortality may be due to the involvement of phosphate in the pathogenesis of vascular calcification. An elevated phosphate is known to induce a change in the phenotype of a cultured vascular smooth muscle cell to an osteoblast-like cell\(^12\) which can then deposit calcium in the vascular wall. This effect is dependent on a sodium-dependant phosphate co-transporter, Pit-1\(^73\) which enables entry of phosphate into cells and is up-regulated in uraemia and calcified arteries; abnormal function may increase the tendency to calcification. Low phosphate has also been shown to affect survival in dialysis patients,\(^10\) but this has not been shown in patients with non-dialysis dependant CKD or in the normal population and may be due to factors specific to dialysis patients.\(^57\) This association may be at least partly related to the link between hypophosphataemia and malnutrition.
There is now increasing evidence that subtle differences in a person’s phosphate, even within the normal range, are associated with increasing cardiovascular risk in normal populations without overt renal disease. The Framingham offspring study found an increased cardiovascular risk with higher ‘normal’ levels and the ARIC study has also found an increased risk of cardiovascular events and death associated with baseline serum phosphate in the higher ‘normal’ range in community dwelling adults. Analysis of the CARDIA study data has found an association between the presence of left ventricular hypertrophy (LVH) and high-normal phosphate levels in a normal population of young adults followed for an average of 5 years. Analysis of the same population out to 15 years with CT scanning has shown that vascular calcification is predicted by similar high-normal baseline values of phosphate. Tonelli et al found an association with increases in normal range phosphate and cardiovascular events in patients who had previously suffered a myocardial infarction.

Calcium is involved in multiple processes including hormone release, neurotransmission, muscle contraction, blood clotting, enzymatic reactions, as a mediator for other hormonal effects and also as a major ion in the structure of bone. In view of this pivotal role in so many processes it is crucial for the plasma level of calcium to be tightly regulated. As renal function declines levels of circulating 1,25 dihydroxyvitamin D fall, this can lead to a drop in serum calcium. The subsequent increased PTH levels, along with the vitamin D receptor activators and calcium containing phosphate binders used to treat secondary hyperparathyroidism can lead to hypercalcaemia, hence tight control of calcium can be difficult in a renal population. It has been shown in dialysis and non-dialysis dependant CKD patients that hypercalcaemia is associated with an increased risk of death. A potential mechanism for the increased mortality is that, like hyperphosphataemia, elevated calcium may play a role in predisposing to vascular calcification. Hypocalcaemia has also been associated with an increase in mortality in men with CKD and in dialysis patients. The mechanism for this is less clear but as calcium is required for stabilising neuromuscular cells lower plasma calcium could lead to death secondary to cardiac arrhythmias. In view of this, maintenance of calcium within the normal range is imperative.

Abnormal levels of parathyroid hormone (PTH) are common in dialysis patients but levels begin to rise early, when estimated glomerular filtration rate (eGFR) falls below
There is a link between hyperparathyroidism and LVH with high PTH levels being associated with increased left ventricular mass index (LVMI) in healthy and uraemic populations, and parathyroidectomy reducing LVMI at six months despite no change in blood pressure in patients with primary hyperparathyroidism. An association between PTH and mortality has been shown by Kalantar-Zadeh and colleagues who showed a strong association between incremental PTH values and death risk above PTH levels of 300ng/L, and this was significant above 400ng/L.

D2 (Ergocalciferol) or D3 (Cholecalciferol)

25 hydroxylation occurs in the liver
25(OH) Vitamin D stored

Paracrine / Autocrine
1α-hydroxylation occurs intracellularly

1,25(OH)2 vitamin D deactivated intracellularly

Endocrine
1α-hydroxylation occurs within kidney

Circulating 1,25(OH)2 Vitamin D

Figure 8: Summary of vitamin D pathway

Vitamin D is another factor linking CKD-MBD and cardiovascular disease. Vitamin D (D2 or D3) is 25-hydroxylated in the liver and then stored or released into the circulation. 1α-hydroxylation to produce circulating 1,25dihydroxyvitamin D occurs in the kidney and with worsening renal function this hydroxylation is impaired. 1α-hydroxylation also occurs within many cells in the body; 25 hydroxy-vitamin D is taken into cells and the local 1,25 dihydroxyvitamin D produced regulates processes within the cells and is then broken down immediately; adequate extracellular 25 hydroxy-vitamin D is the necessary substrate for
this intra-cellular 1,25 dihydroxyvitamin D formation (see figure 8). Vitamin D (25(OH) and 1,25(OH)\(_2\)) deficiency is common in the general population and has been shown in 57% of medical inpatients\(^{80}\) but in >90% of dialysis patients.\(^{81,82}\) Serum levels of 25(OH) and 1,25(OH)\(_2\) vitamin D fall as eGFR falls.\(^ {48}\) Low levels have been associated with increased vascular stiffness\(^ {81}\) and with all-cause mortality. Low serum levels of 25(OH) but not 1,25(OH)\(_2\) vitamin D have been associated with an increased risk of incident coronary artery calcification.\(^ {83}\) As mentioned previously, low levels of 25(OH) vitamin D may be of great importance because of the impact on many intercellular processes throughout the body.

**Vascular and soft tissue calcification**

Cardiovascular disease was originally thought to be due to abnormal atherosclerosis in chronic kidney disease patients, but evidence points away from this being the pivotal player. The management of lipid-lowering therapy does not seem to improve survival in dialysis patients\(^ {84}\) and the incidence of arrhythmia, cardiomyopathy and sudden cardiac death are much higher in renal patients and cannot be explained by atherosclerosis alone.\(^ {85}\) A study in 2000\(^ {86}\) found that coronary artery calcification is common and progressive in young adults with renal disease. Calcification is now known to occur in both the arterial intima and media in renal disease and both independently predict all-cause and cardiovascular mortality.\(^ {13}\) Intimal calcification is present within the lumen and is associated with traditional atherosclerosis. Medial calcification occurs within the vessel wall and though the lumen may remain patent the artery cannot distend as required, leading to poorer perfusion, arterial stiffness and increased left ventricular work, amongst other abnormalities.

Dialysis patients have been found to have a markedly higher arterial calcification score compared to the general population\(^ {87}\) with coronary artery calcification present in some degree in 54% to 100% in case series. CKD patients with coronary artery calcification have been shown to have a 2- to 5- fold increase in calcification compared to age-matched controls with angiographically proven coronary artery disease.\(^ {88}\)

Medial vascular calcification is a contributory factor to the increased vascular stiffness that is seen in renal patients and the two factors are strongly associated.\(^ {89}\) Vascular stiffness is a
strong predictor of cardiovascular and all cause mortality\textsuperscript{8,90} which may in part be due to limited distension of the large arteries, and greater after-load upon the left ventricle, contributing to LVH. In systole the blood vessels distend and can ‘store’ blood which is then passed to the peripheries during diastole. This enables steadier perfusion at the peripheries and less damage to the smaller vessels. However when the vessels are stiff or calcified this cannot occur. Coronary perfusion is also affected by arterial stiffness as when the pressure wave produced in systole reaches a major junction (e.g. the bifurcation of the aorta) then a reflected pressure wave is produced, which reaches the heart during diastole, enabling perfusion of the coronary arteries. When the blood vessels are stiff then the pressure waves travel faster leading to the reflected wave reaching the heart during systole and leading to abnormal coronary perfusion.\textsuperscript{91}

**Abnormalities in bone maintenance**

Abnormalities in bone turnover, strength, mineralisation, volume and linear growth are also common in dialysis patients and for precise diagnosis of these factors a bone biopsy is required. However, this investigation is not often performed in clinical practice and is mainly performed in the research setting due to expertise required in interpretation of the results and patient discomfort caused. PTH is the main regulator of bone remodelling and skeletal turnover in CKD patients.\textsuperscript{66} However there is skeletal resistance to PTH in CKD and therefore higher levels are required to maintain normal turnover.\textsuperscript{49} This resistance appears to be related to factors secondary to the renal impairment as patients with idiopathic hypoparathyroidism and normal renal function have preserved or increased bone mass during calcium and vitamin D therapy.\textsuperscript{92}

Historically high levels of PTH lead to high bone turnover and the accumulation of fibrous tissue within the marrow. With the introduction of vitamin D analogues to help control secondary hyperparathyroidism the reverse disorder has become prevalent, low bone turnover (adynamic bone disorder). Additional predispositions for this condition are aging and diabetes,\textsuperscript{93,94} both highly represented in dialysis populations, which are associated with low bone turnover. Adynamic bone disorder leads to the bone being less able to buffer changes in calcium and phosphate occurring within the extra-cellular space, which potentially increases the likelihood of mineral deposition within the blood vessels and vascular calcification.
Abnormalities of bone turnover start early in renal disease and patients with non-dialysis dependant CKD have been shown to have decreasing bone mineral density (BMD) in relation to decreasing eGFR. The association of low BMD with vascular calcification and cardiovascular disease is well established in the general population but is more difficult to clarify in the renal population due to the other bone abnormalities that can occur. Low BMD has been inversely related to carotid intima-media thickness, coronary artery calcification in dialysis patients and histological changes on bone biopsy, and presumably these partly explain how low BMD has been associated with increased mortality risk in CKD patients. Treatment of this is not clear as low bone density does not necessarily relate to a diagnosis of osteoporosis in renal patients. The main treatment for low bone density is bisphosphonates whose mechanism of action is to decrease bone turnover; as there is increasing prevalence of adynamic bone disorder in renal disease there are safety concerns regarding the use of this medication in these patients.

**Management of CKD-MBD and its effects on cardiovascular disease**

Whilst striving to improve the underlying skeletal and metabolic conditions of CKD-MBD its management can involve use of a number of medications which may contribute to cardiovascular risk. To improve hyperphosphataemia phosphate binders are commonly prescribed for renal patients alongside their low phosphate diet. Aluminium hydroxide was originally used and was a highly effective binder but in some patients toxicity occurred, leading to problems including abnormal bone structure and turnover. Calcium-containing phosphate binders became the first-line therapy and a number of different preparations are available. However, some investigators have shown an association between the ingested daily calcium load incurred with phosphate binders and the degree of vascular calcification and presence of adynamic bone disorder. Calcium-free phosphate binders have been available for over a decade, but are expensive in comparison to traditional binders. Sevelamer hydrochloride and Sevelamer carbonate are resin-based binders and Lanthanum Carbonate is a metal based binder. One study suggested that the use of Sevelamer hydrochloride, which leads to lower LDL-cholesterol levels along with controlling phosphate, attenuated the progression of vascular calcification compared to calcium containing binders. Evidence that this might relate more to the lipid-lowering properties of Sevelamer was provided in another study in which calcium containing binders and a statin were used in the control arm. Similar lipid-lowering was reflected in similar
attenuation of vascular calcification with both regimes\textsuperscript{104} though in both studies PTH control was not comparable in each arm, which may have affected their outcomes.

One small study of incident dialysis patients suggested a survival benefit with the use of Sevelamer compared to calcium containing phosphate binders,\textsuperscript{105} but no overall survival advantage was shown when these agents were compared in a large randomised trial setting with prevalent dialysis patients (Dialysis Clinical Outcomes Revisited, DCOR).\textsuperscript{106} However, the DCOR study did show a benefit in a sub-group of patients aged >65 years. There are, as yet, no published studies examining the effect of lanthanum upon survival and vascular calcification.

Other compounds used to control CKD-MBD are vitamin D analogues (alfacalcidol, paracalcitrol, calcitriol) and calcimimetics (cinacalcet). Vitamin D analogues are used to suppress PTH via negative feedback but they can cause increased levels of calcium and phosphate, which potentially could exacerbate calcification as discussed earlier. Paracalcitol, a selective vitamin D analogue (having reduced effects upon gut receptors, and hence calcium absorption), was associated with a reduced incidence of prolonged hypercalcaemia*(see footnote) in comparison to calcitriol.\textsuperscript{107} In retrospective observational studies the use of active vitamin D analogues has been associated with an improvement in all-cause\textsuperscript{71,108} and cardiovascular\textsuperscript{109} mortality but the cause of this has not been examined, and no randomised trial evidence exists.

Cinacalcet, a calcimimetic, acts on the calcium-sensing receptor leading to the receptor sensing more calcium than is present and is used to suppress PTH but it reduces calcium and phosphate levels. This therapy is only licensed in dialysis patients as it can cause marked hypocalcaemia in patients with non-dialysis dependant CKD. A study in rats has shown calcimimetics can halt formation of and regress vascular calcification.\textsuperscript{110} A randomised prospective study (ADVANCE) investigating the effect of Cinacalcet upon

* Addendum to published paper for thesis: There was no difference in incidence of hypercalcaemia and hyperphosphataemia between paracalcitrol and calcitriol in the trial. A difference was only seen when comparing patients who had 2 consecutive episodes of hypercalcaemia; therefore the term prolonged was used within this paper.\textsuperscript{107}
vascular calcification in the dialysis population has been completed with results awaited.\textsuperscript{111} A large survival study (EVOLVE) is also in progress.\textsuperscript{112}

**Conclusion**

Observational studies provide compelling evidence that CKD-MBD and its treatment can be intimately related to cardiovascular disease. Greater perturbations of phosphate, calcium and parathyroid hormone are all associated with worse survival. Arterial stiffness due to vascular calcification is a key component of the cardiovascular risk, and CKD-MBD is also a contributor to the greatly increased prevalence of LVH seen in CKD populations. Due to the complexity of CKD-MBD (Figure 9) the control of one factor alone may not be enough and multiple therapies are probably needed for us to impact on the high mortality suffered by the renal population. However, although control of serum phosphate, calcium and parathyroid hormone, along with replacement of vitamin D, all appear logical actions in the management of CKD-MBD, the area is blighted by a lack of prospective trials, and so any benefit to survival is only speculative at this stage.

![Figure 9: An illustration of the complex interactions between CKD-MBD and cardiovascular risk](image)

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The ADVANCE study has been completed and the results have now been published.\textsuperscript{113} Patients within this trial were randomised to cinacalcet with low dose vitamin D compared to variable dose vitamin D. The primary end-point was stated as the percentage change in Agatston coronary artery calcification score and this did not reach significance ($P=0.07$). However when the volume method of measuring calcification was used a significant difference in calcification progression was seen ($P=0.009$). A difference in baseline serum phosphate was seen between the two arms and when this was incorporated in the statistical model then the primary outcome also became significant ($P=0.006$). Overall this study suggested a benefit with cinacalcet therapy in calcification progression at the coronary arteries, thoracic aorta, and with valvular calcification.

The EVOLVE study was a mortality study comparing cinacalcet and placebo with variable vitamin D doses permitted in both arms. The trial has now concluded and the primary end-point was time to the composite event which incorporated all-cause mortality or first non-fatal cardiovascular event (myocardial infarction, hospitalisation due to angina, heart failure or peripheral vascular event). The pre-specified primary end-point was not significant in the intent-to-treat analysis ($P=0.11$) but when analyses were adjusted for baseline characteristics or took into account the effects of parathyroidectomy, transplantation and on-going study drug use the study suggests that cinacalcet may result in a reduction in the composite primary end-point (relative reduction 10-15%).\textsuperscript{114}
2.4 Bone in renal disease

PTH is known to be the major regulator of bone remodelling and skeletal turnover in CKD patients. PTH promotes recruitment and differentiation of osteoclasts, therefore increasing breakdown of bone mineral and degradation of bone collagen. Osteoclasts, however have no PTH receptors and the PTH effect is mediated via cytokines and other factors released from osteoblasts other local cells. Osteoprotegerin (OPG), RANK (receptor activator of NF-κB) and RANK ligand (RANK-L) have been identified as important in the molecular signalling involved in skeletal remodelling. PTH also promotes bone formation by direct action on osteoblasts, increasing both the number and activity of these cells. It is important to note that in CKD there is skeletal resistance to PTH and therefore higher than normal levels are more likely to be associated with normal bone turnover. This bone resistance to PTH appears to only occur in CKD patients as patients with idiopathic hypoparathyroidism (and normal renal function) have preserved or increased bone mass during calcium and vitamin therapy.

As PTH levels rise well above normal both resorption and formation increase in a coordinated manner leading to increased bone turnover. This process results in structurally weaker woven bone rather than lamellar bone due to abnormalities in the spatial arrangement of collagen fibrils. This process is an integral component of high turnover bone disease seen in CKD patients. At mild- moderate increased levels of PTH the bone structure is not impaired. However, when the SHPT is severe, fibrous tissue accumulates within the marrow accounting for the histopathological term osteitis fibrosa cystica. This was the main bone disease found in dialysis patients a few decades ago along with osteomalacia prior to vitamin D availability. However, low turnover bone disease (adynamic bone disorder) now accounts for two thirds of skeletal lesions on bone biopsy in patients on peritoneal dialysis. In this situation bone turnover and resorption are decreased as a result of decreased osteoblast recruitment and activity and this also leads to defects in bone mineralisation.

Adynamic bone disorder is related more to the treatment of SHPT rather than SHPT itself. Vitamin D sterols may exert a direct inhibitory effect on bone cells and large doses of calcium given as phosphate binders act to reduce PTH levels further. Another potential reason for the increasing prevalence of adynamic bone disorder is an increasing elderly and
diabetic population requiring dialysis (see table 2). Diabetes and increasing age are known to be associated with an increased risk of low bone turnover. Malnutrition or a low albumin may also be important in low bone turnover. Adynamic bone disorder is much more frequently found in patients on peritoneal dialysis and this may be due to lower albumin levels due to increased protein loss and higher glucose levels. However, not all peritoneal dialysis patients show low bone density or evidence of bone loss despite the presence of long-standing adynamic bone. The multiple potential mechanisms for decreased bone formation in chronic kidney disease are summarised in table 2.

<table>
<thead>
<tr>
<th>Cause</th>
<th>Mechanism of decreased osteoclast activity</th>
</tr>
</thead>
</table>
| Low serum 1,25 OH Vit D              | ↓osteoblast differentiation  
|                                      | ↓osteoblast lifespan  
| Metabolic acidosis                   | ↓1,25 OH Vit D production  
|                                      | ↓collagen synthesis  
| High serum phosphate                 | ↓1,25 OH Vit D production  
| Calcium loading /hypercalcaemia      | ↓1,25OH Vit D production  
|                                      | ↑1,25 OH Vit D degradation (CaSR mediated)  
| High serum IL-1, IL-6, TNF           | ↓osteoblast lifespan  
| Low serum IGF-1 activity             | ↓IGF-1 and IGFBP-5 levels  
|                                      | ↑Inhibitory IGFBPs (2,4,6) levels  
|                                      | ↓osteoblast lifespan  
| Malnutrition, proteinuria            | ↓IGF-1  
|                                      | ↓25 OH Vit D levels  
| Diabetes                             | ↓25 and 1,25 OH Vit D levels  
|                                      | ↑advanced glycaemic end-product  
|                                      | ↓osteoblast lifespan  
| Age-related                          | ↑advanced glycaemic end-product  
|                                      | ↓osteoblast lifespan  
| Hypogonadal                          | ↓osteoblast lifespan  
| Uraemic toxins (uric acid)           | ↓1,25OH Vit D production  
|                                      | ↓Vit D receptor activity  
|                                      | ↓osteoblast proliferation  
| Aluminium toxicity                   | ↓osteoblast activity  

Table 2: Causes and proposed mechanisms of decreased bone formation in patients with chronic kidney disease. Adapted from Andress DL; Kidney Int 2008 (with permission)
Abnormalities of bone resorption and formation probably start early in the development of renal impairment. Patients with chronic kidney disease, not on dialysis, have been shown to have decreasing BMD in relation to decreasing eGFR. This reduction in BMD was also associated with an increase in PTH and phosphate and a decrease in calcium and 1,25 dihydroxyvitamin D levels. Serum levels of biochemical markers of bone formation (osteocalcin, bone specific alkaline phosphatase) were also found in higher levels in patients with worsening renal function suggesting increased bone formation.\textsuperscript{95} There are differing results in the literature as to whether mainly cortical or both cortical and trabecular bone are affected by this increased resorption.\textsuperscript{95, 122, 123} Whichever is affected, low BMD has been shown to be associated with an increased risk of death in CKD patients.\textsuperscript{102}

The association between BMD and fracture risk in patients with chronic kidney disease is also unclear.\textsuperscript{124-128} A recent meta-analysis has shown that low BMD is associated with an increased risk of fracture in patients with chronic kidney disease stage 5.\textsuperscript{129} This fracture risk has also been shown to be significantly reduced if patients have a parathyroidectomy.\textsuperscript{130}

There are a multitude of non-invasive methods available to measure bone mineral density but their value in CKD is not well established. There are also inconsistent findings on the correlation with bone mineral density and fracture risk in the CKD population.\textsuperscript{47} The spectrum of bone abnormalities seen in CKD, such as adynamic bone disorder or high turnover disease, can only be diagnosed with a bone biopsy.

\emph{Measurement of bone mineral density:}

\emph{Dual X-ray Absorptiometry (DXA)}

This is the most common method used in clinical practice to measure bone mineral density. It is based on two peaks of photon energy passing through the body\textsuperscript{131} and as they pass through the bone, their intensity is attenuated and absorbed. This change is then used to determine the bone density\textsuperscript{132} and produces a 2-D measurement of bone mineral content in grams per area (in cm\textsuperscript{2}). It is important to note that trabecular and cortical bone cannot be differentiated with this method. As CKD patients are also prone to vascular calcification,
any calcium deposited in the arterial wall of the aorta would also be added to the bone content in a standard posterior-anterior spinal DXA.

**Quantitative Computerised tomography (QCT)**

This measurement utilizes standard computed tomography (CT) which has been modified with specialized software and calibration phantoms. This phantom allows calibration of the bone density measurements to occur. When analysing the results the region of interest is isolated to the inner vertebral body which contains only trabecular bone. The scan therefore has the advantage of avoiding any error due to the presence of aortic vascular calcification.

This method can be used centrally or peripherally (pQCT) in the forearm. The central spine QCT will measure bone density of trabecular bone only whereas the peripheral measurement in the forearm can measure cortical and trabecular bone density.\(^{133}\) This is important as there is a marked decrease in cortical bone in patients with CKD\(^ {123}\) and the ability to differentiate between the two may be important. The distal forearm is also mainly cortical bone so the two measurements represent the two differing types of bone mentioned. BMD measured at the distal radius site (pQCT) has been shown to predictive of fracture risk in a number of studies\(^ {128}\) and is related to PTH levels\(^ {134}\) in haemodialysis patients. These images also enable cortical and trabecular bone to be measured independently.

**Other methods**

The favoured method of investigating bone parameters in renal disease is a bone biopsy but it is used infrequently and is invasive and often painful. This method, however, can provide the researcher with information regarding bone turnover, strength, mineralisation, volume and growth. Other methods available include quantitative ultrasound, radiographic morphometry and radiographic absorptiometry. These methods can also give measurements of bone mineral density but they have not been used in this research.
2.5 Bone and vascular axis

CKD-related bone changes and vascular calcification are thought to represent parts of the same disease spectrum and are both included in the definition of CKD-MBD. Both bone disease and vascular calcification have been associated with derangements of mineral metabolism in renal disease such as phosphate, calcium and PTH. Aside from this their association with each other and with cardiovascular disease is well known in the general population.\(^{96-99}\) BMD has been associated with coronary artery disease found on coronary angiography in a retrospective study by Marcovitz et al.\(^ {135}\) This study of mainly women (89%) found that a low BMD predicted coronary disease defined as ≥ 50% stenosis in at least one major artery.

Bone histological changes in renal disease have been associated with increased vascular calcification.\(^ {101, 136}\) There have been differing reports regarding the association between bone density and calcification though this may be due to the DXA of the spine also analysing vascular calcification as previously discussed.\(^ {137, 138}\) Low BMD has been shown to be inversely related to coronary artery calcification\(^ {88}\) and it is also associated with increased cardiovascular death in the dialysis population.\(^ {102}\)

On a cellular level, a phenotypic change in vascular smooth muscle cells (VSMCs) is seen in the pathogenesis of vascular calcification in renal disease. This change is demonstrated by the reduction of gene expression normally seen in VSMCs (SM alpha actin, SM22) and the gain of gene expression normally seen in an osteoblast like cell (Runx2, osteopontin, Tissue non-specific alkaline phosphatase (TNAP)).\(^ {12}\) This new osteoblast like cell has the ability to form hydroxyapatite and allows mineralisation to occur within the vessel. The RANK-L/OPG pathway has also been implicated in the formation of vascular calcification and is a known key regulatory mechanism in bone homeostasis.\(^ {139}\) As the same regulatory mechanisms are involved then this could explain the link seen between bone turnover and vascular calcification. Bone formation and resorption markers (bone specific alkaline phosphatase and tartrate resistant acid phosphatase 5b) have also been associated with increased cardiovascular events in patients with chronic kidney disease.\(^ {140}\)

In renal disease bone abnormalities and ectopic calcification are now commonly thought to be linked but further trials are required to clarify the link between these disease processes.
2.6 Vascular calcification in chronic Kidney Disease: A Clinical Review

Helen Eddington, Smeeta Sinha, Philip A. Kalra,

This manuscript was published in: Journal of Renal Care 2009; 35(suppl.1): 45–50.
Summary

Vascular calcification, which is associated with arterial stiffness, is now known to be an important predictor of cardiovascular and all-cause mortality in patients with renal disease. This calcification starts developing in the early stages of chronic kidney disease (CKD) and is present in over 50% of patients at the time of dialysis commencement. Once calcification is present it continues to progress, though some medications have been shown to slow this progression. Vascular calcification and bone abnormalities are now both encompassed by the term of CKD - mineral bone disorder and are thought to be part of the same disease process in CKD.

Vascular calcification and arterial stiffness have been extensively researched in the renal population and many factors are known to be associated with their presence and progression. This calcification is an important factor to be considered in the management of the renal patient but there are different methods available for its measurement. These details will be discussed further in this review along with evidence available for management of this important complication of renal disease.

Introduction

There is a significant increased risk of cardiovascular mortality in renal patients compared to the general population\(^5\) and this cannot be explained by an increase in classical risk factors alone, such as hypercholesterolaemia and smoking. There is currently little proof that lipid-lowering medication improves cardiovascular outcomes in dialysis patients\(^8^4\) which opposes the data in the general population. There is also an increased incidence of arrhythmias, cardiomyopathy and sudden cardiac arrest in patients with renal disease and these cannot be explained by atherosclerosis alone.\(^8^5\)

Chronic kidney disease-mineral bone disorder (CKD-MBD) is a term encompassing calcification, bone abnormalities and mineral metabolism derangements in the setting of CKD.\(^4^7\) These factors are all now thought to be linked and part of the same disease spectrum. Bone histology changes have been associated with increased vascular calcification\(^1^0^1\) and low bone mineral density has been shown to be inversely related to coronary artery calcification in patients with end-stage renal disease.\(^8^8\) The connection
between low bone mineral density and calcification is also well known in the general population.\textsuperscript{96-99} Another study has shown an association between low bone mineral density and coronary artery disease found on coronary angiography, though this was a retrospective study in a mainly female population.\textsuperscript{135}

Despite these similarities in the connection between bone and vascular disease, the calcification that occurs in renal disease is thought to be different to that of the general population. Vascular calcification in renal disease can occur in both the intima and the media of the arteries (see Figure 10). Intimal calcification is associated with atherosclerosis though in renal patients the plaques have increased calcification compared to plaques seen in the general population.\textsuperscript{141} Medial calcification occurs in diabetes, ageing and renal disease; the calcification is known to occur in much younger patients than those who develop intimal calcification, and in renal disease it is associated with a longer duration on haemodialysis.\textsuperscript{13} It has also been shown that both sites of calcification are associated with an increased risk of cardiovascular death in dialysis patients.\textsuperscript{13}

Vascular calcification is highly prevalent in renal disease and in case series; coronary calcification was present to some degree in 54–100\% of the dialysis population.\textsuperscript{87} Such calcification can be demonstrated in pre-dialysis patients. Once present the calcification has been shown to progress rapidly\textsuperscript{86, 88, 142} and this progression has been associated with increased phosphate and calcium x phosphate product ([Ca] x [P]).\textsuperscript{86} Therefore controlling secondary hyperparathyroidism is pivotal in the management of this calcification. Increased calcium, phosphate and [Ca] x [P] have all been shown to be associated with mortality in dialysis patients\textsuperscript{62} so maintaining them at required levels is becoming increasingly important. There are many guidelines available on the management on bone chemistry in renal disease. Table 3 depicts a brief summary of the current KDOQI (National Kidney Foundation-Kidney Disease Outcomes Quality Initiative) bone metabolism and disease guidelines for the CKD stages 3-5.\textsuperscript{143} The control of these parameters, such as calcium and phosphate, is difficult, and the medications described later are used in combination to aid reaching these targets. No one factor has been found to control the formation of calcification and it is known to be an active cell mediated process. There is increasing knowledge of the mechanisms involved in the pathogenesis of vascular calcification but these will be covered in another review in this supplement.
Figure 10: Diagrammatic representation of intimal and medial calcification
<table>
<thead>
<tr>
<th>KDOQI</th>
<th>Ca mmol/L (mg/dL)</th>
<th>Phosphate mmol/L (mg/dL)</th>
<th>[Ca]x[P]</th>
<th>Maximum calcium intake recommended as binder</th>
<th>PTH pg/mL (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD stage 3</td>
<td>Normal</td>
<td>0.87-1.49</td>
<td>-</td>
<td></td>
<td>35-70 (3.85-7.7)</td>
</tr>
<tr>
<td>(30-59ml/min)</td>
<td></td>
<td>(2.7-4.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKD stage 4</td>
<td>Normal</td>
<td>0.87-1.49</td>
<td>-</td>
<td></td>
<td>70-110 (7.7-12.1)</td>
</tr>
<tr>
<td>(15-29ml/min)</td>
<td></td>
<td>(2.7-4.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKD stage 5</td>
<td>2.1-2.37</td>
<td>1.13-1.78</td>
<td>&lt;55mg^2dL^2</td>
<td>1.5gm/day</td>
<td>150-300 (16.5-33)</td>
</tr>
<tr>
<td>(&lt;15ml/min)</td>
<td>(8.4-9.5)</td>
<td>(3.5-5.5)</td>
<td>(&lt;4.6mmol^2/L^2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Summary of KDOQI bone metabolism and disease clinical practice guidelines
Vascular calcification is associated with vascular stiffness which is also increased in CKD. It is associated with increased blood pressure, age, diabetes, worsening renal function and mortality.\textsuperscript{2, 6} An artery normally distends to accommodate the volume of blood ejected from the heart, however when the vessels are calcified, especially with medial calcification, the vessel can no longer expand. This leads to the blood moving faster through the blood vessels and this stiffness is most commonly measured using pulse wave velocity which measures the speed of the pressure wave through the vasculature. The stiffness leads to changes in the perfusion of the peripheral vessels and also of the heart. In normal circumstances the heart is perfused by coronary flow during diastole from the reflected wave in the blood vessels. However when the vessels are stiff the blood reaches junctions in the arterial tree faster which lead to earlier reflected waves, these in turn reach the heart during systole and lead to poorer coronary perfusion (see Figure 11).\textsuperscript{91} The stiffer blood vessels also increase the pressure the heart has to pump against leading to left ventricular hypertrophy.\textsuperscript{91}

![Diagram](image)

**Figure 11:** Diagrammatic representation of intimal and medial calcification
Secondary hyperparathyroidism develops as renal function deteriorates due to a number of factors. A simplified diagram of the factors involved in the formation of secondary hyperparathyroidism is shown in Figure 12. In the normal state PTH leads to increased serum calcium secondary to its actions at the bone and kidney.

![Diagram of factors involved in secondary hyperparathyroidism](image)

Figure 12: Factors involved in the formation of secondary hyperparathyroidism

Vitamin D is normally ingested or is synthesised in the skin when exposed to sunlight. This vitamin D is then hydroxylated at the liver to form 25 hydroxy-vitamin D. This is then further hydroxylated at the kidney by the enzyme 1α-hydroxylase to form 1,25 hydroxy-vitamin D, the active form. PTH stimulates the 1 alpha-hydroxylation of vitamin D at the kidney leading to the formation of this active vitamin D. This vitamin D then acts on the gastrointestinal system to increase calcium and phosphate absorption. As renal function deteriorates less phosphate is excreted, there is less hydroxylation of vitamin D and there are less PTH receptors found in bone. These factors lead to increased serum phosphate, low serum calcium, low vitamin D levels, and less negative feedback at the parathyroid gland and hence higher PTH levels. The presence of vascular calcification has been associated with the use of some of the medications we use to treat secondary hyperparathyroidism, such as calcium-based phosphate binders.\(^{13}\) The KDOQI guidelines state that a maximum of 1.5 gm elemental calcium should be given per day as a binder, though this guideline is opinion based.\(^{143}\) There is now some evidence that Sevelamer may
reduce the progression of calcification when compared to calcium binders.\textsuperscript{103} However, this may be due to the lipid-lowering properties of Sevelamer, as when this was compared to calcium binder treatment, with use of similar lipid lowering in both groups due to the addition of a statin, the progression of calcification was similar.\textsuperscript{104} There is some evidence that Sevelamer may also impart a survival benefit in our renal patients compared to calcium binders, but a large prospective randomised control trial showed no overall benefit except in patients over 65 years.\textsuperscript{105, 106, 144} Lanthanum is also now available as a non-calcium phosphate binder; however no trials examining its effects on vascular calcification or survival have been performed.

Vitamin D analogues are well established as treatment for secondary hyperparathyroidism in renal disease. The active vitamin D is prescribed to replace the deficiency seen in renal disease as there is decreased activity of 1α-hydroxylase which is mainly present in the kidney. Most of the available vitamin D treatments in common use, lower parathyroid hormone by action on the vitamin D receptors in the glands, but they also act at the receptor in the gastro-intestinal tract leading to an increase in serum calcium and phosphate.\textsuperscript{145} These vitamin D analogues have been associated with an increase in calcification but whether this is due to the medication or the secondary increase in calcium and phosphate is unclear. Newer analogues are now available (paracalcitrol) which have been shown to have less of an effect on the calcium and phosphate levels\textsuperscript{146, 147} by more selective action on the vitamin D receptors in the parathyroid glands as opposed to the GI tract. Paracalcitrol has been shown to confer a survival benefit but only in retrospective observational studies.\textsuperscript{148} No prospective randomised control trial has taken place as yet.

Calcimimetics (cinacalcet) are also now available for treatment of advanced secondary hyperparathyroidism. This medication reduces parathyroid hormone, calcium and phosphate allowing more patients to reach treatment targets.\textsuperscript{149} In rat studies calcimimetics have been shown to reduce calcification\textsuperscript{110} and prospective studies (e.g. ADVANCE and EVOLVE) examining their effect on calcification and mortality in dialysis patients are ongoing with results available from 2010. There is also some evidence that calcimimetics are beneficial to the bone and there have been some studies which have shown an improvement in bone mineral density and bone turnover with the use of cinacalcet.\textsuperscript{150, 151}
There is now overwhelming evidence that the presence of vascular calcification and vascular stiffness is associated with an increased mortality, but their presence is not always evident clinically at the bedside. There are many methods now used to assess calcification and these have been summarised in Table 4.

The gold standard method is CT (spiral or electron beam) as this gives a quantitative calcification score for the patient. It can be used to scan a variety of vascular sites and trials have used coronary, aortic and femoral artery scores. As this method provides a quantitative score it can be used to monitor progression over time and therefore should be used when monitoring difference in therapies in longitudinal studies.

Other methods available include ultrasound and plain X-rays. These can be used to determine if calcification is present and when multiple sites have been examined a scoring system has been used in cross-sectional observational studies. Although vascular calcification is easy to determine on a plain X-ray, it cannot be truly quantified and so progression cannot be accurately determined by this technique.

Despite the availability of these methods to assess vascular calcification, most are only utilised in the research setting and many patients have calcification that is unknown to their treating physician. All methods, except the ultrasound method, involve exposing the patient to some radiation and therefore this inherently limits their widespread usage in

<table>
<thead>
<tr>
<th>Method</th>
<th>Availability of scanner/software/training</th>
<th>Radiation level</th>
<th>Quantitative</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain X-Ray</td>
<td>** ****</td>
<td>***</td>
<td>If multiple sites— semi quantitative</td>
<td>****</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>** ****</td>
<td>*****</td>
<td>If multiple sites— semi quantitative</td>
<td>*****</td>
</tr>
<tr>
<td>Spiral CT</td>
<td>**</td>
<td>*</td>
<td>yes</td>
<td>***</td>
</tr>
<tr>
<td>Electron beam CT</td>
<td>*</td>
<td>*</td>
<td>yes</td>
<td>**</td>
</tr>
</tbody>
</table>

Table 4: Summary of available methods used to determine presence of calcification. Score system range: * Poor - **** excellent
routine practice. Although, to date, only Sevelamer has been shown to slow progression of calcification, no treatment is known to lead to regression of calcification. For this reason routine screening for vascular calcification is not undertaken in most hospitals, especially as no proven ‘treatment’ is available.

To conclude, vascular calcification and stiffness are of the utmost importance in renal disease and their presence are associated with increased mortality. The calcification is now thought to be linked to renal bone disorders and composes part of the same disease spectrum. Calcification starts early in renal impairment and to improve our patients’ survival and morbidity we need to control serum calcium, phosphate, calcium x phosphate product and PTH as tightly as possible. Hopefully the future will see the introduction of medications that stop the formation of calcification or lead to its reduction.

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2.7 Mechanisms of vascular calcification

The underlying mechanisms of vascular calcification are becoming increasingly understood. There are many circulating factors, ion transporters, metabolic enzymes, matrix and signalling molecules that are now being implicated in the vascular calcification process. These have been summarised in Table 5.

Increased phosphate is a common finding in patients with CKD and is known to be associated with mortality in dialysis patients\textsuperscript{10, 62} as well as pre-dialysis patients\textsuperscript{57, 152}. Elevated serum phosphate has been shown to induce the phenotypic change seen in VSMCs. A sodium dependant phosphate co-transporter (Pit-1) has been found to be necessary for phenotypic modulation and calcification of VSMCs in vitro\textsuperscript{153} and has been found to be up-regulated in uraemic rats with calcified arteries\textsuperscript{154}. TNAP, which is one of the genes expressed on the osteoblast like cell and is up-regulated in CKD, may affect calcification by two methods - increasing phosphate levels and reducing the level of the calcification inhibitor, pyrophosphate\textsuperscript{12}. Pyrophosphate deficiency is known to pre-dispose to vascular calcification; infantile arterial calcification, a genetic disorder, is secondary to a deficiency of PC-1/eNPP-1 (ectonucleotide pyrophosphatase phosphodiesterase) which is responsible for the generation of pyrophosphate\textsuperscript{155}.

Matrix-Gla protein (MGP) is known to be a potent inhibitor of vascular calcification and has carboxylated and under-carboxylated forms. Studies have shown that impaired carboxylation of MGP is associated with medial and intimal calcification in human sclerotic arteries but active, carboxylated MGP is found in healthy arteries\textsuperscript{156}. Warfarin is known to inhibit the carboxylation of MGP and is associated with an increased risk of calciphylaxis, a cutaneous small vessel calcification disorder. There have been no studies looking at the presence or progression of vascular calcification and the carboxylated versus under-carboxylated forms of MGP.
Table 5: Summary of vascular calcification regulatory molecules. Adapted from El-Abbadi and Giachelli\textsuperscript{12}.

<table>
<thead>
<tr>
<th>Regulatory molecules</th>
<th>Effect on calcification</th>
<th>CKD changes in molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Circulating factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>Promoter</td>
<td>Increase</td>
</tr>
<tr>
<td>Calcium</td>
<td>Promoter</td>
<td>Increase</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Promoter</td>
<td>Decrease</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>Inhibitor/promoter</td>
<td>Increase</td>
</tr>
<tr>
<td>Fetuin A</td>
<td>Inhibitor</td>
<td>Decrease/no change</td>
</tr>
<tr>
<td>Pyrophosphate</td>
<td>Inhibitor</td>
<td>Decrease</td>
</tr>
<tr>
<td>FGF-23</td>
<td>Inhibitor</td>
<td>Increase</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Inhibitor</td>
<td>Increase</td>
</tr>
<tr>
<td>HDL</td>
<td>Inhibitor</td>
<td>Decrease</td>
</tr>
<tr>
<td>LDL</td>
<td>Promoter</td>
<td>Increase</td>
</tr>
<tr>
<td>Osteoprotegerin</td>
<td>Inhibitor</td>
<td>Increase</td>
</tr>
<tr>
<td><strong>Ion Transporters/homeostasis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit-1</td>
<td>Promoter</td>
<td>Increase</td>
</tr>
<tr>
<td>TNAP</td>
<td>Promoter</td>
<td>Increase</td>
</tr>
<tr>
<td>ANK</td>
<td>Inhibitor</td>
<td>Increase</td>
</tr>
<tr>
<td>PC-1/NPP-1</td>
<td>Inhibitor</td>
<td>Decrease</td>
</tr>
<tr>
<td>Klotho</td>
<td>Inhibitor</td>
<td></td>
</tr>
<tr>
<td><strong>Matrix molecules</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix Gla-protein</td>
<td>Inhibitor</td>
<td>Increase</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Inhibitor</td>
<td>Increase</td>
</tr>
<tr>
<td><strong>Signalling molecules/pathways</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runx2/Cbfa1</td>
<td>Promoter</td>
<td>Increase</td>
</tr>
<tr>
<td>Msx2/Wnt</td>
<td>Promoter</td>
<td>Increase</td>
</tr>
<tr>
<td>BMP2/4</td>
<td>Promoter</td>
<td>Increase</td>
</tr>
<tr>
<td>BMP7</td>
<td>Inhibitor</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Promoter</td>
<td>Increase</td>
</tr>
</tbody>
</table>

FGF-23: fibroblast growth factor 23; HDL: high density lipoprotein; LDL: low density lipoprotein; Pit-1: sodium dependent phosphate co-transporter; TNAP: tissue non-specific alkaline phosphatase; ANK – major pyrophosphate plasma membrane transporter; PC-1/NPP-1: ectonucleotide pyrophosphatase phosphodiesterase;
Fetuin–A is a calcium binding glycoprotein that is synthesised by hepatocytes and is estimated to contribute about 50% of the precipitation inhibitory capacity of serum. In dialysis patients fetuin–A levels are known to be diminished and have been associated with increased cardiovascular mortality. Bone morphogenetic protein-7 (BMP7) is another calcification inhibitor and is an important regulator of skeletal remodelling and its expression is reduced in renal injury. Interestingly it has been found to reverse hyperphosphataemia and vascular calcification in mice. Additionally BMP7 increases the expression of the VSMC phenotype when added to in vitro human aortic VSMC culture. This could be an exciting potential future treatment for the renal population.

Osteoprotegerin (OPG) is a decoy receptor for the receptor activator of nuclear factor-κB (RANK), and is therefore involved in the prevention of bone resorption and vascular calcification. Activation of RANK has been associated with increased vascular calcification in vitro and there is an increase seen in OPG in the setting of CKD though this could be an adaptive mechanism in response to bone loss secondary to hyperparathyroidism or as an inhibitor of vascular calcification.
2.8 Vascular Stiffness

The artery has two main functions: to deliver an adequate supply of blood to the body tissues and to smooth out the pulsations occurring secondary to ventricular ejection. The ability of an artery to accommodate the volume of blood ejected from the left ventricle is described in terms of distensibility or stiffness. This ability of the aorta and other large arteries is vitally important as only half of the blood ejected during systole reaches the peripheries; the other half is stored and pushed forward during diastole secondary to elastic recoil. This ability to store blood is obviously hindered when arterial stiffness is present.

Arterial stiffness is determined from the pressure-diameter relationship as Young’s modulus which is characteristic of the intrinsic elastic properties of the biomaterials of the arterial wall. Young’s modulus is usually measured in the common carotid artery and is the slope of the relationship between stress and strain of arterial vessels. It is calculated by trans-cutaneous measurements of common carotid internal diameter, wall thickness and carotid pulse pressure (PP). Compliance and distensibility can then be determined from the changes in diameter during systole and by simultaneously measured PP. Clinically arterial stiffness is measured most frequently as pulse wave velocity (PWV) along a given artery (see figure 13). The most widely accepted relationship of PWV and Young’s modulus is the Moens-Korteweg equation:

\[ \text{PWV}^2 = \frac{Eh}{2r\rho} \]

Where E is Young’s modulus, r is the radius, h is the arterial wall thickness and ρ is the fluid density. Therefore the PWV is equal to the reciprocal value of volume distensibility and increases with a decrease in distensibility i.e. increased in stiffness.

Increased vascular stiffness leads to an increased pulse wave velocity as the ejection of blood into the aorta is not cushioned but propagated to other arteries. This pressure wave is then reflected at any points of structural and functional discontinuity of the arterial tree generating a reflecting wave. The forward and reflected waves are in constant interaction and are summed up in a measured pressure wave. This reflection in normal, young adults leads to a boosting effect in early diastole on coronary perfusion without increasing left ventricular after-load. With stiffer arteries this reflected wave is travelling faster and has
Figure 13: Measure of carotid – femoral (aortic) pulse wave velocity

PWV = time delay (Δt) separating the waves recorded over the carotid and femoral arteries, divided by the distance between recording sites. (Printed with permission: London et al Advances in Chronic Kidney Disease 2004:11(2); 202-209)

occurred earlier and therefore impacts on the central arteries during systole amplifying aortic and ventricular pressures during systole and reducing aortic pressure in diastole which in turn affects coronary perfusion. All this leads to increased systolic blood pressure (SBP) and decreased diastolic blood pressure (DBP). The increased after-load induces ventricular hypertrophy and impairs diastolic myocardial function and ventricular ejection. The SBP and wide PP increase left ventricular workload and hypertrophy and also accelerate arterial damage, increase fatigue, degenerative changes and arterial stiffness therefore making the situation worse and ultimately affecting cardiovascular mortality.

Another measure of vascular stiffness is Augmentation index (AIx). This non-invasive measurement is performed at the radial or the carotid artery. The waveform is analysed and the central arterial waveform is determined using a transfer function incorporating certain assumptions such as arterial diameter, wall elasticity, wall thickness and amount of branching of the connecting brachial vascular bed. In Figure 14 the typical arterial waveform can be seen.
Figure 14: Arterial waveform seen when measuring augmentation index.
P1: pressure at first systolic inflection; P2: peak systolic pressure; TR: time of wavefoot to foot of reflected wave; ΔP P2-P1; TTI: time tension index; DTI: diastolic time index

This waveform is detected peripherally using applanation tonometry. The waveform produced is calibrated against the peripheral blood pressure measured with a conventional cuff. An average waveform is then calculated from a series of recorded contiguous pulses. This peripheral waveform is then used to derive the central aortic waveform.

The augmented pressure is the difference between the systolic peak pressure (P2) and the first systolic inflection (P1). Augmentation index (Alx) is the augmented pressure divided by pulse pressure. The reported augmentation index is also corrected for heart rate as pulse pressure amplification between central and radial sites is dependent on heart rate. An increased augmentation index is related to increased vascular stiffness and to increased systolic workload for the heart. The waveform can also produce further information such as central time tension index (TTI: shaded area in Figure 14) which is calculated as the area under the systolic curve and is related to the work of the heart and oxygen consumption. Similarly the central diastolic time index (DTI: shaded area in figure 14) is the area under the diastolic curve and relates to the time and pressure for coronary perfusion. The subendocardial viability ratio (SEVR) or Buckberg Index can be calculated from these areas (DTI/TTI) and represents the ratio of supply and demand of the heart. It has been shown that layers of the subendocardium are under-perfused when the SEVR is less than 100%. AIx can be measured at the carotid or radial artery; the carotid measurements are closer in distance to the derived central pressure and therefore exposed
to less variables suggesting they may be preferable. However a study comparing radial and carotid pressure waveform measurements in 44 patients indicated that radial measurements were superior. This was due to increased inter-observer variability at the carotid, lower achievement of adequate ‘quality index’ and increased beat-to-beat variability.165

Potential underlying mechanisms for the increased stiffness in uraemia include chronic fluid overload, arterial calcifications, micro-inflammation, sympathetic nervous system over-activity, activation of the renin-angiotensin system, increased lipid oxidation, and abnormalities of the nitric oxide system.2 Arterial stiffness has been associated with even a mild deterioration in renal function, independent of blood pressure (BP) and other classical cardiovascular risk factors.166 Arterial stiffness and plasma creatinine have also been found to be positively related in untreated hypertensive patients with preserved renal function (creatinine <135ml/min). This association was independent of 24hr ambulatory BP and age. This suggests an independent link at an early stage of hypertensive vascular disease between these two parameters.167 A further longitudinal study of essential hypertensive patients found that serum creatinine is a major determinant of accelerated progression of aortic stiffness in treated hypertensive patients.168 PWV and Augmentation Index (AIx) are both important in the renal population in assessing cardiovascular risk and mortality. London et al have shown that PWV and AIx are independent predictors of mortality.6, 8 Even a small increase in PWV by 1m/s in one study lead to an all-cause mortality adjusted odds ratio of 1.39. It was found to be the strongest predictor of cardiovascular mortality; patients in the highest tertile of PWV had a 5.9 fold adjusted risk of cardiovascular death compared to patients in the lowest tertile.6 Mineral metabolism abnormalities such as serum calcium, phosphate and [Ca] x [P] have been associated with increased vascular stiffness137, 169 though no studies have shown a similar relationship with PTH.170, 171

Vascular stiffness is also closely correlated to vascular calcification89, 172, 173 especially medial calcification. This is due to the calcification restricting the distensibility of the blood vessels and therefore increasing the vascular stiffness.9 This correlation is important as it is easier, in a clinical setting, to measure vascular stiffness and therefore, give treating physicians an impression of their patients’ mortality risk.
2.9 Carotid Intima-media thickness

Carotid Intima-media thickness (CIMT) is measured using B-mode ultrasound and measures the thickness of the intima and media layers in the carotid artery. The measurement is used as a surrogate marker for atherosclerosis and detects subclinical atherosclerosis.\textsuperscript{174} Cardiovascular disease and CIMT are associated\textsuperscript{175-177} and can be used to identify patients with angiographically defined cardiovascular disease.\textsuperscript{178} This has been confirmed in a meta-analysis which suggests that a 0.1mm increase in CIMT can lead to a 10-15% increase risk of myocardial infarction and a 13-18% increase risk of stroke.\textsuperscript{176}

CIMT has been shown to be reproducible and is non-invasive\textsuperscript{179} though there has previously been a lack of standardisation of CIMT measurement. This could lead to inaccurate assumptions based on progression or regression of CIMT over time.\textsuperscript{174} Carotid intima media border identification software has now been developed\textsuperscript{180} and the measurement was standardised in 2008.\textsuperscript{181}

CIMT is increased in patients with end-stage renal disease, particularly in patients with diabetes.\textsuperscript{182,183} It has also been shown to be associated with mortality in patients with end-stage renal disease.\textsuperscript{184} Serum phosphate has been associated with an increased CIMT\textsuperscript{185} though there have been conflicting results regarding the association with iPTH.\textsuperscript{186,187}

2.10 Cardiac morphology

Cardiac structure and function are affected by the increased arterial stiffness and calcification as stated earlier. Left ventricular hypertrophy is known to predict cardiovascular mortality and is present in 75% of patients commencing dialysis.\textsuperscript{188} The mechanisms leading to poor survival in renal failure may be mediated via left ventricular hypertrophy, coupled with small vessel vasculopathy. These mechanisms alongside vascular calcification are important in the pathological process. Diminished capillary supply to the myocardium with associated fibrosis has been shown to be a predominant histopathological finding in the uraemic heart.\textsuperscript{189} Diffuse late gadolinium enhancement on cardiac magnetic resonance (CMR) scans is speculated to be due to this myocardial fibrosis. This is consistent with histopathological reports of fibrosis in post-mortem
specimens of hypertrophic hearts of patients with end stage renal disease and may relate to poor outcome.\textsuperscript{190, 191}

CMR produces high quality images which allow for accurate measurements of left ventricular mass and function. Most research on cardiac structure has utilized echocardiogram as the method of choice. Echocardiograms are cheaper, more widely accessible and more tolerable to the patient. However, CMR is now widely recognised to be a more accurate and reproducible non-invasive technique than echocardiogram for the assessment of cardiac structure and function. CMR also requires a greatly reduced sample size to detect significant differences.\textsuperscript{192, 193} Differences which cannot be reliably assessed by echocardiogram become technically feasible to detect, such as changes in ejection fraction as small as 3-5\%. Utilisation of CMR to precisely measure left ventricular mass, volumes and function would therefore enable detection of even small changes in the left ventricle in a small population. Studies have found that the normal range of LVMI is much lower when measured by CMR compared to echocardiography and therefore the two measurements are incomparable.\textsuperscript{194}

The use of echocardiogram to measure left ventricular mass is associated with inherent sources of error. Both the M-mode and 2D area-length formulae assume a fixed geometric shape. CMR standard 3D technique has been compared to cube-function and area-length measurements in 212 healthy subjects. The cube-function and area-length formulae and their assumption of geometric shape resulted in significant variation in left ventricular mass estimates compared with direct measurement by CMR using the 3D technique. It was concluded that the 3D technique was preferable in small studies that involved serial measurements.\textsuperscript{195}

Biomarkers suggestive of cardiac damage are increased in renal disease and are associated with abnormal cardiac morphology.\textsuperscript{196, 197} Troponin T has been associated with left ventricular hypertrophy in patients with end-stage renal disease.\textsuperscript{196} An increased level has also been associated with heart failure\textsuperscript{198} and a more recent study suggests that the serum Troponin T level is an independent predictor of cardiac events in patients with CKD.\textsuperscript{199} N-terminal Pro-Brain Natriuretic Peptide (NT-ProBNP) is another important biomarker and is used in the management of heart failure.\textsuperscript{197} NT-proBNP has been shown to be elevated in
stable CKD patients only when there is concurrent left ventricular hypertrophy\textsuperscript{200} and a close correlation between serum BNP and left ventricular mass has been shown in haemodialysis and peritoneal dialysis patients.\textsuperscript{201}
2.11 Clinical management of disturbances of calcium and phosphate metabolism in dialysis patients

Dr James Heaf (Dept of Nephrology B, Herlev Hospital, University of Copenhagen, Denmark) and I originally wrote a review article with this title which was published in Nephrology Dialysis Transplantation Plus in 2009 (2(4): 267-272) but as the medications and evidence have progressed since publication this article has been adjusted and up-dated for inclusion in this thesis.

Vascular calcification has been associated with increased phosphate and ingested calcium load and has also been shown to predict mortality. Although calcium and phosphate regulation is thus an integral part of the management of all the complications and components of CKD-MBD it is not the only answer. The primary objectives are the avoidance of hyperphosphataemia, hypercalcaemia and also hypocalcaemia with its associated risks of cardiac arrhythmias and hyperparathyroidism. Which treatment to use is more debatable due to the interactions with bone and vascular complications. The medications will be discussed in the following sections:

The Case for Calcidiol (25-hydroxy vitamin D)

Native vitamin D precursors are initially ingested or formed in the skin secondary to exposure to UVB light, in the form of D3 (cholecalciferol). D2 (ergocalciferol) is a commonly used artificial dietary substitute. D2 or D3 is then 25 hydroxylated in the liver to 25-hydroxy vitamin D (calcidiol) and stored until required when the highly regulated 1-alpha hydroxylation occurs to form the active 1,25 dihydroxy vitamin D (calcitriol). Renal patients are known to have both calcidiol and calcitriol deficiency which develops over the course of CKD and is highly prevalent in dialysis populations. While normalization of 25-hydroxyvitamin D levels to a level >75 nmol/L (>30 µg/L) is recommended for healthy individuals and patients with CKD stages 3 & 4, supplementation in CKD stage 5 has traditionally not been considered indicated, due to reduced or absent hydroxylation to calcitriol. There are a number of reasons why this attitude may be erroneous. Investigations show that calcidiol and calcitriol concentrations are correlated even in severe uraemia, suggesting that increased substrate will help to normalize calcitriol activity. Furthermore, vitamin D receptors are widely distributed throughout the body, and
extra-renal 1\(\alpha\)-hydroxylase activity is found in several cells including bone, macrophages, endothelium, prostate, breast and brain. In the presence of uraemia, extra-renal production of calcitriol is upregulated. Vitamin D deficiency is associated with a wide variety of diseases including several cancers, type 2 diabetes mellitus, pneumonia, tuberculosis, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, hypertension and atherosclerosis. A randomized controlled trial has demonstrated a protective effect of cholecalciferol against cancer and low levels of 25-hydroxycholecalciferol are associated with excess mortality in dialysis. Cholecalciferol supplementation has been shown to reduce PTH in CKD stage 3 but has little or no effect in stages 4 & 5. However an article has shown that the combination of cholecalciferol and calcium in post-menopausal women can reduce PTH in moderate CKD (stage 3-4); patients with severe renal impairment were excluded. In conclusion, D3 or D2 supplementation may be advantageous, both by stimulating 1,25(OH)\(_2\)D production even in severe uraemia, and in providing a substrate for extra-renal effects independent of renal function and renal 1\(\alpha\)-hydroxylase activity. Caution mandates that normalization of calcidiol levels should also be a goal for CKD stage 5 patients: if it works, it may help prevent atherosclerosis, hypertension, immune deficiency and cancer; if it doesn’t, there is no harm done. The lack of evidence regarding supplementation continues though further research is on-going in many populations.

**Phosphate control**

The first line therapy for managing increasing serum phosphate levels is the initiation of a phosphate restricted diet. However many patients, particularly the young, find this restrictive and interfering with their normal lives. Phosphate binders are then introduced and, depending on the available budget, there are increasing variations to choose from. Table 6 lists the different phosphate binders currently available with a summary of the benefits and problems with each one.

Historically, aluminium hydroxide and calcium containing binders were used to control phosphate. However aluminium is now known to accumulate with prolonged exposure and causes severe toxicity including encephalopathy, myopathy and decrease in bone turnover and is therefore no longer recommended for long term or regular use. Calcium-containing
binders are currently the most common first line phosphate binders used in clinical practice. The ingested calcium load has now been shown to be associated with the presence of increased vascular calcification and adynamic bone disorder.\textsuperscript{101} The National Kidney Foundation - Disease Outcomes Quality Initiative guidelines (K/DOQI),\textsuperscript{143} published in 2003, recommended a maximum intake of 2g elemental calcium per day, of which only 1.5gm should be due to a prescribed phosphate binder (corresponding to about 3 calcium carbonate or 6 calcium acetate tablets daily). This guideline was opinion based as there is no data to suggest that a certain amount of calcium is either safe or detrimental. Secondary to these guidelines a policy of a more sparing use of calcium binders wherever funding allows was followed. This has lead to an increased use of the alternative, more expensive, calcium-free binders which are now available on the market.

The non-calcium containing phosphate binders currently on the market include Fosrenol® (Lanthanum Carbonate), a metal based binder and Renagel® or Renvela® (Sevelamer hydrochloride/carbonate), a resin based binder; the carbonate form launched most recently in 2010 in the UK. Renagel has been shown to attenuate the progression of vascular calcification when compared to calcium based binders.\textsuperscript{103} This could be in part due to the lipid lowering effect of Renagel because, when similar lipid profiles are achieved with a statin, no differences were found between calcium binders and Renagel.\textsuperscript{104} Both of these clinical trials however achieved differing PTH control between each arm and therefore may not be comparable.

One study suggested a survival benefit with the use of Renagel over calcium binders.\textsuperscript{105} However a large randomised trial (Dialysis Clinical Outcomes Revisited, DCOR) showed no survival advantage in the overall prevalent dialysis population but only a benefit in patients older than 65 years.\textsuperscript{106} From the above discussion, one would expect any survival advantage of sevelamer (or lanthanum) to be limited to patients with a tendency to hypercalcaemia; surprisingly, no study analysing this question has yet been published.

Despite these somewhat ambiguous benefits Renagel does have some disadvantages. The hydrochloride compound can cause acidosis especially in pre-dialysis patients. This problem is now overcome with the use of the carbonate form, Renvela. The Sevelamer
<table>
<thead>
<tr>
<th>Phosphate binder</th>
<th>Generic name</th>
<th>Common side effects</th>
<th>Potential disadvantage</th>
<th>Potential benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosex</td>
<td>Calcium acetate</td>
<td>Nausea</td>
<td>Increase calcium load leading to increased calcification</td>
<td>Cost</td>
</tr>
<tr>
<td>PhosLo</td>
<td></td>
<td>Constipation, diarrhoea</td>
<td></td>
<td>Less calcium than other calcium binders</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypercalcaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcichew</td>
<td>Calcium carbonate</td>
<td>Nausea</td>
<td>Increase calcium load leading to increased calcification</td>
<td>Cost</td>
</tr>
<tr>
<td>Calcium 500 Titralac</td>
<td></td>
<td>Constipation, diarrhoea</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypercalcaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium acetate + Magnesium carbonate</td>
<td>Nausea</td>
<td></td>
<td>Lower calcium load than other calcium-containing phosphate binders. Suitable for patients with normo- or hypomagnesaemia, e.g. PD patients</td>
</tr>
<tr>
<td>OsvaRen</td>
<td></td>
<td>Constipation, diarrhoea</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypercalcaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypermagnesaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renagel</td>
<td>Sevelamer hydrochloride</td>
<td>Nausea</td>
<td>Expensive</td>
<td>Attenuates progression of calcification compared to calcium binders</td>
</tr>
<tr>
<td>Renvela</td>
<td>Carbonate</td>
<td>Indigestion</td>
<td>Acidosis in pre-dialysis – hydrochloride form only</td>
<td>Mortality benefit in over 65yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Multiple tablets – compliance poor</td>
<td>Lowers LDL cholesterol</td>
</tr>
<tr>
<td>Fosrenol</td>
<td>Lanthanum carbonate</td>
<td>Nausea</td>
<td>Metal and long term effects unknown</td>
<td>One tablet only per meal increasing compliance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diarrhoea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alucaps</td>
<td>Aluminium hydroxide</td>
<td>Nausea</td>
<td>Aluminium toxicity including encephalopathy and bone disease</td>
<td>Excellent phosphate binder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diarrhoea</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Summary of phosphate binders used in renal disease
tablets are also large and some patients have difficulty tolerating them which can lead to reduced compliance though a powder, dissolvable in water, is also available.

The other non-calcium phosphate binder is lanthanum carbonate and involves one tablet to be taken per meal. This regimen was shown to be more palatable to some patients and may improve compliance.\textsuperscript{215} However lanthanum also has potential disadvantages in that it is based on an elemental metal. Due to the previous problems with aluminium, regulatory authorities and nephrologists alike have been guarded of its potential harm. Lanthanum absorption is very low compared to aluminium and has a hepatic excretory mechanism as compared to aluminium’s renal excretion. It has not been shown to cross the blood-brain barrier\textsuperscript{216} and long-term clinical and bone studies are reassuring.\textsuperscript{217} As yet no large survival or calcification studies have been published examining the effects of Lanthanum.

A recently introduced product, Osvaren®, supplements calcium with magnesium carbonate, achieving equivalent phosphate binding at a lower calcium load; it is thus intermediate between calcium-containing and calcium-free phosphate binders. It is particularly suitable for patients with hypomagnesaemia e.g. peritoneal dialysis patients.

**PTH, vitamin D and calcium control:**

Other compounds used to maintain calcium and phosphate in the recommended target range are vitamin D analogues and calcimimetics. These treatments are mainly used to lower PTH levels but as calcimimetics reduce calcium and phosphate levels and vitamin D analogues increase them these drugs are used in different situations or more commonly synergistically. Calcitriol and 1-alfacalcidol are the traditionally used vitamin D analogues in renal disease providing replacement of the active 1,25 hydroxy vitamin D. They act by restoring a negative feedback to the parathyroid gland, reducing PTH. However they also act at receptors in the gastrointestinal tract which leads to increased absorption of calcium and phosphate. This can lead to less efficient suppression of PTH as the doses are restricted by hypercalcaemia and hyperphosphataemia. A newer vitamin D analogue, paracalcitrol, claims to lead to a lower incidence of these problems. However, in the randomised trial between calcitriol and paracalcitrol the frequency of hypercalcaemia and hyperphosphataemia was similar but suggested paracalcitrol lead to less persistent hypercalcaemia.\textsuperscript{107} This reduction of persistent hypercalcaemia may still have an impact on
morbidity and mortality, one retrospective study has shown a survival advantage in haemodialysis patients over calcitriol. Surprisingly, the excess risk of hypercalcaemia associated with calcitriol may not apply to 1-alfacalcidol: at equivalent doses, intestinal calcium absorption is 80% lower with 1-alfacalcidol treatment compared to calcitriol.

No prospective randomised mortality studies have been completed as yet.

Several retrospective studies have shown a survival advantage of active vitamin D supplementation in dialysis and the advantage is independent of dose. In the study by Teng et al the effect was equally present in patients with hypocalcaemia, hyperphosphataemia and hypoparathyroidism. This raises the interesting possibility that the excess mortality seen in patients with adynamic bone disease is iatrogenic, due to conventional recommendations to deny these patients therapy with active vitamin D sterols. Similarly, both low and high 1,25(OH) vitamin D levels are associated with increased carotid intima-media thickness and calcification in children on dialysis. Thus, the existing epidemiological evidence suggests that all patients with CKD stage 5 should be treated with a small dose of active vitamin D regardless of calcium, phosphate and PTH. However, this does not make the control of calcium and phosphate easier.

**Cinacalcet**

Calcimimetics are a class of drugs which act at the calcium sensing receptor to reduce PTH secretion. Cinacalcet, a type II calcimimetic (synthetic phenylalkylamime), is licensed for use in SHPT. The therapy has the added attraction of lowering serum calcium and potentially serum phosphate and therefore has a role where vitamin D analogues fail. This therapy is only licensed for dialysis patients as it can lead to more severe hypocalcaemia and can increase phosphate in CKD stage 3-4.

The main site of action of the calcimimetics (calcium-sensing receptor agonist) is on the CaSR on the chief cell in the parathyroid gland. The CaSR (see Figure 15) is a G protein-coupled receptor and has 3 main structural domains; an N-terminal extracellular domain which contains the calcium binding site, a trans-membrane domain and an intracellular domain which mediates intracellular signalling. Activation of the CaSR is also known to engage mitogen-activating protein kinase C cascade through both G protein linked phospholipases and tyrosine kinases eventually leading to a decrease in PTH secretion.
The CaSR along with the Vitamin D receptor (VDR), directly mediate the changes in gene transcription and hormone synthesis within the parathyroid gland to facilitate calcium homeostasis. Cinacalcet acts by inducing a conformational change of the receptor leading to enhanced calcium sensitivity.\textsuperscript{226, 227} Both the CaSR and VDR receptors are down-regulated with the formation of parathyroid gland hyperplasia that occurs in the development of secondary hyperparathyroidism.\textsuperscript{228, 229} Calcimimetics are also known to increase the expression of the CaSR and VDR leading to increased responsiveness of the parathyroid cell and to reduce parathyroid cell hyperplasia.\textsuperscript{230-232}

Figure 15: The extracellular CaSR. The CaSR consists of three molecular domains: an extracellular ligand-binding domain; a transmembrane domain and a cytosolic domain, which mediates intracellular signalling. Reproduced from NDT Plus (2008) 1 (suppl 1): i7-i11. doi: 10.1093/ndtplus/sfm038 with permission from Oxford University Press on behalf of the ERA-EDTA.\textsuperscript{225}

The CaSR is present in many cells throughout the body including cardiac myocytes, endothelial cells and vascular smooth muscle cells and hence there is the possibility that increased activation may affect the pathophysiology of cardiovascular disease.\textsuperscript{233}

Cinacalcet is currently the only calcimimetic licensed for clinical use and is rapidly absorbed from the gastrointestinal tract with the maximum plasma concentration being achieved approximately 90mins after oral administration.\textsuperscript{233} Cinacalcet is mainly protein-
bound (93-97%) and is metabolised in the liver via CYP3A4, CYP2D6 and CYP1A2 into inactive metabolites. The half-life of cinacalcet is 30-40 hours but this can be prolonged in the presence of moderate-severe liver impairment and therefore these patients should be closely monitored.

The plasma concentration of iPTH has been shown to decrease by as much as 60-70% within a 2-4 hour period after a single oral dose of cinacalcet. The size and length of response is mainly dose dependent. The pharmacokinetic and pharmacodynamic profiles of cinacalcet are not affected by haemodialysis though dosing is often given more than 12 hours before haemodialysis treatment to aid PTH monitoring.

Rat studies have shown that calcimimetics can halt the formation and even lead to regression of vascular calcification. In rats, calcimimetics were also found to reduce the progression of renal failure and the development of cardiovascular risk factors and structural abnormalities. Calcimimetics may affect calcification via an indirect or direct mechanism. As calcium, PTH and phosphate have been shown to induce calcification, the reduction of these biochemical markers may aid regression. Direct effects may be via the CaSR and may lead to increased production of MGP or increased activation of phagocytic cells.

In humans, meta-analysis of placebo controlled trials with Cinacalcet have shown a significant reduction in fracture, parathyroidectomy and cardiovascular hospitalisation. The ADVANCE study compared cinacalcet with low dose vitamin D sterols and variable dose Vitamin D sterols and was published in 2009. The primary outcome was percentage change in the agatston score of coronary artery calcification which is a quantitative measure of vascular calcification. The primary outcome did not reach significance (P=0.07) with a 24% change in the cinacalcet arm and 31% change with variable vitamin D though baseline phosphate differed between the arms. In multivariate analyses, adjusting for PTH, calcium, phosphate, years on dialysis and baseline calcification, the difference in coronary calcification between treatment arms was significant (P=0.006). When coronary calcification score was measured by the volume method this also led to a significant difference in calcification between arms (P=0.009). Overall there was a consistent reduction of progression of calcification in coronary arteries, thoracic aorta and valvular calcification in the cinacalcet arm suggesting
a potential benefit. The EVOLVE study\textsuperscript{112,114} has also failed to show a survival benefit with the pre-specified intention to treat analysis ($P=0.11$). Unfortunately there were differences in age despite randomisation and when differences at baseline were adjusted for in the analyses then the cinacalcet arm showed a reduction in the primary end-point which included survival, first myocardial infarction, hospitalisation due to unstable angina, heart failure or peripheral vascular event ($P=0.008$). During the EVOLVE trial there was also substantial dropout from the trial with over 600 patients (440 in the placebo arm) being withdrawn after starting on commercial cinacalcet. When the rate of parathyroidectomy, transplantation and study drug use is taken into account cinacalcet again showed a benefit in reduction in the primary composite end-point ($P=0.03$). This data, although not pre-specified, does suggest some potential cardiovascular benefit with cinacalcet.

Cinacalcet treatment may lead to improvement in bone markers.\textsuperscript{150} There have been two studies in dialysis patients that have shown improvement in bone mineral density with the use of Cinacalcet.\textsuperscript{151,242} In rats, bone volume has also been shown to improve with calcimimetic R-568 though only when given with calcium supplementation.\textsuperscript{243}

As this medication is expensive it is only commonly used in the difficult patients whose secondary hyperparathyroidism cannot be controlled with vitamin D analogues alone, and where the alternative is often parathyroidectomy. The National Institute of Clinical Excellence have not approved its use in dialysis patients unless the iPTH is greater than 800pg/mL and a parathyroidectomy is contraindicated.\textsuperscript{244}

\textit{Parathyroidectomy}

Parathyroidectomy is reserved for these patients who have developed an adenoma and/or have tertiary hyperparathyroidism. Associated hypercalcaemia and hyperphosphataemia will usually be cured by the operation. This procedure has been associated with a survival advantage compared to not having a parathyroidectomy, but not to calcimimetic therapy.\textsuperscript{245} The use of cinacalcet in these severe patients varies from country to country and serum iPTH can be lowered with this therapy also.
Changing the calcium content of dialysate can also be utilized to aid calcium control. The pursuit of plasma calcium targets can lead to use of a high-calcium dialysate (>1.4 mmol/L) in hypocalcaemic patients and conversely low-calcium dialysate in patients with high/high-normal calcium levels.\textsuperscript{143}

Using the medications that were discussed, management of each patient can be individually tailored to control their bone chemistry parameters. However, despite the array available, many patients are still not reaching the targets. This may be due to a number of reasons including inadequate phosphate binder efficacy, suboptimal physician attention to the problem and poor patient compliance.

\textit{Summary}

Good management is still a goal difficult to achieve and no single treatment is the answer. Increased understanding of the underlying mechanisms will hopefully improve with time and our management may change accordingly. There is still much research to be done in this area and many questions remain unanswered.
3. AIMS AND OBJECTIVES
As has been emphasised in the foregoing sections, CKD-MBD is a common problem for the CKD patient and the complications develop early in the course of chronic kidney disease. This complication of renal disease impacts on the morbidity and survival of our patients and yet we still do not understand the full extent of its effects or how to optimally manage the condition. The aim of this thesis is to investigate further the impact of CKD-MBD on the renal population and to establish if there are associations with modifiable risk factors.

The first stage was therefore to perform epidemiological studies to investigate the associations between serum phosphate, a well known factor in the development of vascular complications, and survival. Modifiable risk factors associated with increased augmentation index were also explored. Thereafter the focus changed to determine the effects of calcimimetics on bone and cardiovascular complications in the setting of a randomised trial. This data was also used to explore whether tight control of SHPT by any treatment suggested any further cardiovascular benefits.

If we are to improve clinical outcomes we also need to know what the laboratory measures and where the ‘targets’ should be set for the management of CKD-MBD. Given the variability of intact PTH assays, the impact of the variation on regional audit performance was explored to determine if clinical management did affect performance.

**Experimental chapter 1:**

Given the association of serum phosphate and mortality in dialysis populations I was keen to explore this further in the pre-dialysis and earlier CKD settings. The KDOQI guidelines use studies in dialysis patients to determine target ranges for pre-dialysis patients. Dialysis patients are thought to show a U-shaped relationship between phosphate and survival though this may not exist in early CKD. The aim was to use the Chronic Renal Insufficiency Standards Implementation study (CRISIS) to explore this relationship.
Experimental chapter 2:

Vascular stiffness as measured by pulse wave analysis and pulse wave velocity have been associated with an increased mortality in dialysis patients.\(^6,8\) There have also been some data suggesting that arterial stiffness changed with declining renal function.\(^{166, 246, 247}\) We decided to investigate the CRISIS data to determine if phenotypic and biochemical features were associated with an increasing radial AIX.

Experimental chapter 3:

The aim of this open-label randomised controlled trial was to examine the effect of cinacalcet with standard therapy compared to standard therapy alone on bone and cardiovascular parameters in haemodialysis patients by achieving equivalent control of secondary hyperparathyroidism between treatment arms.

The primary objective was to determine if cinacalcet with standard therapy for CKD-MBD slowed progression of vascular calcification compared to standard therapy alone.

The secondary objectives were to determine if cinacalcet with standard therapy for CKD-MBD reduced progression of vascular stiffness, CIMT, left ventricular hypertrophy, and bone mineral density changes compared to standard therapy alone. Changes of biomarkers were also analysed between treatment arms.

Experimental chapter 4:

As equivalent control of iPTH and phosphate was achieved between treatment arms in the randomised controlled trial the data was re-analysed to determine if targeting tight control of intact PTH and phosphate led to an improvement in cardiovascular outcomes. This was performed as post-hoc analysis which might then provide hypothesis-generating information.

The impact on survival of baseline cardiovascular parameters and the change in biochemical parameters was also explored.
Experimental chapter 5:

In this study I aimed to highlight the iPTH assay variability in the North West of England and to determine if this variability would affect clinical management. I also explored whether conversion factors could be established to align the different assay methods used in the North West and whether these would be consistent with other published conversion factors. Further analyses were performed to determine if the variation in iPTH targets achieved in regional audit data could be attributed to the iPTH assay used and whether, if corrected, this would alter performance of individual renal units.
4. GENERIC METHODS
These methods incorporate methodology relevant to more than one experimental chapter. The Chronic renal insufficiency standards implementation study relates to experimental chapter 1 & 2. Experimental chapters 1-4 have used some or all of the investigations that are detailed below.

Further methods unique to each experimental chapter are detailed within the chapter where necessary.

4.1 Chronic Renal Insufficiency Standards Implementation Study

Ethics approval

The CRISIS study was approved by the Salford and Trafford Local Research and Ethics Committee and the Salford Royal Hospital NHS Foundation Trust Research and Development department in 2002.

Study design and methodology

CRISIS is designed and run as a prospective observational epidemiological study which continues to collect data annually from non-dialysis patients with Chronic Kidney Disease stage 3-5 who are attending the renal out-patient department of Salford Royal Foundation Trust. The study started in 2002 and recruits patients who have any form of kidney disease and who are able to give informed consent. The study provides high quality data which can be examined to determine contributing factors and associations with cardiovascular events and risk and progression of renal disease.

At recruitment patients undergo a detailed questionnaire which collects data regarding co-morbidities, functional assessments, quality of life and previous hospitalisations. All medication history is recorded. Blood samples are taken, some are processed for routine laboratory markers as per normal clinical practice and further samples are taken for DNA and biomarker analysis and are processed and stored at -80°C.

Since 2005 patients have also undergone a vascular stiffness assessment by measuring augmentation index (Alx) at the radial artery using the SphygmoCor® (Atcor Medical,
Australia). This measurement was chosen as it can be assessed at the radial artery and does not require the patient to become undressed. This enables the more elderly patients to still have regular measurements and allows a larger number of patients to be studied within the out-patient setting. The AIx assessment along with phenotypic data and plasma / serum samples are repeated annually up to the time at which a patient starts renal replacement therapy, dies or no longer requires secondary care nephrological follow-up. Cause of Death for all patients is accessed via the Office of National Statistics.

**Inclusion Criteria**

- Estimated eGFR >10 ≤60mL/min/1.73m$^2$ at first review.
- Patients need to be able to give written consent to take part

A 50% random sample of all patients referred for a renal opinion to Salford Royal Hospital for an opinion are approached

**Exclusion Criteria**

Patients with previous evidence of renal replacement therapy (dialysis or kidney transplant) are excluded

**Data collection**

Phenotypic data is collected by a face-to-face interview between a trained research nurse and each patient within the out-patient setting. This interview occurs alongside their routine clinic visit. All biochemical parameters are analysed at Salford Royal Hospital laboratories as part of their normal clinical monitoring. Estimated GFR is determined using the abbreviated MDRD equation

\[(186 \times (\text{Creat} / 88.4)^{-1.154} \times (\text{age})^{-0.703} \times (0.742 \text{ if female}) \times (1.212 \text{ if black})^{248}\]

The data is stored within an access database and selected data can be accessed to answer each individual research question.
4.2 Investigations

4.2.1 Calcification score

Coronary and abdominal aorta calcification scores (experimental chapters 3 & 4) were measured using images from CT scans performed by a qualified radiographer on GE CT Lightspeed 16 slice scanner; during this assessment the QCT images were also acquired. To reduce cardiac motion artefacts images were acquired using electrocardiograph (ECG) gating with an exposure time of 100ms. Using the GE software this allowed images to be acquired at the same point in the cardiac cycle, therefore eliminating replicated images. The cardiac images were acquired according to the Central Manchester Foundation Trust Radiology department protocol. The abdominal aortic images were acquired from the QCT images and enabled the radiation dose to be lower.

All the calcification images were analysed using SmartScore software and scored according to the validated Agatston method. The Agatston method uses a threshold of 130 Hounsfield units (HU) to distinguish between calcification and noise produced during the scan. The plaques are ranked according to the HU density (1=130-199HU, 2 = 200-299HU, 3= 300-399HU, 4 ≥ 400HU) and this is then multiplied by the area of the plaque. The score for each patient is determined by the sum of all plaque numbers. Although a calcification score of 0HU is normal there is no documented value at which a person is counted as abnormal. I propose a calcification score of <30HU would suggest minimal calcification was present.

All scans were reported by 2 independent people who were blinded to the randomisation outcome and blood results. The scores were reviewed to ensure agreement between the reports and scans were re-reviewed if there was a >10% variation in results. The average of the two results for each area (coronary and aorta) was calculated and used for further analysis. Total calcification score was calculated was the sum of the coronary and aortic calcification score.
4.2.2 Augmentation Index measurement at Radial Artery

In the CRISIS study (experimental chapter 2) patients were settled in a quiet room for 10 minutes after which two blood pressure measurements were taken via the cuff and Korotkov method. If blood pressure readings differed by more than 5mmHg, a 5 minute interval was introduced and further BP readings were performed. The second BP value was then entered into the SphygmoCor® (AtCor Medical) device. All AIX measurements were performed on the radial artery of the non-dominant hand. A Millar micromanometer was used to measure pressure change within the artery using applanation tonometry. The software automatically adjusts the AIX measurement to a heart rate of 75 beats per minute. Consecutive readings were taken and to ensure results reach quality standards the following criteria were applied:

- Waveform recordings were visually satisfactory
- A minimum of 11 seconds recording time was required

Readings were valid if:

- Pulse height, diastole length and pulse length differences were ≤ 5% variation
- Mean pulse height > 80mV (quality index 80% from the SphygmoCor software)
- Aortic T1 was between 80-133 ms and ejection duration between 200-400ms.
- A minimum of two good quality AIX readings that were within 10% of each other.

The average of the two readings was taken as the representative value for the patient. The readings for the CRISIS study were taken by a qualified nurse or medical persons trained in using the equipment.

During the cinacalcet RCT (experimental chapters 3 &4) the patients had their weight and height recorded prior to the measurement. The measurement was taken post dialysis at the patients’ dialysis unit. The patient lay semi recumbent and relaxed for 10 minutes prior to the recordings being taken. The post dialysis blood pressure was utilized for the scans and the measurements were taken from the radial artery of the non-dominant arm. If an arterio-
venous fistula was present on the non-dominant arm the dominant arm was utilized. The same method and quality measures were used as described above. These scans were performed by me and I was not blinded to the randomisation.

4.2.3 Pulse wave velocity

The same software and equipment used for the pulse wave analysis was used to perform a carotid-femoral pulse wave velocity measurement (experimental chapters 3 & 4). Height, weight and blood pressure measurements were inputted as before. ‘Carotid’ was selected as the proximal artery and ‘femoral’ as the distal artery. The proximal measurement was the distance measured in millimetres from the sternal notch to the site of the probe on the carotid artery using tape measure. The distal distance was the distance from the sternal notch to the groin crease at the point where the femoral artery was studied. The software required entry of these measurements and subtracted proximal from distal automatically to give the path length. Three ECG leads were attached to the participant’s chest (positions A, B and C: figure 16) to ensure that the main electrical deflection is positive (i.e. upwards). If the ‘R’ wave did not have a positive deflection then the electrode at the C position and another electrode were exchanged to produce a positive deflection.

Figure 16: Electrode placement to enable ECG tracing to be acquired during measurement of carotid-femoral pulse wave velocity

The intersecting tangent algorithm was used and this determines the point yielded by the intersection of a line tangent to the initial systolic upstroke of the pressure tracing and a horizontal line through the minimum point. A measurement of the carotid artery waveform followed by a measurement of the femoral waveform was performed and the capture time was 10 seconds for both sites. For discerning the quality of the PWV waveforms, the ‘Fem-Car’ distance calculation in m/s was less than 6. Two measurements were taken and were required to be within 10% variation with all quality parameters.
satisfied. If these quality assurance measures were not reached then further measurements were taken until achieved. For the RCT this measurement was performed by me and I was not blinded to the randomisation.

4.2.4 Cardiac Magnetic Resonance scan

The CMR scans were performed at the Wellcome clinical research facility and results shown in experimental chapters 3 & 4. Each CMR scan was performed by a qualified radiographer using a Philips Intera 1.5T imager (release 11). This utilised a phased array chest coil and was ECG gated with retrospective ECG triggering using fast imaging (short repetition time <3.5ms) with steady state progression. The Philips cardiac software package was used for the acquisition of images. All scans were performed on a non-dialysis day and all occurred at about the same time of day for all participants (12-3pm). If participants were unable to enter the CMR scan due to claustrophobia then they were allowed to continue in the study.

The sequence of images was as follows:

1. Scout images: Axial scout images used to obtain left ventricular vertical long-axis cine by aligning the LV apex with the centre of the mitral valve at end expiration. (see Figure 17: Still image showing ventricular long axis 4 chamber view of the heart)

2. Volumetric data: A short axis view was obtained parallel to the mitral valve, halfway between mitral annulus and LV apex at end expiration and was aligned passing just above the inferior papillary muscle and through the lower anterior right ventricular free wall where it met the inferior wall. A diastolic image at end-expiration provided the reference image on which a stack of contiguous short-axis slices were positioned, with the first slice positioned at the atrio-ventricular ring, and the last slice covering the apex. Breath-hold short-axis cine sections were acquired from the atrio-ventricular ring to the apex, with 6.0-mm section thickness and a 4.0-mm gap and one or 2 sections per breath hold depending on patient abilities and scanner speed. End-systole was defined visually as the phase with the smallest LV volumes. In selection of the most basal slice for left ventricular analysis, slices were considered to be within the left ventricle if the blood volume was surrounded by 50% or more of ventricular myocardium. The apex was the most apical slice in that
myocardium remained visible for analysis. On each end-diastolic frame, endocardial and epicardial borders were manually traced, and with an endocardial border was traced on the end systolic frame. Papillary muscles and trabeculations were included with the left ventricular mass while right ventricular trabeculations arising from the interventricular septum were excluded. Right ventricular volumes were obtained from true trans-axial cine sections. From these values end-diastolic and end-systolic volumes were calculated as well as left ventricular myocardial mass determined by multiplying myocardial tissue volume by the specific gravity of 1.05.

Figure 17: Still image of ventricular long-axis showing 4 chamber view of the heart

All images were reported by a single reader who was qualified to interpret the scans and was blinded to the randomisation output and blood results for each participant.
4.2.5 *Carotid intima-media thickness*

The participant was examined while lying semi-recumbent post dialysis within their dialysis unit (experimental chapters 3 & 4). The study number and date of examination were recorded on the SonoSite® machine. After palpation of the right carotid pulse, the SonoSite vascular probe was placed transversely to first identify the bifurcation of the right carotid artery. The probe was then rotated to image the carotid artery 1cm below the bifurcation. This image was then enlarged and the carotid-intima media thickness measurement was taken. The measurement was between the 2 echogenic lines seen which have been validated to be the lumen-intima interface and the media-adventitia interface.\(^2\)\(^5\)\(^1\)\(^,\)\(^2\)\(^5\)\(^2\) The image was labelled as ‘right’ and then the data transferred to computer. This process was repeated for the left carotid artery. The average of the left and right measurement was used in the data analysis.

The scans were reported by me and I was not blinded to randomisation.

4.2.6 *Bone Mineral Density measurements*

The DXA and pQCT scans were performed in the Clinical Radiology Department of the University of Manchester. The QCT of the spine was performed at the Central Manchester Foundation Trust Radiology department. All scans were performed by a qualified radiographer.

All patients were scanned according to the standard procedures for each department. Ionising Radiation (Medical Exposure) Regulations were followed at each institution.

On arrival in the Clinical radiology department the participants completed a questionnaire and height and weight were recorded. All participants were then scanned using a Lunar Prodigy DXA machine. Images were acquired of the hip; unless contra–indicated (e.g. hip replacement) the left hip was routinely selected. The subject was asked to lie supine, in the midline of the scanning table. The arms were placed on the subject’s chest away from the area to be scanned. A translucent positioning aid was placed between the subject’s feet to abduct the legs approximately 15° from the midline. The radiographer internally rotated
the leg through 25°. To maintain the leg in this position, the foot was secured to the positioning aid using Velcro straps. Using the positioning lights, the scanner arm was centred over the femur, 2cm below the symphysis pubis of the pelvis. The radiographer then began the scan sweep to cover an area 20mm below the lesser trochanter to 30 mm above the greater trochanter.

The spine was then scanned, from the supine position, with the patient lying along the midline of the scanner table; the subject’s knees flexed over a 90°- support pad. The subject’s arms were positioned by their side. Using the positioning lights, the scanner arm was centred in the midline 1.5cm below the iliac crests of the pelvis. The radiographer then began the scan sweep to start from the middle of the 5th lumbar vertebra and to end at the 12th thoracic vertebra, once ribs have been identified.

The pQCT was performed with the participant sitting using a Stratec XCT 2000 machine. The non-dominant arm of the participant was used for the scan unless an arterio-venous fistula was present on that arm in which case the dominant arm was utilised. The patients muscle strength was assessed and the length of forearm measured from the ulna styloid process to the tip of the elbow, using a standard measuring device. This was then used to determine the 50% and 4% positions for the scan. The subject sat with selected arm resting on the scanner arm support, with the wrist in the centre of the scanner gantry and the hand resting on the hand support. The elbow is flexed at right angles and rests in the elbow support. The scanner gantry is moved until the positioning light is approximately 1cm proximal to the ulnar styloid process. A clamp is applied to the wrist and a Velcro strap placed around the elbow for further support and to prevent movement. After this scan is completed the supports are removed and the arm is placed further through the gantry until the marked 50% line is level with the positioning light. The clamp is again placed around the arm for support and to prevent movement. After data acquisition, the subject’s arm is removed from the scanner gantry.

The pQCT of the radius also provided information on cortical area and thickness. Other parameters derived from pQCT include periosteal circumference, endosteal circumference, moment of inertia, stress-strain index, cross-sectional muscle area and total (radius and ulna) bone area.
The QCT occurred at the same time as the calcification quantification assessment. The images were acquired using a GE Lightspeed 16 slice scanner and results analysed with the Mindways software.

All bone mineral density results were reported by one qualified professional who was blinded to the randomisation outcome and blood results. T score indicates the number of standard deviations that the patient lies from an average young adult of the same sex. The Z score is the number of standard deviations away from an average value for someone the same sex and age.

4.2.7 Laboratory blood and urine analysis

All biochemical samples, except iPTH, were analysed using Roche modular analyser with creatinine being measured using the compensated kinetic Jaffe method. Intact PTH was measured using the Diagnostic Products Corporation Immulite 2000 for experimental chapters 1-4.

All haematology samples were analysed using the Sysmex XE-2100 analyser except ESR which was measured using Starrsed compact method.

Between 2005 and 2006 the routine clinical measurement of proteinuria changed from 24 hour urine collections to spot urine Protein: Creatinine Ratio (PCR). A study was performed\textsuperscript{253} during the change between methods to measure proteinuria to ensure that spot urine PCR at different time points throughout the day was equivalent to a 24 hour urine collection. This confirmed that significant proteinuria could be excluded using a random urine sample.

4.2.8 Serum and plasma biomarker analysis

For the patients in the randomised controlled trial (RCT) serum and plasma samples were collected from a dry needle as a pre-dialysis sample (experimental chapters 3 & 4). They were then allowed to settle for between 30mins and 120minutes. The samples were then spun at 2800rpm for 10 minutes (Hettich Rotina 420R centrifuge) to allow separation of the supernatant to occur. The supernatant was then transferred into smaller storage tubes.
with a minimum of 0.5ml per tube. A total of 5 serum and 5 plasma storage tubes were collected at baseline and at 12 months for each patient and placed in a -80°C freezer for storage. These samples were all labelled and logged onto the freezer storage software. The samples were split between two freezers for storage in case of freezer failure. Nevertheless, all freezers had fail-safe mechanisms in place in case of freezer or power failure.

Samples were sent to the Royal Liverpool Clinical Biochemistry department for analysis on dry ice and in an appropriate container. A temperature monitor was sent in the container with the samples and was returned on receipt to ensure the samples could not have defrosted on transfer. This biochemistry department is an accredited laboratory and all tests were performed by certified trained personnel due to the guidance relating to clinical trials of investigative medicinal products.

The biomarker analyses were as follows:

25-hydroxy Vitamin D

25(OH) vitamin D concentrations were measured on serum/plasma using high performance liquid chromatography (HPLC) tandem mass spectrometry (MS) calibrated using a National Institute of Standards and Technology (NIST) aligned international standard material incorporating an internal deuterated standard (Chromsystems, Manchester UK). The assay meets the performance targets set by the Vitamin D External Quality Assessment Scheme (DEQAS) Advisory Panel for 25(OH) vitamin D assays. Following protein precipitation using zinc sulphate 25(OH) vitamin D₂, 25(OH) vitamin D₃ and the deuterated internal standard (d₆ 25 OH D₃) were extracted from serum/plasma samples using Isolute C18 solid phase extraction cartridges. Potential interfering compounds were removed by initial elution with 50% methanol followed by elution of the vitamins using 10% tetrahydrofuran in acetonitrile. Dried extracts were reconstituted prior to injection into a HPLC tandem MS (Micromass Quatro Ultima Platinum, Manchester, UK) in the multiple reaction mode (MRM). The MRM transitions (m/z) used were 413.2 > 395.3, 401.1 > 383.3 and 407.5 > 107.2 for 25(OH) vitamin D₂, 25(OH) vitamin D₃, and hexa deuterated (OH) vitamin D₃ respectively. Coefficients of variation for the assay were <10% across a working range of 2.5 nmol/L to 624nmol/L for both 25(OH) vitamin D₂ and 25(OH)
vitamin D₃. The lower limit of detection (LOD) of the assay is 0.5 nmol/L for 25 (OH) vitamin D₂ and 0.1 nmol/L for 25 (OH) vitamin D₃.

1,25 dihydroxy Vitamin D

1,25 (OH)₂ vitamin D was measured in plasma/serum using a commercial assay (Immuno Diagnostic Systems, Boldon, Tyne & Wear, UK). Immunoextraction of 1,25(OH)₂ vitamin D was performed using a mini column containing a solid-phase monoclonal antibody followed by radioimmunoassay (RIA) using a 125I-labelled 1,25(OH)₂ vitamin D derivative tracer and Sac-cell separation. The mean recovery of 1,25(OH)₂ vitamin D₃ was 101%, linearity was excellent, inter- and intra-assay coefficients of variation were 9, 8 and 13% and 11, 10 and 14% at low, medium and high concentrations of 1,25 (OH)₂ vitamin D₃, respectively. The cross-reactivity of vitamin D metabolites was < 0.0015% for 25(OH) vitamin D₃, 24, 25 (OH)₂ vitamin D₃ and dihydrotachysterol and 0.54% for 1 alpha calcidol. 1,25 (OH)₂ vitamin D₂ cross-reactivity was 79%. The detection limit of the assay was 5 pmol/L. Comparison with a commercial radio receptor assay (RRA) and an in-house RIA gave regression equations of y = 0.94x + 11.8 (r = 0.98) and y = 0.91x-1.7 (r = 0.95), respectively, with no major discrepancies between the methods in all patient groups studied.²⁵¹ Plasma concentrations of 1,25(OH)₂ vitamin D obtained with the assay were as follows: normal, unsupplemented subjects: mean 88, range 48-155 pmol/L, n = 68, patients with chronic renal failure: mean 11, range 3-36 pmol/L, n = 27, primary hyperparathyroidism: mean 198, range 130-299 pmol/L, n = 23, Paget's disease: mean 92, range 42-149 pmol/L, n = 24, osteomalacia: mean 43, range 27-61 pmol/L, n = 9.

Osteoprotegerin

Osteoprotegerin (OPG) was measured in plasma using an enzyme linked immunosorbent assay (ELISA) supplied by Immuno Diagnostic Systems (IDS) (Boldon, Tyne&Wear, UK). The assay has a sensitivity of 0.14 pmol/L established from precision profiles (22% coefficient of variation of duplicates) and a CV of <8% across the range 1-30 pmol/L. Using the IDS OPG ELISA kit a monoclonal anti-OPG antibody was coated onto the inner surface of polystyrene microtitre wells (the solid phase or capture antibody). The sample (50uL) was then incubated, together with a biotinylated polyclonal anti-OPG antibody, in the antibody coated wells overnight at 4°C. The wells were washed and enzyme
(horseradish peroxidase) labelled streptavidin, was added and binded selectively to complexed biotin and, following a further wash step, colour was developed using a chromogenic substrate tetramethylbenzidine (TMB). The absorbance of the stopped reaction mixtures were read in a microtitre plate reader, colour intensity developed being directly proportional to the concentration of OPG present in the sample.

**FGF 23 Immutopics Assay**

The method for measuring FGF-23 was a 2nd generation, two-site ELISA supplied by Immutopics Inc (San Clemente, CA, USA). Two affinity purified goat polyclonal antibodies have been selected to detect epitopes within the carboxyl-terminal (C-Term) portion of FGF-23 beyond amino acid in position 179 at the enzyme cleavage point. One antibody was biotinylated for capture and the other antibody was conjugated with the enzyme horseradish peroxidase (HRP) for detection. These antibodies bound to both the intact molecule and large C-Term fragments of FGF-23. The FGF-23 molecule appears to be unstable resulting in decreased immunoreactivity over time. Sample collection and storage procedures were carried out in an expeditious manner. Due to the variable lability of the molecule, measurement of the FGF-23 concentration was made using EDTA plasma or cell culture media.

A sample containing human FGF-23 was incubated simultaneously with the biotinylated capture antibody and the HRP conjugated antibody in a streptavidin coated microtitre well. FGF-23 contained in the sample was immunologically bound by the capture antibody and the detection antibody to form a “sandwich” complex (see Figure 18).

At the end of this incubation period, the well was washed to remove any unbound antibody and other components. The enzyme bound to the well was incubated with a substrate solution in a timed reaction and then measured spectrophotometrically. The enzymatic activity of the antibody complex bound to the well is known to be directly proportional to the amount of FGF-23 in the sample. A standard curve was generated by plotting the absorbance versus the respective FGF-23 concentration for each standard on linear or logarithmic scales. The concentration of human FGF-23 in the samples was determined directly from this curve.
Sandwich complex where:

- = Streptavidin coated onto well
- = Biotin–Anti human FGF-23 (C-terminal) complex
- = Human FGF-23 (C-terminal)
- = Anti human FGF-23 (C-terminal)–HRP complex

Figure 18: Diagrammatic representation of the ‘sandwich principle’ showing FGF23 bound by capture antibody and detection antibody

The LLOD is 1.5 RU/mL (20 duplicate determinations of the zero standard) and the intra/inter CV of the assay is < 8% across the assay range 18-445 RU/mL. Parallelism on dilution was between 92-114% and recoveries have varied between 91-116% for samples with varying from low (375 RU/mL) to high (1125 RU/mL) concentrations.

4.2.8.5: NT Pro BNP

NT-ProBNP was measured in plasma/serum using an electrochemiluminescent immunoassay (ECLIA) on a Modular Analytics E170 analyser (Roche Diagnostics, Lewes, UK). The assay was based on the sandwich principle (See Figure 18) and employed in the primary incubation a biotynilated monoclonal antibody (Ab) specific for NT-ProBNP and a ruthenium complex labelled monoclonal Ab to NT-ProBNP forming the sandwich complex. Streptavidin coated microparticles added in the second incubation stage then bound to the biotin and these complexes were magnetically captured onto an electrode where after washing, an applied voltage generated the chemiluminescent emission measured by a photomultiplier with the signal generated directly proportional to the concentration of analyte in the sample.

The assay LLOD was 5pg/mL (0.6 pmol/L) (20 duplicates of the zero standard), sensitivity at a CV of 20% for duplicates was 50 pg/mL (6 pmol/L) and the inter/intra assay CV was < 3.5% across the range 47-32930 pg/mL (5.43-3885 pmol/L). Parallelism on dilution can vary by 25% around the mean value and recoveries have varied between 88-119% for samples with varying from low (110pg/mL) to high concentrations (33000 pg/mL). (Conversion factors pmol/L x 8.457= pg/mL, or pg/mL x 0.118= pmol/L)
**Troponin T**

Troponin T was measured in plasma/serum using an electrochemiluminescent immunoassay (ECLIA) on a Modular Analytics E170 analyser (Roche Diagnostics, Lewes, UK). The assay was based on the sandwich principle (See Figure 18) and employed in the primary incubation a biotynilated monoclonal antibody (Ab) specific for Troponin T and a ruthenium complex labelled monoclonal Ab to Troponin T forming the sandwich complex. Streptavidin coated microparticles added in the second incubation stage bound to the biotin and these complexes were magnetically captured onto an electrode where, after washing, an applied voltage generates the chemiluminescent emission measured by a photomultiplier with the signal generated directly proportional to the concentration of analyte in the sample.

The assay LLOD was 0.010 µg/L (20 duplicates of the zero standard), sensitivity at a CV of 20% for duplicates was 0.035 µg/L and the inter/intra assay CV was < 10.0% across the range 0.060-6.000 µg/L. Parallelism on dilution can vary by 12% around the mean value and recoveries have varied between 93-108% for samples with varying from low (0.050 µg/L) to high concentrations (5.000 ug/L).

**Fetuin-A**

Fetuin-A was measured in serum/plasma using an ELISA supplied by BioVendor GmbH (Heidelberg, Germany). The assay was based on the sandwich principle (See Figure 18) and a 96 well plate was employed in the primary 60 minute incubation; the sample to be tested and a polyclonal Ab specific for Fetuin-A immobilised to the well. After washing a second polyclonal Ab to Fetuin-A conjugated to horseradish peroxidase was added to the wells and incubated to allow attachment to the captured Fetuin-A. After washing a signal was generated by adding hydrogen peroxide and TMB with the absorbance signal directly proportional to the concentration of analyte in the sample (read at 450 nm compared to a reference of 630nm).

The assay LLOD is 0.104 ng/mL (20 duplicates of the zero standard), sensitivity at a CV of 20% for duplicates was 0.320µg/L and the inter/intra assay CV was < 6.0% across the
range 2.0-100 ng/mL. Parallelism on dilution varied from 102-114% and recoveries ranged between 104-109% for samples varying from low (50 ng/mL) to high concentrations (2000 ng/mL).
5. EXPERIMENTAL CHAPTERS
5.1 EXPERIMENTAL CHAPTER 1:

Serum phosphate and mortality in patients with chronic kidney disease


This manuscript was published in: Clin J Am Soc Nephrol 2010 Dec; 5(12): 2251-7
5.1.1 Detailed methods:

**Ethics and funding**

The main CRISIS ethics application allowed for this data to be accessed from the database and no further ethics or research and development approval was required. Funding for this project was provided by an unrestricted educational grant from Genzyme.

**Inclusion Criteria**

All generic CRISIS inclusion criteria apply:

Study specific Inclusion criteria:
- To be included in the 12 month time averaged serum phosphate analysis at least 2 serum phosphate measures were required during the first 12 months of inclusion in CRISIS

**Exclusion Criteria**

All Generic CRISIS exclusion criteria apply

Study specific exclusion criteria:
- Patients lost to follow up from the CRISIS study
- Patients with no serum phosphate at recruitment to CRISIS

**Study Design**

Serum Phosphate is strongly associated with an increased risk of mortality in the dialysis population. This association has a U-shaped relationship and we were keen to explore the relationship between phosphate and survival within the non-dialysis dependent CKD stage 3-5 population. Of the published papers examining the relationship between phosphate and survival none were prospective studies and therefore we felt we could add to the literature.
Data included within the CRISIS database for patients recruited before January 2008 was used for this study. Of the 1390 patients that were consented at that time, only 1203 patients were included. 77 patients were lost to follow-up and 110 patients had no baseline phosphate available.

Phenotypic data including previous co-morbidities and medications were utilised within the analyses. Cardiovascular disease was deemed to be present at baseline if patients had experienced a previous myocardial infarction, coronary angioplasty, coronary artery bypass graft, stroke, angina or had peripheral vascular disease.

The average of all serum phosphate values analysed at Salford Royal Hospital within the first 12 month of their inclusion within the CRISIS study generated the 12 month time-averaged phosphate value.

Estimated eGFR was calculated using a modified MDRD (Modification of Diet in Renal Disease) equation\(^{255}\). Change of estimated GFR over 1 year was calculated using the following equation:

\[
\frac{(\text{eGFR}_1 - \text{eGFR}_0)}{(T_1 - T_0)} \times 365.25
\]

**Data collection**

Data was extracted from the CRISIS access database and exported into SPSS software to allow statistical analysis.

**Statistical methods**

On initial examination of the data there was a strong and anticipated relationship between phosphate and eGFR (see figure 19). Estimated GFR is well known to be strongly associated with an increased mortality\(^3, 256, 257\) and this could potentially affect the results of this study. We therefore utilised a similar method to that published by Kestenbaum et al\(^{57}\) and used the Loess smoothing technique to remove random variation. This method is a regression modelling technique that combines multiple regression models in a local area thus producing a line through 50% of the data. This allowed us to separate the population
firstly in half and then into quartiles of phosphate relative to eGFR. After this we therefore had quartiles of phosphate for any eGFR; these quartiles did not have fixed phosphate concentrations as they vary as the eGFR changes. This allows the effect of renal function within the analyses to be reduced significantly. The Loess smoothing was performed using SigmaPlot 2001 software.

Figure 19: Graph to show the association between phosphate and renal function. This confirms a non-linear association.

Parameters were assessed for association with the quartiles of phosphate relative to eGFR and this was done with Analysis of Variance or Chi-squared tests where appropriate. This was performed for the whole population and according to eGFR category (CKD stage 5 and CKD stage 3-4). Analyses were performed with the recognition that the patients with CKD stage 5 who were close to requiring renal replacement therapy or had chosen conservative care may have had differing associations with phosphate and mortality. Mortality studies in dialysis patients have shown a U-Shaped association with serum
phosphate\textsuperscript{10, 62} however studies in patients with CKD\textsuperscript{57} have shown a potential linear relationship where the lower serum phosphate levels are associated with a better outcome.

Survival analyses were performed using cox regression and were adjusted for age, gender, proteinuria, diabetes, haemoglobin, systolic blood pressure, smoking status, cardiovascular disease, eGFR and the use of vitamin D analogues and phosphate binders at baseline. The analyses were performed on the population as a whole and split according to CKD stage.

Further survival analyses were performed using 12 month time-averaged serum phosphate and baseline serum phosphate according to the targets defined by the National Kidney Dialysis Outcomes Quality Initiative (KDOQI)\textsuperscript{143} and the UK Renal Association.\textsuperscript{258} These guidelines were chosen as they were in use at the time of the study. The serum phosphate level ‘in target’ depends on the eGFR and this is shown in more detail in Table 7.

<table>
<thead>
<tr>
<th>Phosphate ‘targets’ (mmol/L)</th>
<th>CKD stage 3-4</th>
<th>CKD stage 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal Association\textsuperscript{258}</td>
<td>0.90 - 1.50</td>
<td>1.10 – 1.80</td>
</tr>
<tr>
<td>KDOQI\textsuperscript{143}</td>
<td>0.87 - 1.48</td>
<td>1.13 – 1.78</td>
</tr>
</tbody>
</table>

Table 7: Published target range for serum phosphate according to CKD stage and guideline

All analyses were performed using SPSS 14.0 and a p value of <0.05 was considered significant.
Abstract:

Background and Objectives:

Higher phosphate is associated with mortality in dialysis patients but few prospective studies assess this in non-dialysis patients managed in an out-patient nephrology clinic. This prospective longitudinal study examined whether phosphate level was associated with death in a referred population.

Design, setting, participants and measurements:

1203 non-dialysis CKD patients in the Chronic Renal Insufficiency Standards Implementation Study were assessed. Survival analyses were performed for quartiles of baseline phosphate relative to GFR, 12 month time-averaged phosphate, and baseline phosphate according to published phosphate targets.

Results:

Mean (SD) eGFR was 32 (15) ml/min/1.73m², age 64 (14) yrs, phosphate 1.2 (0.30) mmol/L. Cox multivariate adjusted regression in CKD stage 3-4 patients showed an increased risk of all-cause and cardiovascular mortality in the highest compared to lowest quartile of phosphate (HR 1.8 (1.1, 3.1) & HR 2.8 (1.3, 6.4) respectively). No association was found in CKD stage 5 patients. Patients who had values above recommended targets (KDOQI) for phosphate control had increased risk of all-cause and cardiovascular death (HR 2.7 (1.3, 5.7) & HR 4.0 (1.4, 11.9) respectively) compared to patients below target. The highest compared to lowest quartile of 12-month time-averaged phosphate was associated with an increased risk of mortality HR 1.8 (1.1, 2.9).

Conclusions:

In CKD stage 3-4 patients, higher phosphate was associated with a stepwise-increase in mortality. As phosphate levels below published targets (as opposed to within them) are associated with a better survival this suggests that guidelines for phosphate in non-dialysis CKD patients could be re-examined. Intervention trials are required to determine if lowering phosphate will improve survival.
**Introduction:**

Higher serum phosphate is associated with mortality in haemodialysis patients.\(^{10, 11, 259}\) There have been three studies in patients with CKD, not on dialysis, evaluating the association of serum phosphate with mortality; two of these found a positive association.\(^{57, 58, 260}\) The relationship between serum phosphate and mortality in patients with CKD stage 3-5 (eGFR <60ml/min/1.73m\(^2\)) who are not on dialysis, and who are under regular nephrological review, has not previously been examined in a prospective systematic way. Survival data according to follow-up phosphate results are also lacking in CKD patients.

The aims of this single-centre study were to investigate whether an association of serum phosphate with all-cause and cardiovascular mortality could be shown prospectively in out-patients with advanced CKD (stage 5) not receiving dialysis and also in those with earlier (stage 3 and stage 4) CKD. The relationship of 12-month time-averaged phosphate and mortality and the influence upon survival of a serum phosphate within guideline targets was examined in this population.

**Methods:**

The Chronic Renal Insufficiency Standards Implementation Study (CRISIS) is a single-centre prospective epidemiological study of non-dialysis dependent patients with an eGFR<60ml/min/1.73m\(^2\), under review in the out-patient clinics of Salford Royal Foundation NHS Trust. A random 50% sample of patients with eGFR <60ml/min/1.73m\(^2\) are approached for study entry, and those who provide informed consent are enrolled. The study has been recruiting since 2002 and its primary function is to determine factors influencing outcomes and progression of renal disease in CKD patients. Of the 1390 patients enrolled in CRISIS at the time of this analysis 1203 had data that was available for analysis; 77 patients were excluded due to loss of follow-up, and 110 patients were excluded due to lack of available baseline phosphate. All patients were followed-up from recruitment into the study until either death or datalock (January 2008). Cause of death was obtained from UK Office of National Statistics. All biochemical samples were analysed in a single laboratory using a Roche Modular analyzer (Creatinine using the compensated kinetic jaffé method), with the exception of intact parathyroid hormone (PTH) which was analysed with a second generation assay on a Diagnostic Products Corporation Immulite
2000. Proteinuria was determined with either 24 hour urine collections or urine protein: creatinine ratio, depending on routine clinical practice at recruitment. Baseline clinical data determined at recruitment were collected for analysis including co-morbidities and current medication. Cardiovascular disease was defined as previous myocardial infarction, coronary-artery bypass graft, coronary angioplasty, peripheral vascular disease, stroke or angina.

Twelve month time-averaged phosphate was determined as the average of all serum phosphate results in the first twelve months since recruitment. All patients who had two or more phosphate results available during the 12 month period were included. Change in eGFR over one year (ΔeGFR) was calculated using the following equation: (eGFR₁ – eGFR₀/ T₁-T₀) x 365.25.

The CRISIS study has ethical approval and investigations were performed in accordance with the principles of the Declaration of Helsinki as revised in 2000.

**Statistical analysis:**

Due to the strong link between serum phosphate, eGFR, and mortality, the phosphate results were reported relative to renal function. Phosphate results were divided into quartiles relative to eGFR (calculated using a modified MDRD equation)²⁵⁵ (see Figure 20) and adjustment was effected using a ‘local smoothing’ technique in SigmaPlot 2001. This technique maintains the integrity of the data while reducing the ‘noise’ and provides a ‘smooth line’ through the middle of the data. This was then performed in the upper and lower halves of the phosphate data range to produce quartiles. This method has previously been published in studies examining phosphate and mortality.⁵⁷ ANOVA and chi-square tests were used to examine associations with potentially influential parameters across the quartiles; these were repeated according to eGFR category (15-59ml/min/1.73m², and <15ml/min/1.73m² –see below). All cox regression survival analysis performed in this study were adjusted for age, gender, proteinuria, diabetes, haemoglobin, systolic blood pressure, smoking status, cardiovascular disease, eGFR and the use of vitamin D analogues and phosphate binders at baseline. Survival analyses were performed on baseline serum phosphate relative to eGFR in the whole population. As there is increasing evidence of
reverse cardiovascular epidemiology in patients with advanced (stage 5) CKD,\textsuperscript{261,262} a group of patients who tend to have extensive co-morbidities and multiple competing cardiovascular risk factors, analysis was also stratified for eGFR both above and below 15\text{ml/min/1.73m}^2.

![Figure 20: Phosphate quartiles generated relative to eGFR using local smoothing technique](image)

Survival analyses on 12 month time-averaged phosphate were performed from study baseline. Survival analyses were also performed on phosphate according to categories defined by the National Kidney Dialysis Outcomes Quality Initiative (KDOQI) (the guideline in use at the time of data lock) and UK Renal Association (UKRA) guidelines.\textsuperscript{143,258} These were determined according to stage of CKD i.e. CKD stage 3-4 patients were ‘in target’ with serum phosphate 0.87-1.48mmol/L (KDOQI) or 0.9-1.5mmol/L (UKRA), CKD stage 5 patients were ‘in target’ with serum phosphate 1.13-1.78mmol/L (KDOQI) and 1.1-1.8mmol/L (UKRA). All analyses were performed using SPSS 14.0 and a \( p \) value of <0.05 was considered significant.
**Results:**

Mean (SD) eGFR of the population was 32 (15) ml/min/1.73m$^2$, age 64 (14) years, corrected calcium 2.29 (0.14) mmol/L and phosphate 1.2 (0.30) mmol/L. Cardiovascular disease was present in 32% of patients at enrolment and 32% were diabetics. Only 7% (n=89) of patients were receiving a phosphate binder and 17% (n=199) were receiving a vitamin D analogue at baseline. Due to these small numbers further analyses of binder and vitamin D analogue usage was not performed, although baseline usage was adjusted for in survival analyses. Due to the strong associations between eGFR and both mortality and phosphate, analyses were performed on phosphate relative to eGFR in order to overcome this potentially confounding relationship, and the demographics according to these quartiles are shown in Table 8. This demonstrated that baseline eGFR related phosphate quartiles were positively associated with female gender, proteinuria, diabetes and current smokers. A negative association was seen with age, haemoglobin, weight, BMI, and cardiovascular disease. There was no significant difference in mean days of follow-up from lower to higher quartiles of phosphate (P=0.8) and these were 1079, 1055, 1035, and 1036 days respectively.

Death occurred in 22% (n=271) of the total population, with 9% (n=109) due to cardiovascular causes, in a median follow up period of 37.2 (0.5-64) months. There was a higher percentage of deaths in CKD stage 5 patients (62/167; 37%) compared with those with CKD stages 3-4 (209/1036; 20%, P<0.001). 59% of CKD stage 5 patients progressed to renal replacement therapy during the follow-up period compared to only 9% of CKD stage 3-4 patients (P<0.001). There was no significant difference in clinical characteristics between the study population and those patients who had been excluded from study (data not shown).

For each 0.323mmol/L (1mg/dL) increase of baseline phosphate a 26% increase in all-cause mortality (HR 1.3 (1.1, 1.5) P=0.01) and a 50% increase in cardiovascular mortality (HR 1.5 (1.2, 2.0) P=0.002) was observed for the entire population (CKD stage 3-5) in multivariate analyses.
Table 8: Demographic data across quartiles of phosphate adjusted for eGFR.

eGFR: estimated glomerular filtration rate; PTH: parathyroid hormone; CxP: calcium phosphate product; CVD: cardiovascular disease; DM: diabetes; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; BMI: body mass index; Non-ca: non calcium containing
Cox regression showed no significant difference in mortality across phosphate quartiles relative to eGFR (HR 1.4 (0.9, 2.2) *P*=0.1) when comparing the highest to the lowest quartile in CKD stages 3-5. Cox regression stratified according to CKD stage 3-4 (n=1036) and those with CKD stage 5 (n=167) showed an increase in risk of all-cause mortality in CKD stage 3-4 patients across the phosphate quartiles with the highest quartile having a significantly increased hazard ratio of 1.8 (1.1, 3.1; *P*=0.02) compared to the lowest quartile (Table 9). Cardiovascular mortality risk was also increased in CKD stage 3-4 (HR 2.9 (1.3, 6.4) *P*=0.01). No such association was observed for patients with stage 5 CKD (HR 0.6 (0.2, 1.4) *P*=0.2).

Table 9: Cox regression: Hazard ratios across quartiles of phosphate relative to eGFR in patients with CKD stage 3-4.

<table>
<thead>
<tr>
<th>N=1036</th>
<th>All-cause mortality</th>
<th>Cardiovascular mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate quartiles according to eGFR</td>
<td>Hazard ratio (95%CI)</td>
<td><em>P</em> value</td>
</tr>
<tr>
<td>1st quartile (low)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2nd quartile</td>
<td>1.6 (1.00,2.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>1.6 (0.99,2.5)</td>
<td>0.06</td>
</tr>
<tr>
<td>4th quartile (high)</td>
<td>1.8 (1.1 – 3.1)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Analysis adjusted for age, gender, proteinuria, diabetes, haemoglobin, systolic blood pressure, current smoking status, cardiovascular disease, eGFR, renal replacement therapy and vitamin D analogue and phosphate binder use.

Cox regression survival analysis of the twelve month time-averaged phosphate results for CKD stages 3-5 showed an increase in mortality across the quartiles with the upper quartile having a significantly increased all-cause mortality compared to the lowest quartile (HR 1.8(1.1, 2.9) *P*=0.01) (Table 10). Cardiovascular mortality also increased but did not reach significance (HR 1.8 (0.9, 3.9) *P*=0.1). When one year δeGFR was included in the multi-factorial model then the relationship between time-averaged phosphate and mortality
became non-significant (HR 1.4 (0.8, 2.6) \(P=0.3\)). Multi-factorial analyses incorporating baseline phosphate with δeGFR and δphosphate were performed. This suggested that baseline phosphate (HR 2.2 (1.1, 4.3) \(P=0.03\)) and δeGFR (HR 1.2 (1.0, 1.3) \(P=0.05\) (per 5ml/min/1.73m²/year change)) were significant factors affecting mortality whereas δphosphate showed no association (HR 1.1 (0.9, 1.3) \(P=0.4\)).

Table 10: Results of survival analysis for quartiles of 12-month time-averaged phosphate for all patients (CKD stages 3-5)

<table>
<thead>
<tr>
<th>Quartiles: 12-month time averaged phosphate (mmol/L)</th>
<th>All-cause mortality</th>
<th>Cardiovascular mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio (95%CI)</td>
<td>(P) value</td>
</tr>
<tr>
<td>&lt;1.02</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1.02 – 1.15</td>
<td>1.2 (0.8, 1.9)</td>
<td>0.4</td>
</tr>
<tr>
<td>1.16–1.34</td>
<td>1.2 (0.8, 1.8)</td>
<td>0.5</td>
</tr>
<tr>
<td>&gt;1.34</td>
<td>1.8 (1.1, 2.9)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Analysis adjusted for age, gender, proteinuria, diabetes, haemoglobin, systolic blood pressure, current smoking status, cardiovascular disease, eGFR and vitamin D analogue and phosphate binder use.

Cox regression was also performed according to whether or not patients had a phosphate level within the KDOQI\textsuperscript{143} or UKRA\textsuperscript{258} targets for phosphate control (Table 11). Phosphate within target was associated with nearly double the mortality risk compared to levels below target in patients with CKD stages 3-5 for both guidelines (UKRA HR 1.7 (1.0, 2.8) \(P=0.05\), KDOQI HR 1.8 (1.0, 3.8) \(P=0.06\)). However phosphate above the target range conferred a 2.5-3 fold increased risk of death (UKRA HR 2.8 (1.5, 5.4) \(P=0.002\), KDOQI HR 2.7 (1.3, 5.7) \(P=0.009\)) compared to serum phosphate below the lower limit of the target range. The risk of cardiovascular death was even greater for patients above target compared to below target (UKRA HR 4.1 (1.5, 10.6) \(P=0.004\), KDOQI HR 4.0 (1.4, 11.9) \(P=0.01\)). The Kaplan-Meier survival curve according to attainment of KDOQI guidelines\textsuperscript{143} is shown in Figure 21; the hazard ratios stated are multi-factorial adjusted analyses.
Table 11: Cox regression: Hazard ratios across international targets of serum phosphate for CKD stage 3-5

<table>
<thead>
<tr>
<th></th>
<th>UK Renal Association&lt;sup&gt;258&lt;/sup&gt;</th>
<th>KDOQI&lt;sup&gt;143&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All cause mortality</td>
<td>CV mortality</td>
</tr>
<tr>
<td>Hazard ratio (95%CI)</td>
<td>$P$</td>
<td>Hazard ratio (95%CI)</td>
</tr>
<tr>
<td>Below target</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>In target</td>
<td>1.7 (1.0, 2.8)</td>
<td>0.05</td>
</tr>
<tr>
<td>Above target</td>
<td>2.8 (1.5, 5.4)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

RA target range CKD stage 3-4<sup>258</sup>: 0.9-1.5mmol/L, CKD stage 5: 1.1-1.8mmol/L.

KDOQI target range CKD stage 3-4<sup>143</sup>: 0.87-1.48mmol/L, CKD stage 5: 1.13-1.78mmol/L.

Analysis adjusted for age, gender, proteinuria, diabetes, haemoglobin, systolic blood pressure, current smoking status, cardiovascular disease, eGFR and vitamin D analogue and phosphate binder use.
Figure 21: Survival according to phosphate levels relative to K/DOQI guidelines

Discussion:

The aim of this study was to determine, in a large non-dialysis CKD population, whether an association exists between serum phosphate and mortality in patients under regular outpatient nephrological care. The CRISIS data was used which is collected with the primary purpose of prospectively examining outcomes and progression of non-dialysis CKD. We found that higher serum phosphate, even within the normal laboratory range, was associated with an increase in mortality in patients with CKD stages 3-4. More than 90% of our CKD stage 3-4 patients had a serum phosphate within the normal laboratory range and this is consistent with other studies. Evidence from the Framingham offspring study suggests that differences within the normal range of phosphate are associated with increased cardiovascular risk in the general population. Analyses of the ARIC study have also found an increased risk of cardiovascular events and death in association with higher ‘normal’ range baseline serum phosphate in community-dwelling adults. Although other studies have also examined the relationship of phosphate to mortality in patients with non-dialysis treated CKD, our study differs in that it is the first single-centre prospective study that has investigated non-dialysis patients with CKD stage 3-5 who are under regular out-
patient nephrological follow-up. The study population was large and patients received focused management of their renal disease suggesting that even when under regular specialist care the mortality risk associated with serum phosphate is still significant.

Kestenbaum\textsuperscript{57} \textit{et al} studied a large US veteran population who had impaired renal function although only 10\% were under regular nephrology care at baseline. The study showed that serum phosphate adjusted for creatinine clearance was associated with mortality. Patients with the lowest serum phosphate had the best survival, as in our study, with a hazard ratio <1, but this did not reach statistical significance. This study also reported a HR1.2 (1.1, 1.4) for each 1mg/dl increase of serum phosphate in multivariate analysis which is comparable to our results (HR1.3 (1.1, 1.5)).

Menon\textsuperscript{260} \textit{et al} investigated the association of serum phosphate and mortality in 840 randomised patients within the MDRD study but showed no association after adjusting for other influential factors such as GFR. Voormolen\textsuperscript{58} \textit{et al} prospectively studied pre-dialysis patients with eGFR<20ml/min/1.73m\textsuperscript{2} and found an association of phosphate with progression of renal disease and mortality. Their population had much higher levels of phosphate compared to our study which is not unexpected given the differences in renal function between the 2 studies (mean eGFR (SD) was 13(5.4) ml/min/1.73m\textsuperscript{2} vs. 32(14) ml/min/1.73m\textsuperscript{2} in our study). 48\% of that study population had a phosphate above target range according to KDOQI guidelines\textsuperscript{143} compared to only 9.7\% of our population; only 2\% were below the target range compared to 12\% in our study. In contrast to the findings of Voormolen \textit{et al} we found that in patients with CKD stage 5, the association of serum phosphate with mortality, when adjusted for GFR, was no longer significant. There are several potential explanations for this finding. Our sample size of CKD stage 5 patients was relatively small. There is no doubt that factors contributing to mortality in CKD stage 5 become more complex even prior to commencement of dialysis. Studies that have investigated modification of other cardiovascular risk factors, such as reducing cholesterol with statins, have shown no benefit to survival in dialysis patients perhaps because of the effects of multiple competing risk factors\textsuperscript{84} (e.g. left ventricular hypertrophy, cardiac dilatation, arterial stiffness). Evidence of this reverse epidemiology is also seen in non-dialysis patients with an eGFR<15ml/min/1.73m\textsuperscript{2}.\textsuperscript{261,262} Our stage 5 CKD group was biased towards even higher risk patients as those patients within CRISIS who opted for conservative care of ESRD were included. As such patients tend to have greater co-
morbidity, are older and more malnourished than their counterparts who had chosen to receive renal replacement therapy, or than those with higher levels of eGFR, it would be expected that in this sub-group a lower phosphate might confer a higher risk of death, conflicting with the evidence found in the larger CKD stage 3-4 population. Block et al\textsuperscript{10} have shown lower phosphate to be associated with increased mortality in haemodialysis patients, presumably partly explained by malnutrition in the sicker patients.

The twelve-month time-averaged phosphate data showed a relationship between persistent elevation of phosphate and mortality. This association became non-significant when \( \Delta eGFR \) was included in the multi-variate model. This could be due to the deterioration of renal function being such a major risk factor for mortality that increased numbers or longer follow-up would be needed to show the effect of phosphate. Also, the time-averaged phosphate included all phosphate results analysed within the first year after enrolment, including those during hospital admissions and in patients with acute renal impairment, whereas \( \Delta eGFR \) only involved two measurements and so may miss important additional changes which had occurred during the year.

A pathogenetic link might explain the associations between mortality and higher phosphate found in this study. One plausible explanation is the involvement of phosphate in the pathogenesis of vascular calcification which has been well documented in the literature.\textsuperscript{264-267} Other CKD-related disturbances can also potentially explain the association. Serum phosphate level has been associated with CKD progression\textsuperscript{58} and left ventricular hypertrophy,\textsuperscript{268} which are known to contribute to cardiovascular disease and death. Our results confirm an association of increasing phosphate with declining eGFR. Native vitamin D\textsuperscript{48} and the phosphatonin, fibroblast growth factor 23 (FGF23),\textsuperscript{269} are factors thought to have regulatory roles in mineral metabolism and abnormalities occur early in CKD prior to changes in calcium, phosphate and PTH. FGF23 is now thought to be the main regulator of phosphate homeostasis and increasing levels have been associated with mortality in haemodialysis patients, an effect independent of serum phosphate.\textsuperscript{53} The mechanisms of this association with mortality remain unknown.

Diet could be a further explanation for the association of higher phosphate and mortality in both the general and CKD population, with higher serum phosphate perhaps reflecting a
more unhealthy high phosphate diet. Conversely, an increased risk of death is seen in dialysis patients with low serum phosphate,\textsuperscript{10, 62} the relationship thought to reflect malnutrition or co-existing illness. This association was not apparent in our study of non-dialysis patients and, importantly, lower serum phosphate (i.e. even below the current target ranges for CKD) seemed to be associated with improved survival. This suggests that malnutrition may be less of a problem in the non-dialysis population with earlier CKD, and interestingly we found that patients with higher serum phosphate actually had lower weight and BMI.

It is possible its relationship with mortality may simply represent an association, with phosphate being co-localised with other more important factors. For example, genetic influence of phosphate control may be associated with a pre-disposition to cardiovascular disease, or higher phosphate may just be a marker of some other cause of cardiovascular risk and mortality. This may explain the recent finding in the CARDIA population that higher serum phosphate even within the normal range was associated with greater likelihood of coronary calcification at 15 yrs in a healthy young population.\textsuperscript{61} More research is needed to investigate these possibilities.

It was interesting to note that patients who had serum phosphate below the targets recommended in the KDOQI and the UKRA guidelines\textsuperscript{143, 258} had the best survival. It should not be overlooked that guidelines for serum phosphate in CKD were devised using only studies involving dialysis patients,\textsuperscript{143, 258} a potential shortcoming for guidelines which have been followed internationally and generalised to non-dialysis CKD patients. Our study would suggest that in non-dialysis CKD patients a lower limit, as opposed to an upper limit, for serum phosphate is not required in the guidelines.

Our study does have several limitations. Patients have been recruited into the CRISIS study consecutively since 2002, and so the length of follow-up period was not consistent. The CRISIS population is 98% Caucasian, indicative of our catchment population, and our results may not be generalisable to CKD patients of other ethnic origins. Although we assessed 12 month time-averaged phosphate we were unable to correct for an equivalent 12 month time-averaged eGFR, hence we could not correct for similar episodes of ill health during the 12 month period. As the majority of the patients had a phosphate within the normal laboratory range and <10% of patients were prescribed binder therapy, data relating
to the effect of phosphate binder usage in the study was not analysed, but the multivariate analyses were corrected for their use.

In conclusion, this large prospective study of CKD patients not receiving dialysis, but managed in a secondary care renal service, suggests that all-cause and cardiovascular mortality risk independently increases as serum phosphate increases in patients with CKD stages 3-4. Patients with phosphate levels even within contemporary CKD guideline targets were associated with poorer outcome than those with serum phosphate below the target range. This suggests that even in earlier stages of renal dysfunction a lower phosphate is associated with a better survival. Intervention trials that investigate whether lowering phosphate will improve survival for patients with earlier stages of CKD are indicated.
5.2 EXPERIMENTAL CHAPTER 2:

Factors Associated with Vascular Stiffness: Cross-Sectional Analysis from the Chronic Renal Insufficiency Standards Implementation Study

Helen Eddington, Smeeta Sinha, Elizabeth Li, Janet Hegarty, Jeanette Ting, Beverley Lane, Constantina Chrysochou, Robert Foley, Donal O’Donoghue, Philip A. Kalra, Rachel Middleton.

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Abstract

Background:
Vascular stiffness is associated with increased cardiovascular risk. This study aimed to identify factors associated with vascular stiffness in a cohort of chronic kidney disease (CKD) patients.

Methods:
The Chronic Renal Insufficiency Standards Implementation Study is a prospective epidemiological study of CKD patients not on dialysis, who are managed in a clinic setting. Phenotypic parameters were collected annually, and vascular stiffness was assessed using augmentation index (AIx). Cross-sectional analysis was performed across quintiles of AI to evaluate factors associated with vascular stiffness.

Results:
Mean patient age was 66.1 ±14.1 years and estimated glomerular filtration rate (eGFR) was 31.2 ± 5.7 ml/min. Corrected calcium was 2.26 ± 0.2 SD mmol/L, phosphate 1.2 ± 0.4 SD mmol/L and intact parathyroid hormone 94 ± 96 SD pg/mL; 18.3% of patients had cardiovascular disease. Increased age and systolic blood pressure were associated with increased AIx (all p < 0.001). No statistical association was present between AIx and eGFR, intact parathyroid hormone, phosphate or protein excretion.

Conclusion:
This study identified blood pressure as a potentially modifiable risk factor associated with AIx, whereas eGFR was not associated with increased AIx in a population of CKD stage 3-5 patients. Further knowledge of factors which influence progression of vascular stiffness will be important in risk quantification and management.
Introduction:

Chronic kidney disease (CKD) has major public health implications especially as CKD patients have considerable cardiovascular morbidity and mortality. Cardiovascular risk is associated with the degree of CKD; therefore, patients with end-stage renal disease have the worst outcomes. This association between reduced glomerular filtration rate (GFR) and mortality is poorly explained by classic cardiovascular risk factors, and non-classic risk factors such as perturbations in bone mineral chemistry and hyperparathyroidism are thought to play an important part. Hence, the increased cardiovascular risk and mortality in CKD is associated with increased vascular stiffness, vascular calcification and left ventricular mass.

Patients with end-stage renal disease have two- to five-fold more coronary artery calcification than age- and sex-matched individuals who have coronary artery disease and the degree of coronary artery calcification is correlated with increased risk of cardiovascular events and death. Furthermore, increased vascular calcification is closely associated with vascular stiffness in patients with CKD and end-stage renal disease.

Vascular stiffness can be assessed using validated methods such as pulse wave velocity (PWV) and augmentation index (AIX). Blacher et al. and London et al. have shown that increased PWV and AIX are independent predictors of mortality in the renal population.

Studies of vascular stiffness in a variety of patient populations have shown an association with age, hypertension, diabetes and coronary artery disease. In addition, other physiological, genetic, cardiovascular and systemic diseases are also associated with increased vascular stiffness. Several authors have shown a relationship between increasing vascular stiffness and declining renal function in small patient cohorts. However, no large studies have been undertaken in patients with CKD. This study aimed to identify factors associated with increased vascular stiffness as measured by AIX in a large representative outpatient CKD stage 3-5 population, none of whom were receiving dialysis. Such information is of importance in the identification of risk factors that may be amenable to treatment.
Methods

The Chronic Renal Insufficiency Standards Implementation Study (CRISIS) is an ongoing prospective epidemiological study of patients with predominantly CKD stage 3-5, undertaken exclusively in an outpatient clinic setting. Patients on dialysis are excluded from enrolment into the study. The study has full ethical approval and recruitment into CRISIS commenced in October 2002, with all patients giving full informed consent. Patients underwent collection of phenotypic and biochemical parameters at baseline and at annual follow-up, and from March 2005 all patients have had vascular stiffness assessed by AIx. AIx was derived by applanation tonometry at the radial artery. AIx derived at the radial artery has been shown to correlate with carotid-femoral PWV.\textsuperscript{281} We have confirmed this correlation in our own patient population, by performing simultaneous carotid-femoral PWV and AIx derived at the radial artery of 106 patients with CKD. The two parameters were found to be significantly correlated ($R^2 = 0.1$, $p < 0.001$). Due to the practical advantages of performing AIx at the radial artery in an outpatient setting, and especially when a significant proportion of study subjects were elderly and immobile, this technique was chosen to study vascular stiffness in the CRISIS population. All patients recruited into CRISIS who had AIx measured between March 2005 and January 2007 were included in this study. Recruitment and data collection occurred in the renal outpatient clinics at one large renal centre, Hope Hospital, Salford Royal Foundation Trust. Phenotypic data were collected in a face-to-face interview and all biochemical parameters collected were analysed in the local pathology laboratories as part of routine clinical monitoring. Estimated GFR (eGFR) was determined using the four-variable MDRD equation $[(186 \times \text{creat}/88.4)^{1.154} \times \text{(age)}^{0.203} \times 0.742 \times \text{female} \times 1.212 \times \text{black})].\textsuperscript{248}$

AIx was measured by one specialist nurse trained in the use of SphygmoCor \textsuperscript{®} System (Atcor Medical, Sydney, Australia). Patients were rested for 10 minutes prior to measurements being taken. Blood pressure was then measured twice using a calibrated sphygmomanometer, and the second reading was recorded. AIx was performed with the patient seated. The transducer was then placed over the radial artery on the non-dominant arm to analyse the radial pulse wave. The SphygmoCor system calculates the AIx by using an inbuilt transfer factor to reconstruct the central aortic waveform from the radial pulse wave analysis. Recordings were taken when a high-amplitude reproducible signal was obtained for at least 10 consecutive beats. The assessment was completed when two AIx
measurements corrected for heart rate were within 10% of each other and all quality control indicators were satisfied. AIx measured at the patients’ first visit when enrolling into the study was used for statistical analysis.

**Statistical Analysis**

The aim of this study was to investigate risk factors associated with increased vascular stiffness within the CKD 3-5 cohort. The study population was arbitrarily divided into quintiles of AIx with equal numbers of patients in each. ANOVA was used to test the relationship between baseline clinical and laboratory parameters and AIx across the quintiles. Multivariate linear regression was then performed to investigate whether individual parameters were independently associated with vascular stiffness. A hierarchal analysis was performed with forced entry for age, gender, diabetes, cardiovascular disease, height and heart rate. Other factors were then introduced in a stepwise manner. A p value of <0.05 was considered significant.

**Results**

A total of 597 consecutive patients were recruited into the study between March 2005 and January 2007. Patients with CKD stage 3-5 accounted for approximately 94% of the cohort (Figure 22). The baseline characteristics are shown in tables 12 and 13. The mean age of our population was 66.1 ±14.1 years and 34.5% were females. The mean eGFR was 31.2 (15.7) ml/min/1.73m². Aetiology of renal disease included diabetic nephropathy (15.2%), vascular disease and hypertension (26.9%) and glomerulonephritis (14.6%). The cause of renal disease was unknown in 9.9% of the population (data not shown). Pre-existing cardiovascular disease was present in 18.3% of patients and 36.3% had diabetes. The mean BMI was 27.6 ± 5.4. The majority of the population were receiving angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (53.6%) and 63.2% were prescribed a statin. Mean AIx was 26.98 ±11.47%.
\textit{AIx Analysis by Quintiles}

Threshold values corresponding to quintiles of AIx were as follows: \(<16.9, \geq16.9 <25.4, \geq25.4 <30.7, \geq30.7 <36.6\) and \(\geq36.6\%\). Table 12 compares patient characteristics according to quintile of AIx and shows that increasing age, proportion of females, height, weight, systolic blood pressure, pulse pressure (Figure 23a,b) and beta blocker usage, as well as reducing heart rate, were all associated with increasing AIx \((P < 0.0001)\). Increased use of aspirin or clopidogrel \((P <0.05)\) and reduced haemoglobin level \((P <0.01)\) were also associated with increased AIx (Table 13).

No association was observed between AIx and eGFR or creatinine (fig. 23 c, d), and there was also no association of AIx with diastolic blood pressure, diabetes, composite cardiovascular disease history, smoking, body mass index, corrected calcium, phosphate, calcium \(\times\) phosphate product, C-reactive protein, intact parathyroid hormone, protein excretion or use of statins. Primary renal disease, previous history of myocardial infarction, stroke, coronary artery bypass graft, congestive cardiac failure, peripheral vascular disease were also not associated with AIx (data not shown).
### Table 12: Comparison of patient characteristics by quintiles of A1x

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>&lt;16.9%</th>
<th>≥16.9, &lt;25.4%</th>
<th>≥25.4, &lt;30.7%</th>
<th>≥30.7, &lt;36.6%</th>
<th>≥36.6%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>597</td>
<td>119</td>
<td>119</td>
<td>120</td>
<td>119</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Age, Years</td>
<td>66.1 (14.1)</td>
<td>61.9 (59.4-64.4)</td>
<td>63.4 (60.9-65.9)</td>
<td>67.9 (65.4-70.4)</td>
<td>68.3 (65.8-70.8)</td>
<td>69.1 (66.7-71.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female sex, %</td>
<td>34.5</td>
<td>16.8</td>
<td>27.7</td>
<td>35.8</td>
<td>39.5</td>
<td>52.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.7596</td>
</tr>
<tr>
<td>Type 1</td>
<td>7.5</td>
<td>7.6</td>
<td>8.4</td>
<td>8.3</td>
<td>8.4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Type 2</td>
<td>28.8</td>
<td>33.6</td>
<td>26.9</td>
<td>29.2</td>
<td>26.1</td>
<td>28.3</td>
<td></td>
</tr>
<tr>
<td>CV disease, %</td>
<td>18.3</td>
<td>19.3</td>
<td>14.3</td>
<td>16.7</td>
<td>19.3</td>
<td>21.7</td>
<td>0.6306</td>
</tr>
<tr>
<td>Smoking %</td>
<td>14.1</td>
<td>16.0</td>
<td>18.5</td>
<td>10.8</td>
<td>11.8</td>
<td>13.3</td>
<td>0.4209</td>
</tr>
<tr>
<td>Height, cm</td>
<td>169 (12)</td>
<td>172 (18)</td>
<td>171 (8)</td>
<td>168 (9)</td>
<td>168 (10)</td>
<td>165 (10)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>80 (14)</td>
<td>85 (23)</td>
<td>83 (18)</td>
<td>80 (15)</td>
<td>79 (15)</td>
<td>72 (14)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>27.6 (5.4)</td>
<td>28.3 (27.2-29.5)</td>
<td>27.7 (26.6-28.9)</td>
<td>27.5 (26.2-28.8)</td>
<td>28.0 (26.8-29.2)</td>
<td>26.3 (25.1-27.5)</td>
<td>0.1875</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>134.2 (21.4)</td>
<td>126.7 (123.0-130.5)</td>
<td>133.9 (130.1-137.6)</td>
<td>132.8 (129.1-136.5)</td>
<td>134.0 (130.2-137.7)</td>
<td>143.8 (140.1-147.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>71.3 (11.4)</td>
<td>71.2 (69.1-73.2)</td>
<td>73.0 (71.0-75.1)</td>
<td>69.3 (67.2-71.3)</td>
<td>70.5 (68.4-72.5)</td>
<td>72.6 (70.6-74.6)</td>
<td>0.0652</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>63.0 (18.3)</td>
<td>55.5 (52.4-58.7)</td>
<td>60.8 (57.7-64.0)</td>
<td>64.1 (60.9-67.2)</td>
<td>63.5 (60.3-66.7)</td>
<td>71.2 (68.0-74.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ACEI or ARB, %</td>
<td>53.6</td>
<td>59.7</td>
<td>52.1</td>
<td>56.7</td>
<td>52.1</td>
<td>47.5</td>
<td>0.3766</td>
</tr>
<tr>
<td>Beta Blocker, %</td>
<td>35.9</td>
<td>22.7</td>
<td>23.5</td>
<td>31.7</td>
<td>44.5</td>
<td>56.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Calcium channel blocker, %</td>
<td>50.4</td>
<td>57.1</td>
<td>52.9</td>
<td>47.5</td>
<td>43.7</td>
<td>50.8</td>
<td>0.2850</td>
</tr>
<tr>
<td>Aspirin or clopidogrel, %</td>
<td>40.7</td>
<td>38.7</td>
<td>30.3</td>
<td>50.0</td>
<td>37.8</td>
<td>46.7</td>
<td>0.0168</td>
</tr>
<tr>
<td>Statin, %</td>
<td>63.2</td>
<td>60.6</td>
<td>58.8</td>
<td>65.0</td>
<td>67.2</td>
<td>59.2</td>
<td>0.5420</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>69.1 (14.1)</td>
<td>81.7 (79.6-83.8)</td>
<td>71.6 (69.4-73.7)</td>
<td>68.9 (66.7-71.0)</td>
<td>64.7 (62.6-66.8)</td>
<td>58.7 (56.6-60.8)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

CV = Cardiovascular; ACEI = angiotensin-converting enzyme inhibitors; ARB = angiotensin receptor blockers
Table 13: Comparison of patient biochemical characteristics by quintiles of augmentation index

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>&lt;16.9%</th>
<th>≥16.9,&lt;25.4%</th>
<th>≥25.4,&lt;30.7%</th>
<th>≥30.7, &lt;36.6</th>
<th>≥36.6</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>597</td>
<td>119</td>
<td>119</td>
<td>120</td>
<td>119</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>eGFR, ml/min</td>
<td>31.2 (16)</td>
<td>30.6 (17)</td>
<td>33 (17)</td>
<td>32.4 (15)</td>
<td>31.8 (15)</td>
<td>28.8 (14)</td>
<td>0.306</td>
</tr>
<tr>
<td>Creatinine, mmol/L</td>
<td>237 (144;68-1,133)</td>
<td>264 (163;88-1,042)</td>
<td>248 (159;68-860)</td>
<td>218 (110;85-613)</td>
<td>220 (132; 77-1,133)</td>
<td>235 (147; 86-975)</td>
<td>0.069</td>
</tr>
<tr>
<td>Corr calcium, mmol/L</td>
<td>2.26 (0.17)</td>
<td>2.27 (2.24-2.30)</td>
<td>2.29 (2.26-2.32)</td>
<td>2.26 (2.23-2.29)</td>
<td>2.25 (2.22-2.28)</td>
<td>2.25 (2.22-2.28)</td>
<td>0.2828</td>
</tr>
<tr>
<td>Phosphate, mmol/L</td>
<td>1.21 (0.37)</td>
<td>1.20 (1.13-1.26)</td>
<td>1.20 (1.14-1.28)</td>
<td>1.19 (1.13-1.26)</td>
<td>1.21 (1.13-1.27)</td>
<td>1.23 (1.17-1.30)</td>
<td>0.9143</td>
</tr>
<tr>
<td>Ca x PO4, mmol/L²</td>
<td>2.7 (1.1)</td>
<td>2.7 (2.5-2.9)</td>
<td>2.8 (2.6-3.0)</td>
<td>2.7 (2.5-2.9)</td>
<td>2.7 (2.5-2.9)</td>
<td>2.8 (2.6-3.0)</td>
<td>0.9208</td>
</tr>
<tr>
<td>iPTH, ng/L</td>
<td>93.7 (95.8)</td>
<td>100.0 (82.0-118.1)</td>
<td>94.4 (76.0-112.8)</td>
<td>85.0 (66.8-103.1)</td>
<td>89.1 (70.6-107.7)</td>
<td>99.7 (81.4-117.9)</td>
<td>0.7317</td>
</tr>
<tr>
<td>Bicarbonate, mEq/L</td>
<td>22.3 (3.8)</td>
<td>22.1 (21.4-22.8)</td>
<td>22.5 (21.8-23.2)</td>
<td>22.3 (21.6-23.0)</td>
<td>22.5 (21.8-23.2)</td>
<td>22.0 (21.3-22.7)</td>
<td>0.7597</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>43.0 (3.8)</td>
<td>43.0 (42.3-43.6)</td>
<td>43.1 (42.4-43.7)</td>
<td>43.3 (42.6-44.0)</td>
<td>42.8 (42.0-43.4)</td>
<td>43.2 (42.5-43.9)</td>
<td>0.7984</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>7.5 (17.5)</td>
<td>5.3 (2.2-8.5)</td>
<td>8.2 (5.1-11.4)</td>
<td>8.5 (5.4-11.7)</td>
<td>8.0 (4.9-11.2)</td>
<td>7.2 (4.1-10.4)</td>
<td>0.6212</td>
</tr>
<tr>
<td>Proteinuria, g/day</td>
<td>0.8 (1.3)</td>
<td>0.9 (0.6-1.2)</td>
<td>0.9 (0.6-1.2)</td>
<td>0.8 (0.6-1.1)</td>
<td>0.6 (0.3-0.8)</td>
<td>0.8 (0.5-1.0)</td>
<td>0.3980</td>
</tr>
<tr>
<td>Haemoglobin, g/L</td>
<td>124.8 (21.9)</td>
<td>124.0</td>
<td>129.5</td>
<td>127.9</td>
<td>118.9</td>
<td>123.8</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>(120.0-127.9)</td>
<td>(125.6-133.4)</td>
<td>(124.0-131.8)</td>
<td>(115.0-122.8)</td>
<td>(119.9-127.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.5 (2.0)</td>
<td>4.6 (4.3-5.0)</td>
<td>4.6 (4.2-5.0)</td>
<td>4.3 (3.9-4.7)</td>
<td>4.4 (4.0-4.8)</td>
<td>4.7 (4.3-5.1)</td>
<td>0.5207</td>
</tr>
</tbody>
</table>

iPTH = Intact parathyroid hormone; CRP = C-reactive protein; CA x PO4 = calcium x phosphate product.
Figure 23: Dot plots of correlation between AIx and systolic blood pressure, $R^2 = 0.063$, $p < 0.0001$ (a), pulse pressure, $R^2 = 0.083$, $p < 0.0001$ (b), creatinine, $R^2 = 0.007$, $P=0.069$ (c) and eGFR, $R^2 = 0.001$, $P=0.306$ (d)
**Linear Regression Analysis**

The significant results of the linear regression analysis are shown in table 14. This analysis demonstrated that age, female sex, systolic blood pressure, and pulse pressure all had independent positive interactions with increased AIX, whereas reducing heart rate and lower plasma calcium were also independently associated with increased AIX.

No association was observed between AIX and the following variables: diabetes, cardiovascular disease, smoking, angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use, calcium channel blocker use, body mass index, total cholesterol, bicarbonate, intact parathyroid hormone and albumin. In particular, increasing AIX was not associated with eGFR, creatinine, urinary protein excretion, phosphate or calcium × phosphate product.

Table 14: Adjusted association with linear regression corrected for age, gender, height, cardiovascular disease, diabetes and heart rate.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Unstandardised beta coefficient</th>
<th>Confidence interval</th>
<th>Standardised beta coefficient</th>
<th>R²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.163</td>
<td>0.098, 0.229</td>
<td>1.190</td>
<td>0.435</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female sex</td>
<td>5.914</td>
<td>3.775, 8.053</td>
<td>0.233</td>
<td>0.435</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.116</td>
<td>0.081, 0.150</td>
<td>0.209</td>
<td>0.477</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>0.141</td>
<td>0.1, 0.183</td>
<td>0.223</td>
<td>0.478</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Heart rate</td>
<td>-0.474</td>
<td>-0.535, -0.412</td>
<td>-0.582</td>
<td>0.435</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Calcium</td>
<td>-4.598</td>
<td>-8.937, -0.259</td>
<td>-0.67</td>
<td>0.448</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Variables in table 14 were tested for association in models adjusted for age, gender, height, cardiovascular disease, diabetes and heart rate; only those with p value <0.05 were included.
Discussion

Many previous studies have shown an association between each of age, female gender, systolic and diastolic blood pressure, pulse pressure and heart rate with increased AIx in both the general population and in cohorts of dialysis patients.\textsuperscript{166, 247, 280, 282} We have confirmed that these relationships also exist in a large population of stage 3-5 CKD patients who were not receiving dialysis. However, contrary to a small number of other studies in the non-dialysis CKD population, we did not find an association between increased AI and declining eGFR.

Although carotid-femoral PWV is considered the gold standard non-invasive assessment for arterial stiffness,\textsuperscript{279} AIx derived at the radial artery is also a useful reproducible method for measuring vascular stiffness.\textsuperscript{283} Like PWV, AIx has been shown to be associated with increased cardiovascular and all-cause mortality in end-stage renal disease,\textsuperscript{8} and both AIx and PWV have been shown to be related measures in vascular stiffness in a CKD population\textsuperscript{281} and in healthy individuals.\textsuperscript{282} We have confirmed that carotid-femoral PWV is significantly correlated with AIx derived at the radial artery in the CRISIS population. However, it is important to note that PWV is predominantly determined by aortic stiffness, whereas AIx is also determined by peripheral pulse wave reflections. Whilst both PWV and AIx are pressure dependant, AIx is also pulse rate dependent. Consequently, it is essential that AIx is corrected for heart rate as in this study. The factors associated with increased AIx specifically are clinically relevant. It has already been shown that increased AIx is associated with increased cardiovascular and all-cause mortality even in the presence of normal PWV in end-stage renal disease.\textsuperscript{8} In addition, AIx measurement at the radial artery using the SphygmoCor system has recently been shown to produce highly reproducible results in a CKD stage 3-5 cohort.\textsuperscript{284} As AIx is technically easy and a rapid method for assessing vascular stiffness in large populations,\textsuperscript{279, 283} we felt that this was the most applicable assessment tool in our outpatient CKD population, many of whom were elderly and immobile. Furthermore, the measurement of AIx at the radial artery is potentially amenable to future use in day to day clinical practice, in comparison to PWV.

Ageing has been associated with vascular stiffness since 1922, and consequently the link between ageing and arterial stiffness is well known.\textsuperscript{285, 286} The positive association between systolic blood pressure and vascular stiffness related to the underlying physiology and
anatomy of blood vessels. An association was seen with systolic blood pressure and pulse pressure but not diastolic blood pressure. Shinohara et al. also showed that systolic, but not diastolic blood pressure was associated with aortic PWV in haemodialysis patients, and Wang et al. found similar results in a CKD population. The generally shorter stature of females is thought to lead to earlier aortic wave reflection and so to increased AIX. This hypothesis is supported by our data showing an increase in AIX in females and an inverse correlation with height. Yasmin and Brown have also shown the same association. We also found an association with weight, although there was no association with BMI, diabetes, previous cardiovascular history or smoking, which may appear surprising. However, recent studies have also shown no association between AIX and BMI in haemodialysis patients or patients with metabolic syndrome. An association between PWV and obesity in hypertensive patients has been reported, but Wang et al. found no association between PWV and BMI. We also found an association of AIX with gender and heart rate which also can be explained by physiological mechanisms. Heart rate is thought to affect the AIX due to changes in the timing of reflected pressure waves and the duration of systole, and hence a lower heart rate is association with increased AIX. Beta blocker usage was associated with increased AIX in cross-sectional analysis, but this effect was lost when the analysis was adjusted for heart rate. Several other drugs, such as calcium channel blockers and statins, have been shown to attenuate vascular stiffness in the general population. However, there was no association between these agents and AIX in our study population. This may be a result of the fact that many of our patients were receiving several different classes of antihypertensives and other drugs concomitantly, such that the vasoactive effects of individual classes could have been obscured.

No statistical association was observed between AIX and eGFR in our study, which is in contrast to several recent reports in the literature which suggests that arterial stiffness is association with severity of CKD. Briet et al. showed CKD patients had increased carotid-femoral PWV in comparison to hypertensive and normotensive patients. Multivariate analysis of their study population showed an association with gender, mean blood pressure, heart rate, age, and eGFR. However, they did not find a significant relationship between eGFR and PWV within the CKD population when this was analysed alone, which supports the findings from our study. The majority (almost 80%) of our patients has CKD stages 3 and 4 (eGFR 15-60 ml/min), a group that has not been
extensively studied in the literature. Wang et al.²⁸⁰ conducted a study of 102 CKD patients (stages 1-5) which showed an association between eGFR and PWV. They showed a slight increase in PWV between CKD stages 3 and 4 (PWV 10.0 and 10.4 m/s, respectively). Mourad et al.¹⁶⁶ and Ohya et al.²⁴⁷ have also shown an association with declining eGFR, but they studied very few patients with impaired renal function. Mourad et al.¹⁶⁶ studied 1,290 hypertensive patients who underwent PWV assessment, but the majority of their patients had an eGFR of >60 ml/min. Similarly, approximately 96% of patients studied by Ohya et al.²⁴⁷ had an eGFR greater than 60 ml/min. These data suggest that declining renal function is associated with vascular stiffness in the earlier stages of CKD, and additionally, that patients with CKD have increased vascular stiffness in comparison to the general population and hypertensive population. Our data, which were obtained in a large cohort of patients with moderate and advanced CKD, suggest that with declining eGFR other factors are likely to determine the degree of vascular stiffness in a given individual. Patients with advancing CKD are exposed to a multitude of metabolic and clinical risk factors, and evidence suggests that many of these can influence vascular structure and function. It is inevitable that a combination of abnormalities contributes to the CKD population’s increased vascular stiffness, and each of these may not necessarily progress at the same rate as eGFR declines. Hence, with advancing CKD the association between AIx and eGFR may be distorted by the influence of unquantifiable risks such as duration of hypertension and also CKD vintage, perturbations in bone mineral chemistry and by the influence of the many therapeutic agents that these patients are prescribed. The presence of an arterio-venous fistula has been associated with increasing AIx in transplant recipients. In our study, the majority of patients had CKD stage 1-4 (84%) but 16% of the patients had CKD stage 5 and a proportion of these may have had a functioning fistula and consequent increased AIx. These data were not collected. However, if the presence or absence of a fistula were to be confounder in our population, we would expect that the relationship between declining GFR (i.e. stage 5) and increased AIx, if it were to exist, would, if anything, be strengthened - which it was not. Haemodialysis vintage has been shown to be a major factor in determining cardiovascular risk and vascular stiffness, and it is likely that a similar effect applies in patients with advance CKD not yet on dialysis. Unfortunately, it is difficult to accurately ascertain when a patient develops CKD as many patients present to renal physicians with established renal disease. Long-term longitudinal studies may help answer this question.
Our study did not show an association between AIx and certain risk factors known to be associated with increased cardiovascular mortality such as diabetes, phosphate, or protein excretion. Wang et al. also showed no association between PWV, diabetes, previous cardiovascular history or smoking in patients with CKD. However, we did show a negative association with serum corrected calcium, which may seem hard to explain as calcium load and calcium × phosphate product have been associated with vascular calcification and increased mortality in dialysis populations. In contrast to this literature, Foley et al have previously identified an association between hypocalcaemia and increased adverse events in the dialysis population. Hence, although we might have expected a higher corrected calcium to be associated with increased vascular stiffness, our findings may reflect the complexity of the interaction between underlying metabolic complications, particularly CKD-mineral and bone disorder, and their treatments in these patients. CKD-mineral and bone disorder is associated with low calcium, high phosphate and high parathyroid hormone and has been shown to be associated with increased vascular stiffness and vascular calcification. In addition, the relationship between measured bone biochemistry and subsequent management is complex and the patients in our study were not treated to a formal protocol. Whether the negative association between AIx and corrected calcium can be explained by CKD-mineral and bone disorder or as a result of treatment is uncertain. To explore this further, we would need to investigate patients with CKD in a longitudinal study with a well-defined bone mineral treatment protocol.

This study has highlighted blood pressure as a potential modifiable association, whereas eGFR was not associated with increased AIx in our CKD stage 3-5 population. Further knowledge of factors which influence progression of vascular stiffness will be important in risk quantification and appropriate management, again indicating the importance of undertaking longitudinal studies in these patients.
5.3 EXPERIMENTAL CHAPTER 3:

A Randomised control trial to examine the effects of cinacalcet on bone and cardiovascular parameters in haemodialysis patients with uncontrolled secondary hyperparathyroidism

Helen Eddington, Constantina Chrysochou, Darren Green, Ibi Erikoisima, Osvaldo Espinosa, Alastair Hutchison, Abdalla Bubtana, Janet Hegarty, Andy Vail, William D Fraser, Grant Heatlie, Paul Taylor, Judith Adams, Philip A Kalra
5.3.1 Detailed methods

These methods relate to the experimental chapters 3 and 4 and will incorporate an overview of the trial, justification of the inclusion and exclusion criteria and how screening and recruitment was performed. Further methods relating to the trial are incorporated within the papers. The trial was registered on the international standard randomised clinical trials register (ISRCTN81718275).

Study Overview

This trial was designed and started in 2005 when cinacalcet had just been licensed in the United Kingdom. At that time very little information regarding the effects of cinacalcet on bone and cardiovascular disease was available. This open-label randomised controlled trial was therefore designed to investigate effects of cinacalcet on bone and cardiovascular health in a haemodialysis population. Patients whose iPTH concentration was above the KDOQI upper limit of 300pg/mL were recruited. Once consented, patients were randomised to cinacalcet alongside standard therapy or standard therapy alone. All patients underwent investigations at baseline and at 12 months. As the patients started the trial they underwent an intensive 12 week period where the patient and their management was reviewed fortnightly with the aim to achieve the KDOQI calcium, phosphate and PTH targets. This guideline was chosen as these were the most current at the start of the study.

This study aimed for equivalent control between treatment arms therefore any additional effect could be due to the cinacalcet.

A detailed diagram showing the pathway of this open label randomised control trial is shown within the paper (Figure 24, Page 151) and the study protocol is attached in Appendix 1.
**Ethics and regulatory approval:**

Ethics approval was granted by the Salford and Trafford Local research ethics committee in 2005 (Reference number: 05/Q1404/216). Medicines Health and Regulatory Authority approval was also granted in 2005 (EudraCT number: 2005-003871-19).

**Inclusion criteria:**

The inclusion criteria for the trial are listed below and their justification is discussed:

- iPTH ≥ 300pg/mL
- Corrected calcium ≥ 2.1mmol/L
- Age 18-75
- Dialysis for >90 days

These factors were chosen for a number of reasons. When designing the trial, contemporary evidence suggested that a ‘normal’ PTH for the general population (i.e. 10-65pg/mL) was too low for renal patients and was associated with an increased vascular calcification\(^{13}\) and mortality.\(^ {296, 297}\) The UK Renal Association guidelines\(^ {258}\) were under review and no lower limit for PTH had been stated in their previously published guidelines. The most up-to-date guidelines available were devised by the National Kidney Foundation KDOQI and advised maintaining the PTH between 150-300 pg/mL.\(^ {143}\) We therefore decided to recruit patients with an iPTH ≥300pg/mL.

The normal range of corrected calcium at Salford Royal Hospital was 2.1-2.6mmol/L. As hypocalcaemia is a known side-effect of cinacalcet\(^ {223}\), it was therefore important for patients to be at least at the lower level of normal calcium at recruitment. This also ensured that hypocalcaemia was not a long-term problem and that calcium could be maintained in the normal range. Age of the participants was restricted due to the high mortality of renal patients. The age range was originally set at 18-70 years but was changed with an amendment to an upper age of 75 to aid recruitment. Patients over the age of 70 have a much shorter life expectancy; for this reason we had excluded them to reduce our drop-out rate. This was important as there were only a small number of patients in the study and a larger than expected mortality rate would have led to it being underpowered.
The patients were also required to have been on dialysis for greater than 90 days. This criterion was included to ensure that patients were stable on dialysis at the time of recruitment and avoided patients with an acute presentation of their renal impairment which can be associated with higher PTH levels and early mortality. According to the inclusion criteria haemodialysis or peritoneal dialysis patients could have been recruited to the study but only haemodialysis patients were recruited. This was important as peritoneal dialysis patients have been found to be more prone to adynamic bone disease which is associated with an increased calcification risk.\textsuperscript{121}

**Exclusion Criteria**

The exclusion criteria for the trial are listed below and their justification is then discussed:

- Atrial fibrillation
- Any contraindications to magnetic resonance (CMR) scan or ability to cooperate with scan
- Any factors which would influence the CT e.g. artificial heart valves, previous sternotomy wires, stents
- Contraindication to cinacalcet e.g. pregnant, breast feeding, known reaction
- Moderate to severe liver disease (Alanine transaminase > x3 normal range)
- Have a poor record of compliance with medication
- Have participated in a study involving an investigational drug during the 30 days prior to the 1\textsuperscript{st} visit.
- Be involved in any other research study which exposed the patient to radiation.

These exclusion criteria were required due to the investigations involved in the trial. The CT and CMR scans involved cardiac gating which synchronises the image acquisition to a set point in the cardiac cycle. Therefore the scanner was unable to produce accurate images in patients with atrial fibrillation. Patients who have artificial heart valves and sternotomy wires, etc were also excluded as the metal leads to scatter on the CT images which would have hindered the accurate calculation of calcification score. The patient was required to lie flat for both the CT and the CMR scan and also needed to be able to perform breath holds for the scans. If the patients were unable to perform the necessary breath holds then
this will lead to inaccuracy or to no images being acquired. The CT scan involved exposing the patient to radiation which involved a theoretical risk of increased chance of malignancy. For this reason we did not include any patient who had been involved in any other trial which involved the patient being exposed to radiation. The other criteria were important as this was a clinical trial of a medicinal product. The exclusion criteria of prescribing cinacalcet as stated in the British National Formulary were included. Cinacalcet is eliminated via the liver hence patients with moderate to severe liver disease were also excluded.

**Screening**

Initially only Salford, Bolton and Wigan dialysis units were recruitment sites in the trial. Through the course of the trial further recruitment sites were added to include Rochdale, Tameside, Wythenshawe, Chorley and Preston haemodialysis units. Every month I screened all haemodialysis patients at the recruitment sites as potential participants for the trial. All patients underwent monthly blood tests as part of their routine clinical care which included corrected calcium and a 3-monthly iPTH measurement. These results were monitored and if a patient was found to fit the criteria they then entered the next stage of screening. The next stage involved a review of their medical notes and discussion with medical and/or nursing staff involved in the regular care of the patients. This stage identified patients who were outside of the age criteria, had any medical conditions which excluded them from the study and any other social and recent episodes which meant that they would be inappropriate to be approached at that time.

**Recruitment**

If all criteria were satisfied then patients were approached while attending haemodialysis. The patients were informed as to why the study was devised and why we felt it was an important study. They were informed of the risks and benefits of taking part and were then left a patient information leaflet to read. The patients were then re-approached after a minimum of 5 days to allow the patient time to read the leaflet and discuss with their family whether they were willing to take part. Three consent forms were completed as per protocol and one returned to the patient on completion. As part of the consent process a
screening form was also completed to ensure that the patient fulfilled all the inclusion criteria and did not have any exclusion criteria.

After a patient was consented screening bloods were taken to ensure that initial blood tests, analysed at Salford Royal Hospital, still fulfilled the criteria of inclusion to the study. Some patients were excluded from the study due to these results. As there were many different iPTH assays available it was felt important that all iPTH measurements were performed in the same laboratory for the study. All patients in the study had a hospital number for Salford Royal Hospital generated to allow samples to be processed.
5.3.2 History and development of trial

The trial began recruitment in 2006 but despite very simple inclusion criteria the exclusion criteria led to many patients not being suitable. The scan burden also limited recruitment as it deterred patients due to the time required. In view of this a number of amendments to expand and improve recruitment were completed throughout the trial.

Amendment 1: August 2006. Upper age limit increased from 70 to 75 years

Amendment 2: October 2006. Inclusion criteria changed as local laboratory lower limit for corrected calcium changed from 2.2 to 2.1 mmol/L. Documentation of calcium dialysate was added to the questionnaires and the number of patients to be recruited increased from 40 to 48. The numbers to be recruited increased as this would have therefore increased our power in the study. At this point recruitment was steady and we felt that this aim was a possibility.

Amendment 3: November 2006. We requested to minimally extend the MR scan time in a sub-group of controls to facilitate methodological developments of new emerging pulse wave analysis techniques in vascular disease. A sub-group (n=10) of patients would undergo an extra 10 minutes of MR scan time to allow imaging of aortic blood flow. In addition, a non-invasive pulse wave assessment of the wrist artery was to be performed at the same time as the MR scan. These extra measurements would have been performed at inception and repeated after 1 year. This amendment was refused due to the scan burden the patients were already undergoing and the ethics committee felt that this should run as a separate study.

Initially recruitment only involved Salford Royal Hospital NHS Trust and its satellite units at Wigan and Bolton. As the trial progressed further R&D approval was sought to extend to Rochdale dialysis unit which is also a satellite unit of Salford Royal Hospital.

Amendment 4: October 2007. To extend recruitment further the name of the Manchester Royal Infirmary site was changed from Manchester Royal Infirmary to Central Manchester and Manchester Children’s Hospital NHS Trust to allow recruitment of the
satellite unit patients at Wythenshawe and Tameside dialysis units. Lancashire Hospital NHS Trust was also added to allow further recruitment at Chorley and Preston.

**Safety**

There were no new regulations regarding the already licensed product, Cinacalcet, in dialysis patients between commencement and completion of the trial. Adverse events and serious adverse events were recorded and reviewed by the data monitoring committee every 6 months. The adverse events due to the drug were expected and stated in the patient information leaflet; they were not at a higher incidence than expected. All the serious adverse events reported were not felt to be related to the trial or Cinacalcet and included admissions for renal related problems or infections. All events are shown within the experimental chapter. There were no Suspected Unexpected Serious Adverse Reactions during this trial.

**Medicines and Health Regulatory Authority: Breach of GCP guidance**

During the trial a ‘serious breach’ of GCP guidance occurred. This was noted during a routine monitoring visit from the sponsor in 2009. The pharmacy documentation regarding the trial was inadequate leading to poor pharmacovigilance. Procedure was followed and the Medicines and Health Regulatory Authority (MHRA) were informed within 7 days. The breach was rectified and the MHRA were satisfied with the actions taken to rectify and the matter was closed by the MHRA. The changes in policy that were needed in Pharmacy and research and development were put in place. The serious breach report sent to the MHRA is attached as Appendix 2.

**MHRA Inspection**

This trial was also inspected as part of a routine MHRA inspection in 2009. Professor Philip A Kalra and I were interviewed. After the interview the MHRA were satisfied with our conduct of the trial and made a few suggestions for improvement. These included ensuring verbal and email discussions related to the trial were appropriately documented and stored, advice was given regarding analysis and archiving of trial data and advised that expansion of the statistical section of the protocol would be advised.
Personal Trial Experience:

I had very limited experience of clinical trials on starting this research. At the beginning my task was to write the protocol and all supporting documents necessary to set up a clinical trial of an investigational medicinal product. I had no idea that this would be a 6 month process involving more forms than I thought possible. Firstly I sought ethics approval; in retrospect this was reasonably easy despite the lengthy form. There were many obstacles; one of these was adverse event recording of which I had no experience and I found the research nurses who were involved in running the commercial trials invaluable for advice regarding this. The trial also involved exposing patients to radiation above what was required for their clinical care. Understanding the regulations and achieving a signature to approve this, took many weeks. I attended the ethics committee meeting where the trial was discussed and managed to answer many questions at that time which allowed the trial to be approved.

The Medicines Health and Regulatory Authority approval was much more complicated. At the time of the trial set up the research and development department consisted of one member of staff; I was informed that I would need MHRA approval but no further advice was available as to how to complete this. I realise that this is no longer the case and the department have many people who can help guide young naïve researchers as I was then. The MHRA website was difficult to navigate and I made many phonecalls to the ‘helpline’ at the MHRA to help me find the relevant paperwork, form, guidance, payment section of the website. The main MHRA application was designed for large pharmaceutical companies performing early studies with unlicensed products and therefore most of the application was irrelevant and difficult to navigate. They asked for the file in a rare file format and this minor fact took many hours and days to overcome though, once I was successful, I am not sure why it took so long. Many instances occurred during my research journey where time seemed to stand still and progress on a trial matter seems to take much longer than you ever thought possible. This is one of the main facts I impart to researchers at the start of their journey. Life in research seems to travel slower and the pace achieved in clinical medicine does not occur in research, no matter how hard you try.

Once the trial was approved I thought the research journey would be much easier. There are many GCP regulations associated with running a clinical trial of an investigational
medicinal product and to the new researcher they can feel cumbersome. During this trial I was the principal investigator, the research nurse, the taxi, the administrator and the analyst which can be complicated to balance while trying to recruit patients. I was also very naïve to the process of recruitment; even to this day I can determine during a conversation whether a fellow clinician has ever tried to recruit to or had experience of a clinical trial by their reaction to the 36 patients I personally recruited to this trial. If they empathise, they have had experience; the people that ask why there were not larger numbers rarely have.

While writing this PhD I have deliberated whether I would brave the process of a clinical trial of an investigational medicinal product again. This trial has given me invaluable experience in the field of clinical research which cannot be learnt from a lecture or from a textbook and therefore I do not regret any decisions and would definitely do it again as I have learnt so much; it would be a waste not to.
Experimental Paper:

Abstract:

Introduction:
This open-label randomised controlled trial compared progression of cardiovascular and bone parameters in 36 haemodialysis patients whom were treated for secondary hyperparathyroidism with either cinacalcet and standard therapy or standard therapy alone.

Methods:
Inclusion criteria included >90 days on dialysis, iPTH >300pg/mL, calcium >2.1mmol/L and age 18-75years. Patients were randomised and all patients underwent an intensive 12 week period of titration to improve control aiming for iPTH 150-300pg/mL. Calcification score was assessed at the coronary arteries and abdominal aorta using CT Agatston score. Cardiac MR was used to assess Left ventricular mass index (LVMI). Arterial stiffness measurements and carotid intima media thickness were measured. Bone mineral density was measured using DXA, QCT and pQCT. Serum and plasma were analysed for a series of biomarkers. All investigations were performed at baseline and at week 52. The primary endpoint was absolute change in total calcification at 1 year.

Results:
The difference in change of calcification between baseline and 1 year was not different between treatment arms (Median change (IQR) cinacalcet: 488 (0, 1539); standard therapy: 563 (50, 1214)). LVMI reduced in both arms (median change (IQR): cinacalcet -22g/m² (-59, -2); control -4g/m² (-32, 11)) despite progression of calcification and vascular stiffness but was not significantly different. In the cinacalcet group a non significant decrease in blood pressure and FGF23 were seen at 1 year.

Conclusion:
No significant benefit in bone and cardiovascular markers was seen with the addition of cinacalcet to standard therapy over a 1 year period. Tight control of secondary hyperparathyroidism may lead to a reduction in LVMI but this needs further investigation.
**Introduction:**

The management of Chronic Kidney Disease – Mineral Bone Disorder (CKD-MBD) can be challenging. Studies have shown that derangements of parathyroid hormone (iPTH), phosphate and calcium are linked to increased mortality. iPTH has also been associated with increased calcification and cardiomyopathy. The introduction of cinacalcet enabled increasing numbers of patients to achieve targets for CKD-MBD though there continues to be restrictions on its use. Since the start of this trial there has been increasing evidence that calcimimetics may reduce progression of vascular calcification and studies are ongoing to assess its impact on survival. Calcification progression has been studied with cinacalcet and other CKD-MBD medications but many randomised trials allowed a disparity of parathyroid hormone or phosphate control between arms leaving the question whether it is the medication or the control that has an effect.

We performed a small open-label randomised control trial in haemodialysis patients to examine the effects of Cinacalcet with standard therapy compared to standard therapy alone. The primary aim was to determine if Cinacalcet treatment attenuated progression of calcification when equivalent control of secondary hyperparathyroidism was achieved between treatment arms. Secondary outcomes included bone mineral density, cardiovascular structure, vascular stiffness and biomarkers. This trial had begun prior to the ADVANCE trial announcement. This was a global study examining the effects of cinacalcet on vascular calcification but this trial differed as it aimed for equivalent biochemical targets. This trial also did not stipulate a maximum vitamin D dose to be used alongside cinacalcet.

**Methods:**

87 patients were approached to take part in this trial; 37 refused, 11 failed initial screening and 3 more patients withdrew prior to randomisation. Therefore 36 haemodialysis patients from North West England were included after providing informed written consent. The inclusion and exclusion criteria are shown in table 15.

An overview of the trial is shown in Figure 24.
Figure 24: Diagram showing the patient flow through the randomised control trial
Inclusion Criteria | Exclusion Criteria
--- | ---
Intact Parathyroid Hormone >300pg/mL | Atrial fibrillation
Corrected calcium >2.1mmol/L | Contraindication for MR scan
Age 18-75 yrs | Past history which affected CT scan readings
On dialysis for >90 days | (artificial heart valves, coronary stents, sternotomy wires)

Table 15: Inclusion and exclusion criteria for randomised controlled trial

Investigations:

The investigations were performed at baseline and at 12 months and are listed below. They have been described previously in the generic methods chapter. Serum and plasma samples were also taken and stored at baseline and at 12 months and analysed as previously described.

Cardiac and abdominal aorta vascular calcification score was determined using a GE CT Lightspeed 16-slice scanner. The cardiac images were acquired during a breath hold with ECG gating. The Aortic images were acquired from images produced during acquisition of the Quantitative Computed Tomography (QCT) bone density measurement. Calcification score was determined using the Smartscore software and the Agatston and volume methods were used. A cardiac magnetic resonance (CMR) scan provided information on cardiac structure and function including left ventricular mass index (LVMI) using the Philips cardiac software package. This was performed using a Philips Intera 1.5T imager (release 11) using the phased-array chest coil and ECG gated imaging. The MR scans were performed on a non-dialysis day within 24 hours of the patients’ previous dialysis session. Bone mineral density measurements were performed and included QCT spine (GE scanner using Mindways software), Dual X-Ray Absorptiometry (DXA) of Hip and spine and peripheral QCT (pQCT) of forearm. The pQCT also provided measurements of bone quality.
The above scans were performed on the same day and interpreted by independent qualified people blinded to randomisation and blood results. The calcification scores were determined independently by 2 people and results were rechecked if more than 10% variation between results.

I performed the following investigations immediately after dialysis with the patient at their dry weight as determined by their medical team and was not blinded to randomisation or blood results:

- Post dialysis blood pressure
- Carotid-femoral pulse wave velocity (cfPWV) (SphygmoCor® (Atcor Medical)).
- Augmentation index (Alx) at the radial artery (SphygmoCor® (Atcor Medical)).
- The carotid-intima media thickness (CIMT) was measured 1cm below the bifurcation of the carotid artery on both sides.

Biomarkers were assessed using the following methods:

- 25(OH) vitamin D: High performance liquid chromatography tandem mass spectrometry
- 1,25(OH)_{2} vitamin D: Radioimmunoassay (Immuno Diagnostic Systems, Boldon, Tyne &Wear, UK);
- NTProBNP: electrochemiluminescent immunoassay (ECLIA) on a Modular Analytics E170 analyser (Roche Diagnostics, Lewes, UK);
- Troponin T: ECLIA on a Modular Analytics E170 analyser (Roche Diagnostics, Lewes, UK),
- FGF23: 2nd generation, two-site enzyme-linked immunosorbent assay supplied by Immutopics Inc (San Clemente, CA, USA);
- Fetuin A: Enzyme Linked ImmunoSorbent Assay (ELISA) supplied by BioVendor GmbH (Heidelberg, Germany);
- Osteoprotegerin: ELISA supplied by Immuno Diagnostic Systems (IDS) (Boldon, Tyne&Wear, UK).
Randomisation occurred when investigations were completed.

Patients underwent a detailed questionnaire at baseline and at 12 months which collected phenotypic data along with New York Heart Association (NYHA) classification of heart failure and the Canadian Cardiovascular Society (CCS) grading system score to describe angina severity. The baseline questionnaire is included in Appendix 1. Adequate dialysis was determined as a Kt/v >1.2 and / or a URR > 65%.

Biochemical and haematology samples were processed at 12 time points through the trial; fortnightly for the first 12 weeks then every 8 weeks until week 52. Samples were taken pre-dialysis and transported within a 2 hour period to the Salford Royal Hospital Laboratories. The samples were then processed and analysed; biochemistry samples using the Roche modular analyzer and iPTH measured using the Diagnostic Product Corporation (DPC) immulite 2000 chemiluminescent immunoassay, haematology results using Sysmex XE-1200.

**Randomisation**

This was an open-label study. Patients were randomised to cinacalcet therapy alongside standard therapy or standard therapy alone. Randomisation was performed via colour coded envelopes prepared by an independent person not involved in the recruitment of patients into the study. The randomisation was stratified by diabetic status (yes / no) and baseline pulse wave velocity (<11 m/sec and ≥ 11m/sec). This was to aim for equivalence of cardiovascular risk between the 2 groups at baseline in view of the small numbers involved in the trial.

The randomisation procedure used a random number table and block randomisation.

**Treatment during the 12 months**

Once randomisation had occurred all patients underwent a 12 week intensive management phase. This involved calcium and phosphate concentrations measured fortnightly and the iPTH was measured at week 2 and at week 12. During this time patients treatment was adjusted and patients were educated in a low phosphate diet. This was performed by me to
ensure all patients received similar advice and management. All phosphate binders were available for prescription including sevelamer hydrochloride and lanthanum carbonate. Phosphate binders and 1 alphacalcidol were provided by the patients’ general practitioners. Cinacalcet prescriptions were provided through the out-patient pharmacy at Salford Royal Hospital using trial-specific prescriptions.

After this period patients were reviewed every 8 weeks until the final visit at week 52 unless a medication change, or biochemical result, warranted management to be assessed more frequently for safety. Biochemical and haematology tests were measured at each time-point and medication altered at the patient review where necessary depending on the results.

All patients received a minimum of 12 consultations, one per time point, throughout the trial.

Regulatory Authority approval and funding

All documents were submitted for approval from Salford and Trafford local research ethics committee (LREC) for this trial and this was granted on the 15th November 2005. Medicines and Health Regulatory Authority approval was also granted in 2005 and the authority was informed of all amendments during the course of the trial.

All Research and Development departments of the involved hospitals also approved the trial. A list of the departments is below:

- Salford Royal Hospital NHS Foundation Trust
- Central Manchester and Manchester Children’s Hospital NHS Trust
- South Manchester Hospitals NHS Trust
- Tameside Hospital NHS Trust
- University of Manchester
- Pennine Acute Hospital NHS Trust
- Lancashire Teaching Hospital NHS Foundation Trust
The sponsor of this trial was Salford Royal Hospital NHS Foundation Trust. This trial was mainly funded by an unrestricted educational grant from Amgen whom also provided the cinacalcet for the trial. A grant awarded from the Translational Imaging Unit at Manchester University funded the CMR scans throughout the trial.

**Primary outcome**

- The primary outcome was the absolute change in total calcification score between the two arms of the randomised trial

**Secondary outcomes**

- The difference in the mean cfPWV at 1 year compared between the two treatment arms
- The difference in the mean AIx at 1 year compared between the two treatment arms
- The difference in the mean LVMI at 1 year compared between the two treatment arms
- The difference in the mean carotid intima-media thickness at 1 year compared between the two treatment arms
- The difference in bone mineral density measurements at 1 year compared between the two treatment arms
- The difference of biomarker concentrations at 1 year compared between the two treatment arms

**Statistics:**

To assess iPTH and phosphate control between the two treatment arms area under the curve was calculated using each time point. An unpaired t-test of the area under the curves for each individual were performed.

Demographic data between the arms were described. Normally distributed data were reported as means with standard deviation, skewed data was reported as median and range and categorical data will be reported as absolute number with percentage.
Categorical data was analysed using chi-square analysis. Continuous data was assessed for normality using graphical means. Where necessary, data were transformed using logarithmic or square root calculations depending on the parameter. All data were then analysed using linear regression adjusted for randomisation, diabetes and PWV as per randomisation stratification.

**Power calculation**

This was performed with a medical statistician from the University of Manchester. A sample size of 20 per treatment arm with an expected 20% loss due to mortality or transplantation would leave 16 per group. This would give an 80% power at the 5% significance level to detect a difference of 1 standard deviation in the absolute change in calcification score. This was calculated using the calculation for comparing means of a normally distributed outcome as no data regarding the effects of cinacalcet were available at the time of trial development.

The assumptions made in this calculation were that the outcome would be normally distributed. Other assumptions used were the difference in means in calcification are 10% and the standard deviation of this population is 10%. No previous trial at the time of design had examined calcification with the use of cinacalcet so therefore no data was available for comparison.

**Results:**

Figure 25 shows a diagrammatic overview of trial recruitment, 36 patients were recruited into the trial but only 2 patients did not complete the trial. Due to the lower dropout than expected the targeted power of 80% was achieved. Demographics of the enrolled population and cardiovascular results at baseline are summarised in Table 16 and 17. The mean age was 51 (15) years, 64% were male and 39% had known cardiovascular disease. The population was mainly Caucasian (83%) and only 11% were diabetics. The median iPTH at baseline was 703 pg/mL (range 318-1586) and the mean phosphate was raised at 1.91 (0.58) mmol/L. Four patients did not undergo any CMR investigations in the study due to claustrophobia but continued in the study. The mean pulse wave velocity, total calcification score and left ventricular mass index were increased at baseline as would be
expected in a haemodialysis population. Diabetes, Smoking status, blood pressure and cardiovascular disease were similar between the treatment arms at baseline.

Figure 25: A consort diagram detailing the recruitment and trial completion

There was no statistical difference of the baseline parameters between treatment arms but the calcification score at baseline, iPTH and cfPWV were higher in standard therapy arm. NTproBNP, Troponin T, FGF23 and OPG concentrations were higher than would be expected in a normal population. Conversely Fetuin A and 25(OH) vitamin D and 1,25(OH)₂ vitamin concentrations were below normal. These results are consistent with other haemodialysis populations. 15 patients were randomised to cinacalcet with standard therapy compared to 21 with standard therapy alone; the difference due to the stratification at randomisation. Two patients in the standard therapy arm did not complete the study; one died and the other patient withdrew in week 51.
### Table 16: Demographics overall and per treatment arm at baseline.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>All patients (n=36)</th>
<th>Cinacalcet (n=15)</th>
<th>Control (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>51 (15)</td>
<td>45 (16)</td>
<td>54 (13)</td>
</tr>
<tr>
<td>Male</td>
<td>23 (64%)</td>
<td>11 (73%)</td>
<td>12 (57%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>30 (83%)</td>
<td>14 (93%)</td>
<td>16 (76%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (11%)</td>
<td>1 (7%)</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>9 (25%)</td>
<td>5 (33%)</td>
<td>4 (19%)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166 (11)</td>
<td>169 (11)</td>
<td>163 (11)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73 (15)</td>
<td>74 (15)</td>
<td>72 (15)</td>
</tr>
<tr>
<td>BMI</td>
<td>26 (5)</td>
<td>25 (5)</td>
<td>26 (5)</td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>131 (26)</td>
<td>133 (27)</td>
<td>130 (26)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>72 (13)</td>
<td>72 (13)</td>
<td>72 (13)</td>
</tr>
<tr>
<td>MI</td>
<td>5 (14%)</td>
<td>2 (13%)</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Angina</td>
<td>7 (19%)</td>
<td>5 (33%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Stroke</td>
<td>3 (8%)</td>
<td>0</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>TIA</td>
<td>7 (19%)</td>
<td>2 (13%)</td>
<td>5 (24%)</td>
</tr>
<tr>
<td>All CVD</td>
<td>14 (39%)</td>
<td>6 (40%)</td>
<td>8 (38%)</td>
</tr>
<tr>
<td>NYHA score</td>
<td>1: 26 (72%)</td>
<td>1: 10 (67%)</td>
<td>1: 16 (76%)</td>
</tr>
<tr>
<td>CCS score</td>
<td>0: 33 (92%)</td>
<td>0: 14 (93%)</td>
<td>0: 19 (91%)</td>
</tr>
<tr>
<td>Renal history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate dialysis</td>
<td>23 (80%)</td>
<td>8 (73%)</td>
<td>15 (83%)</td>
</tr>
<tr>
<td>Dialysis duration (m)</td>
<td>38 [6-319]</td>
<td>33 [6-319]</td>
<td>43 [10-222]</td>
</tr>
<tr>
<td>Previous transplant</td>
<td>9 (25%)</td>
<td>4 (27%)</td>
<td>5 (24%)</td>
</tr>
<tr>
<td><strong>Biochemical results at baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iPTH (pg/mL)</td>
<td>730 [318,1586]</td>
<td>665 [353,1586]</td>
<td>806 [318,1096]</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.30 (0.14)</td>
<td>2.32 (0.16)</td>
<td>2.29 (0.12)</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.91 (0.58)</td>
<td>1.91 (0.58)</td>
<td>1.91 (0.59)</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>124 (15)</td>
<td>124 (18)</td>
<td>124 (14)</td>
</tr>
<tr>
<td>CRP</td>
<td>11 [0-97]</td>
<td>16 [0-97]</td>
<td>7 [0.5-52]</td>
</tr>
<tr>
<td>Hyperparathyroidism medication at baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calcium binder</td>
<td>15 (42%)</td>
<td>7 (47%)</td>
<td>8 (38%)</td>
</tr>
<tr>
<td>Non-calcium binder</td>
<td>30 (83%)</td>
<td>13 (87%)</td>
<td>17 (81%)</td>
</tr>
<tr>
<td>VDRA</td>
<td>35 (97%)</td>
<td>15 (100%)</td>
<td>20 (95%)</td>
</tr>
</tbody>
</table>

MI: myocardial infarction; TIA: transient ischaemic attack; CVD: cardiovascular disease; NYHA: New York Heart Association heart failure score; CCA: Canadian Cardiovascular Society angina score; VDRA: vitamin D receptor analogue; iPTH: intact parathyroid hormone; CRP: C-reactive protein; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: Body mass index.
<table>
<thead>
<tr>
<th>Vascular stiffness</th>
<th>Cinacalcet (n=15)</th>
<th>Control (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cfPWV (m/s)</td>
<td>7.8 (2)</td>
<td>9.2 (3)</td>
</tr>
<tr>
<td>Augmentation index (%)</td>
<td>21 [-5.0, 37]</td>
<td>26 [-10.0, 53]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Carotid intima media thickness</th>
<th>All patients (n=36)</th>
<th>Cinacalcet (n=15)</th>
<th>Control (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average CIMT (mm)</td>
<td>0.04 (0.01)</td>
<td>0.04 (0.01)</td>
<td>0.04 (0.01)</td>
</tr>
</tbody>
</table>

| Agatston calcification score  | 204 [0, 4075]       | 96 [0, 4075]      | 260 [7.5, 3785]|
| Aortic calc score             | 892 [0, 16466]      | 302 [0, 13063]    | 2090 [0, 16466]|
| Total calc score              | 2174 [0, 16635]     | 814 [0, 15090]    | 3401 [7.5, 16635]|

<table>
<thead>
<tr>
<th>Cardiac magnetic resonance imaging results</th>
<th>All patients (n=32)</th>
<th>Cinacalcet (n=12)</th>
<th>Control (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass index (g/m²)</td>
<td>109 (39)</td>
<td>120 (43)</td>
<td>103 (37)</td>
</tr>
<tr>
<td>LV stroke volume (mL)</td>
<td>92 (22)</td>
<td>102 (22)</td>
<td>87 (19)</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>68 (11)</td>
<td>70 (7)</td>
<td>67 (13)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bone (n=36)</th>
<th>Cinacalcet (n=15)</th>
<th>Control (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXA spine BMD g/m²</td>
<td>1.1 (0.2)</td>
<td>1.1 (0.1)</td>
</tr>
<tr>
<td>DXA hip BMD g/m²</td>
<td>0.9 (0.1)</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>DXA femoral BMDg/m²</td>
<td>0.8 (0.1)</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>QCT BMD g/m³</td>
<td>126 (48)</td>
<td>134 (38)</td>
</tr>
<tr>
<td>pQCT50 cortcnt g</td>
<td>80 (30)</td>
<td>86 (23)</td>
</tr>
<tr>
<td>Stress strain index</td>
<td>215 (99)</td>
<td>227 (68)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Cinacalcet (n=15)</th>
<th>Control (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTproBNP (pg/mL)</td>
<td>2945[332,161680]</td>
<td>2483[679,80840]</td>
</tr>
<tr>
<td>25(OH)Vitamin D (nmol/L)</td>
<td>23[34,132]</td>
<td>16[6,59]</td>
</tr>
<tr>
<td>1,25 (OH)₂ Vitamin D (pmol/L)</td>
<td>66[34,132]</td>
<td>62[34,130]</td>
</tr>
<tr>
<td>Osteoprotegerin (pmol/L)</td>
<td>7.7[3.2,23.4]</td>
<td>7.8[3.3,17.4]</td>
</tr>
<tr>
<td>FGF23 (RU/mL)</td>
<td>13794</td>
<td>13793</td>
</tr>
<tr>
<td>Fetuin A (ng/mL)</td>
<td>342[190,588]</td>
<td>363[290,562]</td>
</tr>
</tbody>
</table>

Table 17: Baseline investigations overall and per treatment arm.

cfPWV: carotid-femoral pulse wave velocity; CIMT: carotid intima-media thickness; calc: calcification; LV: left ventricular; DXA: Dual X-Ray Absorptiometry; BMD: bone mineral density; QCT: quantitative computerised tomography; pQCT50: peripheral QCT at 50% of forearm; crtden: cortical density; NTproBNP: N-Terminal Pro Brain Naturetic peptide; FGF23: fibroblast growth factor 23.
Adverse events were similar in both arms during the trial as shown in table 18. There was a higher incidence of hypercalcaemia and less hypocalcaemia in the standard therapy arm compared to patients taking cinacalcet as would be expected.

<table>
<thead>
<tr>
<th>Description</th>
<th>Cinacalcet No of pts (no of events)</th>
<th>Control No of pts (no of events)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>2 (3)</td>
<td>6 (7)</td>
<td>0.43</td>
</tr>
<tr>
<td>renal transplant</td>
<td>1</td>
<td>2 (2)</td>
<td>0.7</td>
</tr>
<tr>
<td>Vascular access procedures</td>
<td>4 (11)</td>
<td>1</td>
<td>0.06</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>brain tumour</td>
<td>0.4</td>
</tr>
<tr>
<td>Parathyroidectomy</td>
<td>1</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Low calcium</td>
<td>11(20)</td>
<td>4 (5)</td>
<td>&lt;2.1mmol/L 0.01</td>
</tr>
<tr>
<td>High calcium</td>
<td>2(5)</td>
<td>9 (17)</td>
<td>&gt;2.6mmol/L 0.06</td>
</tr>
<tr>
<td>Claustrophobia</td>
<td>3</td>
<td>1</td>
<td>In MR scan 0.4</td>
</tr>
<tr>
<td>Indigestion</td>
<td>3</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Other</td>
<td>11(16)</td>
<td>12 (20)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 18: Overview of all adverse events reported during the trial.

Figure 26 shows the median iPTH during the study; similar iPTH control was seen in both arms. Area under the curve was calculated with no significant difference found between the groups (Cinacalcet 21133 (8306-53182) pg/ml/year; Standard therapy alone 28088 (7962-112132) pg/ml/year; B=-19 (-55, 16) P=0.3). Similarly no significant difference was seen with phosphate control (Cinacalcet 87 (47-129) mmol/L/yr; standard therapy 87 (45-132) mmol/L/yr; B=-0.2 (-1.0, 0.6) P=0.6). There was no significant difference in change or final vitamin D dose, calcium binder dose or calcium dialysate between groups. 12 patients in each treatment arm were prescribed a non-calcium phosphate binder (P=0.3).
Figure 26: Median iPTH (range) at time-points during the trial. Percentage within iPTH target of 150-300pg/mL for each treatment arm is shown. Equivalence is seen between each treatment arm.

The primary outcome was absolute change of total calcification score from baseline to 1 year (figure 27) and the difference between arms was not significant (B=5.0 (-0.1-10, P=0.053). When areas of calcification were analysed separately no significant change was seen with either coronary (B=-0.9 (-5.9, 4.1) P=0.7) or aortic calcification (B=4.6 (-0.5, 9.7) P=0.08). 4 patients who completed the trial had a total calcification score <30 at baseline; 3 of which still had a calcification score <30 at 12 months.

Median change in calcification scores are shown in Table 19. The median percentage progression of total calcification in all study patients was 20% (IQR 0, 72%) (coronary 11% (IQR 28, 52%); aortic 12% (IQR 0, 63%)). A post-hoc analysis examining change of vitamin dose and progression of calcification was performed and this showed no statistically significant difference but this can be seen further in table 19.
Figure 27: Change of calcification score using the Agatston method. No significant difference is seen between treatment arms.

<table>
<thead>
<tr>
<th>Median change in calcification (IQR)</th>
<th>All trial subjects</th>
<th>Week 52 iPTH 150-300pg/mL</th>
<th>Week 52 iPTH &lt;150, &gt;300pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cinacalcet n=15</td>
<td>Cinacalcet n=4</td>
<td>Cinacalcet n=11</td>
</tr>
<tr>
<td></td>
<td>Control n=19</td>
<td>Control n=5</td>
<td>Control n=14</td>
</tr>
<tr>
<td>Coronary</td>
<td>43 (-30, 186)</td>
<td>30 (-28,77)</td>
<td>903 (198,1141)</td>
</tr>
<tr>
<td></td>
<td>(-34,903)</td>
<td>(198,1141)</td>
<td>(-30, 524)</td>
</tr>
<tr>
<td></td>
<td>207 (0, 1263)</td>
<td>203 (-47,1048)</td>
<td>239 (-206,1157)</td>
</tr>
<tr>
<td></td>
<td>(5, 688)</td>
<td>(-206,1157)</td>
<td>(0,1491)</td>
</tr>
<tr>
<td></td>
<td>488 (0, 1539)</td>
<td>244 (-74, 1114)</td>
<td>675 (349,2174)</td>
</tr>
<tr>
<td></td>
<td>(50,1214)</td>
<td>(-74, 1114)</td>
<td>(732 (0, 2295)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(412 (-76, 1244)</td>
</tr>
<tr>
<td>Aortic</td>
<td>293 (-5, 688)</td>
<td>239 (-47,1048)</td>
<td>394 (-10,728)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-206,1157)</td>
<td>(-10,728)</td>
</tr>
<tr>
<td>Total</td>
<td>563 (50,1214)</td>
<td>675 (349,2174)</td>
<td>732 (0, 2295)</td>
</tr>
<tr>
<td></td>
<td>(0, 1539)</td>
<td>(349,2174)</td>
<td>(412 (-76, 1244)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alphacalcidol dose (IQR)</td>
<td>3 (1.5,6)</td>
<td>4.6 (3.3,5.8)</td>
<td>2.3 (1.5,9.0)</td>
</tr>
<tr>
<td></td>
<td>3 (0.8,7)</td>
<td>(3.3,5.8)</td>
<td>(0.6,9.0)</td>
</tr>
<tr>
<td>Median wk 52</td>
<td>3 (0.8,3.5)</td>
<td>0 (-1.4,5)</td>
<td>1.25 (-2.0,3.0)</td>
</tr>
<tr>
<td>Median change</td>
<td>1 (-0.8,3.5)</td>
<td>0 (-1.4,5)</td>
<td>0 (-0.8,5.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0 (-0.8,5.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0 (-1.3,5.6)</td>
</tr>
</tbody>
</table>

Table 19: Change of calcification score and alphacalcidol dose by arm and by iPTH control achieved at trial completion.
Interestingly, despite an increase in calcification and vascular stiffness there was a reduction in LVMI in both treatment arms. No significant change was seen for other cardiac parameters measured and there was no change in blood pressure control or haemoglobin levels throughout the study. The difference between arms was not statistically significant (see Figure 28 and Table 20).

The cfPWV and radial AIx increased during the study despite improved control of secondary hyperparathyroidism. The progression was lower in the cinacalcet arm but this was not significant (see Table 20). There was no significant difference in the change in CIMT. When bone data was examined the bone mineral density showed minimal changes in both arms. The cortical density measured by pQCT decreased in both treatment arms but there was no significant difference between arms. The stress strain index improved overall in the cinacalcet arm but not in the standard therapy arm however this did not reach statistical significance.

The biomarkers were analysed and the concentration of proBNP and FGF23 reduced in the cinacalcet arm but no significant differences were seen (see Table 20).

Figure 28: Both treatment arms showed a reduction in LVMI though there was no significant differences in this study.
Table 20: Details of final study parameters and biomarkers and the median change seen between treatment arms.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cinacalcet Mean at 52wks</th>
<th>Cinacalcet Mean change (SD)</th>
<th>Control Mean at 52wks</th>
<th>Control Mean change (SD)</th>
<th>B (CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>131 (21)</td>
<td>-2 (21)</td>
<td>136 (21)</td>
<td>5 (34)</td>
<td>-0.3 (-18.12)</td>
<td>0.7</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>55 (16)</td>
<td>-6 (17)</td>
<td>59 (17)</td>
<td>2 (23)</td>
<td>0.3 (-0.03, 0.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>Radial AIX (%)</td>
<td>16 (15)</td>
<td>-3 (14)</td>
<td>26 (12)</td>
<td>5 (18)</td>
<td>-8.4 (-18.3, 1.6)</td>
<td>0.1</td>
</tr>
<tr>
<td>cf PWV (m/s)</td>
<td>8 (2)</td>
<td>0.4 (1.4)</td>
<td>9.5 (2.6)</td>
<td>0.1 (3)</td>
<td>-0.2 (-1.6, 1.2)</td>
<td>0.8</td>
</tr>
<tr>
<td>CIMT (mm)</td>
<td>0.03 (0.01)</td>
<td>-0.01 (-0.01)</td>
<td>0.04 (0.01)</td>
<td>-0.01 (0.01)</td>
<td>-0.004 (-0.1,0.002)</td>
<td>0.2</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>92 (31)</td>
<td>-28 (32)</td>
<td>90 (20)</td>
<td>-15 (40)</td>
<td>-0.2 (-1.2,0.8)</td>
<td>0.7</td>
</tr>
<tr>
<td>LVEDV (mL)</td>
<td>148 (34)</td>
<td>1 (22)</td>
<td>144 (41)</td>
<td>5 (38)</td>
<td>-5.6 (-31, 20)</td>
<td>0.7</td>
</tr>
<tr>
<td>LVSV (mL)</td>
<td>103 (24)</td>
<td>0.8 (13)</td>
<td>98 (22)</td>
<td>6 (26)</td>
<td>-0.5 (-16, 13)</td>
<td>0.9</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>70 (8)</td>
<td>0 (8)</td>
<td>70 (10)</td>
<td>0 (7)</td>
<td>-0.5 (-7, 6)</td>
<td>0.9</td>
</tr>
<tr>
<td>DXA spine BMD (g/m²)</td>
<td>1.1 (0.1)</td>
<td>0 (0.05)</td>
<td>1.1 (0.2)</td>
<td>0.03 (0.06)</td>
<td>-0.03 (-0.07, 0.01)</td>
<td>0.1</td>
</tr>
<tr>
<td>DXA fem BMD (g/m²)</td>
<td>0.9 (0.1)</td>
<td>0.01 (0.02)</td>
<td>0.8 (0.1)</td>
<td>0.05 (0.1)</td>
<td>-0.01 (-0.04,0.02)</td>
<td>0.6</td>
</tr>
<tr>
<td>DXA hip BMD (g/m²)</td>
<td>0.9 (0.1)</td>
<td>0.02 (0.02)</td>
<td>0.8 (0.2)</td>
<td>0.06 (0.1)</td>
<td>-0.01(-0.03,0.2)</td>
<td>0.4</td>
</tr>
<tr>
<td>QCTBMD (g/m³)</td>
<td>136 (38)</td>
<td>1.1 (12)</td>
<td>120 (40)</td>
<td>9 (19)</td>
<td>-2.7(-14.9,1)</td>
<td>0.6</td>
</tr>
<tr>
<td>Pq50 cortical density (mg/cm³)</td>
<td>1045 (68)</td>
<td>-31 (41)</td>
<td>1008 (58)</td>
<td>-60 (40)</td>
<td>36.6 (8.7, 64.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Stress strain index</td>
<td>244 (66)</td>
<td>6.2 (33)</td>
<td>201.8 (94)</td>
<td>-13 (58)</td>
<td>28(-3.8,59.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>Median at 52wks</td>
<td>Median change (IQR)</td>
<td>Median at 52wks</td>
<td>Median change (IQR)</td>
<td>B (CI)</td>
<td>P</td>
</tr>
<tr>
<td>Pro BNP (pg/mL)</td>
<td>3066 (90,49520)</td>
<td>-583 (-609,2771)</td>
<td>3208 (107,126250)</td>
<td>300 (-638,2846)</td>
<td>0.01 (-0.4,0.4)</td>
<td>0.9</td>
</tr>
<tr>
<td>Troponin T (pg/mL)</td>
<td>30 (4,1768)</td>
<td>0.8 (-6.12)</td>
<td>42 (14,131)</td>
<td>0.5 (-8.8)</td>
<td>0.1 (-0.2,0.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>25(OH) Vitamin D (ng/mL)</td>
<td>16 (4,34)</td>
<td>-3 (-8.3)</td>
<td>13 (4,33)</td>
<td>2 (-5.5)</td>
<td>-0.1 (-0.3,0.7)</td>
<td>0.2</td>
</tr>
<tr>
<td>1,25(OH)₂ Vitamin D (pmol/L)</td>
<td>65 (50,91)</td>
<td>4 (-13,23)</td>
<td>78 (54,122)</td>
<td>10 (-11,39)</td>
<td>-0.1 (-0.3, 0.8)</td>
<td>0.3</td>
</tr>
<tr>
<td>Osteoprotegerin (pmol/L)</td>
<td>8 (3,18)</td>
<td>1.1 (-0.6,2.1)</td>
<td>8 (4,17)</td>
<td>-0.1 (-0.6,1.3)</td>
<td>0.4 (-0.4,0.1)</td>
<td>0.3</td>
</tr>
<tr>
<td>FGF23 (RU/mL)</td>
<td>7422 (328,117011)</td>
<td>-791 (-9273,2382)</td>
<td>11540 (195,143782)</td>
<td>421 (-13430,12338)</td>
<td>-0.06 (-0.6,0.5)</td>
<td>0.8</td>
</tr>
<tr>
<td>Fetuin A (ug/mL)</td>
<td>369 (154,538)</td>
<td>-1 (-39.48)</td>
<td>343 (161,580)</td>
<td>8 (-8.76)</td>
<td>-0.2 (-0.9,0.6)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Bonferonni correction significance at 0.0025
Discussion:

In this study there was no significant difference in progression of vascular calcification with cinacalcet alongside standard therapy compared to standard therapy alone ($P=0.053$). Cinacalcet has been shown in rat studies to prevent or attenuate the progression of vascular calcification.\textsuperscript{110, 235, 237} In the ADVANCE study\textsuperscript{113} there was a suggestion of benefit in the cinacalcet arm; the primary endpoint was negative though the difference reached significance when corrected for baseline phosphate. In ADVANCE the median percentage of coronary calcification progression was 24\% in the cinacalcet arm vs. 31\% in the control arm ($P=0.07$). In this study there was a median total calcification progression of 20\% of the whole study population (coronary 12\%, aortic 13\%) which suggests similar progression of calcification in this study. The slightly lower progression could be explained by the fact that this trial is not only smaller but also included patients whose initial calcification score was less than 30 and therefore includes a different population which may not calcify. This study did not restrict the vitamin D dose in the treatment arm which is different to the ADVANCE trial and this also may have contributed.

Cinacalcet acts by allosteric modification of the calcium sensing receptor (CaSR) resulting in increased receptivity to calcium. There is increasing data about the calcium sensing receptor and its role in the prevention of vascular calcification.\textsuperscript{238, 239, 302} The CaSR has also now been identified on vascular smooth muscle cells,\textsuperscript{15} endothelial cells,\textsuperscript{303} phagocytes,\textsuperscript{237} and other cells\textsuperscript{233} associated with the vascular calcification process and this relationship is under investigation. Despite these potential mechanisms this study showed no difference between treatment arms. This may be due to a number of factors; there was no limit to the dose of vitamin D analogues that could be used within this trial and vitamin D use has been associated with an increase in vascular calcification.\textsuperscript{304, 305} Higher active vitamin D doses were used to achieve the KDOQI targets\textsuperscript{143} and therefore could have negated the result however there was no difference in vitamin D or calcium dose between treatment arms despite the use of cinacalcet. There are conflicting reports and active vitamin D use have been shown to be associated with an increased survival compared to no vitamin D\textsuperscript{71} and a study using a mouse CKD model has shown that the use of calcitriol or paracalcitol was associated with decreased calcification compared to no treatment in the setting of a high phosphate diet.\textsuperscript{306} There was also no difference in the use of non-calcium binders though
the use of sevelamer may have attenuated progression of vascular calcification in this study.\textsuperscript{103}

Within this study no difference in calcification progression was seen depending on iPTH control achievement (iPTH at 52 weeks between 150-300pg/mL). Raised iPTH may be related to increased calcification however patients with an iPTH within target are exposed to iatrogenic causes with higher doses of vitamin D and therefore potentially more frequent episodes of hypercalcaemia and hyperphosphataemia. There was a larger increase in vitamin D use in the patients who had not achieved target and this is probably related to the study design aiming for tight control. This trial was designed and set up prior to the KDIGO guidelines\textsuperscript{17} which suggest a wider target for iPTH and therefore the iPTH target may seem ‘out of date’. A large scale observational study has suggested a survival benefit when patients reach KDOQI targets\textsuperscript{299} and further investigation is still needed in this area.

Despite progression or stability of vascular stiffness and calcification LVMI reduced in both arms in this study. Both phosphate and iPTH reduced and a recent study in rats has shown that iPTH and phosphate are both independently associated with changes associated with cardiac remodelling in uraemia.\textsuperscript{300} High iPTH levels have been shown to be associated with left ventricular hypertrophy (LVH) in haemodialysis patients\textsuperscript{268} and improvement has also been shown post-parathyroidectomy.\textsuperscript{307} The relationship of iPTH and LVH is thought to be independent of blood pressure as patients with primary hyperparathyroidism have shown improvement of cardiac hypertrophy post parathyroidectomy.\textsuperscript{79} As both arms showed a reduction in LVMI no significant difference between treatment arms was seen in this study though there was an increased reduction in the cinacalcet arm. The trend to greater improvement in the cinacalcet arm could be explained by a number of mechanisms.\textsuperscript{233} The CaSR has been identified on cardiac myocytes and shown to affect DNA synthesis\textsuperscript{16} and may affect cell remodelling and growth. This mechanism is also supported by a rat study which suggested cardiac remodelling associated with uraemia was reduced with the use of cinacalcet or parathyroidectomy.\textsuperscript{231} Cinacalcet has also been shown to have blood pressure lowering effects in rat studies\textsuperscript{308} and though the changes in blood pressure in the current study did not reach statistical significance the mean systolic blood pressure had fallen in the cinacalcet arm within this study though this could be due to other, unknown or uncontrolled factors. The changes seen in proBNP could also be potentially explained by
the small differences in blood pressure or LV end-diastolic volumes though this would need further study.

This small study highlights potential benefits, and concerns, when aiming for tight control of CKD-MBD. The results suggest tight control may have benefits on LVMI and CIMT but the potential detrimental effects of the medications used to achieve targets needs to be considered. A proportion of patients within this study also developed low iPTH levels, despite close monitoring and dose adjustments, and therefore had increased likelihood of developing adynamic bone disorder. The variability seen with iPTH measurements could also increase the difficulty in maintaining patients within a small iPTH target range.

FGF23 has been associated with increased mortality in renal patients and JB Wetmore et al have shown a reduction of FGF23 associated with cinacalcet therapy. In this study both treatment arms underwent equivalent phosphate reduction however a trend towards further FGF23 reduction with cinacalcet was seen. This did not reach significance and currently no studies have shown that reduction of FGF23 will improve outcomes. However a study has shown FGF23 levels were associated with left ventricular hypertrophy in a diverse CKD population. Elevated FGF23 was also shown to predict formation of LVH over time in patients who had normal left ventricular geometry at baseline.

Carotid intima-media thickness reduced in both arms with no significant difference seen between arms. CIMT has been shown to predict cardiovascular disease and, more importantly has been shown to predict the coronary artery calcification 3 years later in the general population. This may suggest that longer studies are required when determining a change in calcification. There was no difference in vascular stiffness seen between arms in this study though a trend towards improvement of AIx was seen in the cinacalcet arm. A study of 19 peritoneal dialysis patients also showed no difference with cinacalcet treatment though this was also a small study and further studies in this area should be encouraged.

No effect on bone density or bone strength was seen in this study. Improvement in bone markers has been shown with cinacalcet treatment. Another study has shown some improvement in bone mineral density and a meta-analysis of four trials showed reduction in fracture risk. Sufficient bone changes to show significance may not occur
within a twelve month period and no clear guidance is available to suggest when DXA scans should be repeated. One study suggests that a 5 year time difference is required to see a change in bone mineral density. This study would have been improved with the inclusion of bone biopsies. This investigation was discussed with study collaborators and the ethics committee and it was concluded that this would be too strenuous for the patients, considering the portfolio of other investigations involved and therefore were not performed.

The main limitation of this study was the small sample size. The power calculation was devised with minimal data and led to an under-powered study. Given the results of the ADVANCE study a much larger sample size would be required to show changes in the vascular calcification measured within this study. However this trial would be difficult to replicate with larger patient numbers as the provision of the intense management would be difficult to achieve in normal clinical practice. Nevertheless, some of the results are interesting, if not significant, and they should be used to determine further hypotheses for future studies. Some differences in patient characteristics can be seen at baseline despite stratification at randomisation. The cinacalcet arm had a lower calcification score and higher LVMI at baseline and this may have affected the outcome measures of the trial. These conflicting findings may have been influenced by other unknown factors such as the time taken to develop chronic kidney disease or previous control of hypertension and these are hard to determine in any patient. Another important factor was that patients known to have had intervention for significant cardiac disease such as stent insertion were excluded from the study to allow the primary outcome to be determined accurately. This is an exclusion factor with many calcification studies that utilise CT and should be considered when interpreting the results.

It should be stressed that there is probably a beneficial role for both cinacalcet and vitamin D analogues. Alternative beneficial effects of cinacalcet have already been discussed and there is increasing evidence regarding the broader biological actions of vitamin D, not just in lowering iPTH concentrations.

Cinacalcet has been shown to regress calcification in humans and it does allow SHPT control to be achieved more easily in patients. There is data to suggest cinacalcet may lower blood pressure, improve cardiac morphology and lower FGF23 and this study
has also shown trends consistent with these studies; this may lead to improved patient survival. Patient survival has been investigated in a large international randomised trial, EVOLVE.\textsuperscript{112} The primary end-point of this trial was negative with intention-to-treat analysis but when variation seen in the baseline demographics were included in the analysis a difference in survival was suggested.\textsuperscript{114}

In conclusion, when SHPT control was obtained to equivalent degrees, no difference in change in calcification was seen between the cinacalcet and standard therapy arms. The control of SHPT may be important given the overall reduction in LVMI seen despite progression of other cardiovascular parameters.
5.4 EXPERIMENTAL CHAPTER 4:
Does tight control of secondary hyperparathyroidism lead to cardiovascular or survival benefits in haemodialysis patients?

Helen Eddington, William D Fraser, Grant Heatlie, Paul Taylor, Judith Adams, Philip A Kalra
Abstract

Introduction:
Guidelines have been written regarding the management of CKD-MBD as suggested by the strongest and/or available evidence available at the time. The KDIGO guidelines\textsuperscript{17} suggest an iPTH target of 2-9x upper limit of normal however a large European observational study has shown that patients whose iPTH was within the KDOQI target\textsuperscript{143} of 150-300pg/mL was associated with a survival benefit. This analysis of a RCT aims to investigate the change in cardiovascular parameters when SHPT is treated according to KDOQI guidelines\textsuperscript{143}.

Methods:
36 patients who fulfilled criteria of >90 days on dialysis, iPTH >300pg/mL, calcium > 2.1mmol/L and age 18-75 years were enrolled in a RCT of cinacalcet compared to standard therapy. Patients underwent a CT scan to determine coronary and aortic calcification score, cardiac MR, CIMT and vascular stiffness measurements at baseline and at 12 months. Biomarkers were also measured at both time-points. During the 12 months patients underwent intensive management of secondary hyperparathyroidism. Phosphate and iPTH reduction was equivalent in both arms and therefore analysis in this study incorporates all 36 patients. Survival analyses were also performed on baseline cardiovascular parameters and change in biochemical markers.

Results:
Mean (SD) phosphate reduction of 0.3mmol/L (0.7) and median (IQR) iPTH reduction of 380pg/mL (-754, 120) was achieved during the 12 month trial. Regression of LVMI and CIMT was seen ($P$=0.03 and $P$=0.001) and were significantly associated with change of phosphate on multi-variate analyses. Baseline phosphate and FGF23 were associated with change of coronary calcification but not change of aortic calcification. Survival analysis suggests that patients who underwent a phosphate reduction over the 12 months of the trial had a survival benefit at 5 years.

Conclusion:
This post hoc analysis suggests aiming for tight control of secondary hyperparathyroidism reduces LVMI and CIMT when using whichever treatment. Reduction in phosphate may also improve long term survival.
Introduction:

Cardiovascular disease is the leading cause of death in patients with Chronic Kidney Disease (CKD). The traditional cardiovascular risk factors are present in patients with CKD but there are also renal specific factors that we do not fully understand as yet. The incidence of left ventricular hypertrophy is much higher in the early stages of CKD and dialysis populations compared to the general population and this has been linked with an increased risk of diastolic dysfunction, cardiac failure, arrhythmia and sudden death. Vascular calcification and carotid-intima media thickness are also known to be associated with an increased risk of cardiovascular morbidity and mortality. Serum phosphate and the phosphaturic hormone fibroblast growth factor 23 (FGF23) have been suggested to be cardiovascular risk factors and in the following analysis of a small RCT we explored how these factors may be altered when SHPT is treated intensively.

Secondary hyperparathyroidism (SHPT) is difficult to manage clinically and the targets used to direct our clinical care are based on weak evidence using assays which can produce variable results. The KDIGO guidelines published in 2009 suggest aiming for a intact parathyroid hormone concentration (iPTH) within the range of 2-9x the upper limit of normal depending on the assay used. Since these were published an observational study by Floege et al has shown a survival benefit in patients achieving the tighter KDOQI iPTH target of 150-300pg/mL along with the targets for calcium (2.10-2.37 mmol/L) and phosphate (1.13-1.78 mmol/L). We performed a post-hoc analysis of the 36 haemodialysis patients with advanced SHPT who were managed in the previous randomised controlled trial in which they received either standard therapy (alfacalcidol and phosphate binders) alone or with the addition of cinacalcet. In the study, treatment had been adjusted aiming to achieve KDOQI targets for iPTH and phosphate, and notable control of these parameters was achieved. This analysis aimed to investigate the trial patients as a whole to determine if any differences in cardiovascular end-points might be apparent in association with changes in PTH, phosphate and other biomarkers. We also further investigated whether cardiovascular or biochemical parameters suggest a potential survival benefit.
**Methods:**

The original trial methods have been described in depth previously (see experimental chapter 3). The inclusion criteria included an intact parathyroid hormone (iPTH) >300pg/mL, corrected calcium >2.1mmol/L, age 18-75 years and haemodialysis for >90 days. Exclusion criteria involved patients who have had coronary stents, coronary artery bypass grafts or valve replacements as these would interfere with the coronary calcification score. Other exclusions included atrial fibrillation and severe liver disease. All biochemical samples were analysed in a central laboratory using the Roche modular analyzer and the iPTH was measured using the DPC immulite 2000 chemiluminescent immunoassay. Haematology results were analysed using Sysmex XE-1200.

All patients underwent investigations at baseline and samples were taken to be stored for biomarker analysis. The investigations included coronary and aortic calcification score, cardiac magnetic resonance scan (CMR), and carotid intima media thickness (CIMT). The patients then underwent a 12 week intensive titration phase where blood tests and management of SHPT was reviewed every 2 weeks aiming to achieve KDOQI CKD-MBD targets. 15 of the 36 patients were randomised to cinacalcet. During this time patients also were educated, where needed, to improve adherence to medications; this was performed by a single doctor to ensure reproducibility. Management was then reviewed every 8 weeks or more frequently if dose changes were made. At week 52 the investigations were repeated and further serum and plasma stored.

Biomarkers were assessed using the following methods. 25(OH) vitamin D: HPLC; 1,25(OH)2 vitamin D: RIA (Immuno Diagnostic Systems, Boldon, Tyne & Wear, UK); ProBNP: ECLIA on a Modular Analytics E170 analyser (Roche Diagnostics, Lewes, UK); Troponin T: ECLIA on a Modular Analytics E170 analyser (Roche Diagnostics, Lewes, UK), FGF23: 2nd generation, two-site ELISA supplied by Immutopics Inc (San Clemente, CA, USA), Fetuin A: ELISA supplied by BioVendor GmbH (Heidelberg, Germany); Osteoprotegerin: ELISA supplied by Immuno Diagnostic Systems (Boldon, Tyne & Wear, UK).
All patients gave written consent and the trial had ethical and MHRA approval and followed the 1974 Declaration of Helsinki. A further ethics application was made to facilitate collection of survival data for the trial patients in 2012. This was approved by London Riverside LREC and approval was gained from the relevant research and development offices.

**Statistics:**

In the intention to treat analysis of the trial no statistical difference was shown between the two randomised arms for area under the curve of phosphate and iPTH throughout the 12 months (iPTH: \( P=0.3 \); Phosphate: \( P=0.6 \)) and therefore the 36 enrolled patients were analysed together to examine the overall effects of tight iPTH control. Differences over 12 months were assessed by Wilcoxon signed rank test or paired t-test depending on normality of data. Normality of data was assessed graphically. Multi-factorial linear regression was used to determine further associations using normalised data where required. Change of phosphate, logiPTH, haemoglobin, pulse pressure, NTproBNP and FGF23 were analysed separately and were analysed with consecutive addition of factors.

Survival analyses were performed using Kaplan Meier plots and cox regression analyses adjusted for diabetes, smoking, age, gender and dialysis duration.

A \( p \) value of <0.05 was considered significant.

**Results:**

Baseline demographics are shown in table 21 and confirm the presence of cardiovascular disease in 39% of this population. 64% were male, 11% were diabetic and 25% were active smokers. Table 22 shows the laboratory parameters at baseline and 12 months. Mean (SD) phosphate reduction was 0.3mmol/L (0.7) during the 12 month study and median (IQR) iPTH reduction was 380pg/mL (-754, -120). Table 23 details the cardiovascular parameters at baseline and at 12 months. At the start of the trial, patients have marked vascular calcification and the mean LVMI confirms the presence of left ventricular hypertrophy as defined in previous studies.
Statistically significant reductions were seen for LVMI and CIMT in the complete trial population ($P=0.03$ and $P=0.001$ respectively) between baseline and 12 months. This occurred despite progression of calcification and no significant changes in vascular stiffness, haemoglobin and blood pressure. Change of phosphate ($B=0.006 \pm 0.001, 0.01$ $P=0.03$) and change of NTproBNP ($B=-0.007 \pm -0.002, 0.01$ $P=0.01$) were significantly associated with change of CIMT. However only the relationship with phosphate remained significant on multi-factorial analysis ($B=0.007 \pm 0.0, 013$ $P=0.04$).

Table 21: Baseline demographics of total trial population

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=36)</th>
<th>Adequate dialysis</th>
<th>All patients (n=36)</th>
<th>Dialysis duration (m)</th>
<th>Previous transplant</th>
<th>Calcium binder</th>
<th>Vit D analogue</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>51 (15)</td>
<td>Adequate dialysis</td>
<td>23 (80%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23 (64%)</td>
<td>Dialysis duration</td>
<td>38 [6-319]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>30 (83%)</td>
<td>Previous transplant</td>
<td>9 (25%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (11%)</td>
<td>Calcium binder</td>
<td>15 (42%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>9 (25%)</td>
<td>Vit D analogue</td>
<td>35 (97%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>26 (5)</td>
<td>SBP (mmHg)</td>
<td>131 (26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All CVD</td>
<td>14 (39%)</td>
<td>DBP (mmHg)</td>
<td>72 (13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline Mean (SD)/ Median [IQR]</td>
<td>12 months Mean (SD)/ Median [IQR]</td>
<td>Change Mean (SD)/ Median [IQR]</td>
<td>Percentage Change Mean (SD) Median (IQR)</td>
<td>P value</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>--------------------------</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parathyroid hormone (pg/mL)</td>
<td>730 [485,943]</td>
<td>294 [139,440]</td>
<td>-379 [-754,-120]</td>
<td>-63% [-81, -16]</td>
<td>&lt;0.005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.91 (0.6)</td>
<td>1.62 (0.6)</td>
<td>-0.31 (0.7)</td>
<td>-10% (35)</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected calcium (mmol/L)</td>
<td>2.30 (0.14)</td>
<td>2.37 (0.21)</td>
<td>+0.07 (0.2)</td>
<td>3% (9)</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphate (U/L)</td>
<td>133 [102,230]</td>
<td>88 [67,122]</td>
<td>-44 [-103,-16]</td>
<td>-31% (30)</td>
<td>&lt;0.005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>124 (15)</td>
<td>120 (16)</td>
<td>-5 (20)</td>
<td>-3% (16)</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>5.1 [1.8,9.5]</td>
<td>4.5 [1.3,15.2]</td>
<td>-0.3 [-2.0,+3.3]</td>
<td>-9.7% [-44, 140]</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT proBNP (pg/mL)</td>
<td>2945 [1361,6159]</td>
<td>3208 [1144,11732]</td>
<td>-208 [-981, 2765]</td>
<td>-12% [-44, 113]</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Troponin T (ug/L)</td>
<td>33.2 [23.8, 55.2]</td>
<td>34.4 [21.2,53.5]</td>
<td>0.6 [-6.3,8.5]</td>
<td>1% [-25,32]</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH) vitamin D (nmol/L)</td>
<td>12.6 [8.6, 22.4]</td>
<td>13 [9.3,18.6]</td>
<td>-1.5 [-5.1,4.2]</td>
<td>-11% [-31, 40]</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,25(OH)2 vitamin D (pmol/L)</td>
<td>66 [59,75]</td>
<td>71 [53,112]</td>
<td>4 [-11,31]</td>
<td>7% [-15,50]</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoprotegerin (pmol/L)</td>
<td>7.7 [5.5,9.6]</td>
<td>8.0 [6.4,11.3]</td>
<td>0.3 [-0.5,1.8]</td>
<td>3% [-7, 27]</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGF23 (RU/mL)</td>
<td>13795 [1522, 30371]</td>
<td>8410 [1244, 33010]</td>
<td>-33 [-9244, 9944]</td>
<td>-3%[-49, 85]</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetuin A (ng/mL)</td>
<td>342 [292, 387]</td>
<td>347 [309,427]</td>
<td>6 [-28, 67]</td>
<td>1% [-7, 18]</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 22: Laboratory parameters measured at baseline and at 12 months
Table 23: Cardiovascular parameters measured at baseline and at 12 months

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline Mean (SD)/ Median [IQR]</th>
<th>12 months Mean (SD)/ Median [IQR]</th>
<th>Change Mean (SD)/ Median [IQR]/ median%</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary calcification score</td>
<td>204 [35, 1143]</td>
<td>361 [37,1477]</td>
<td>45 [-31,493] (12%)</td>
<td>0.007</td>
</tr>
<tr>
<td>Aortic calcification score</td>
<td>892 [75, 4439]</td>
<td>1397 [195,5713]</td>
<td>267 [0,744] (13%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total calcification score</td>
<td>2174 [180, 5174]</td>
<td>2939 [304,7708]</td>
<td>545 [0,1364] (20%)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LVMI g/m$^2$</td>
<td>109 (39)</td>
<td>91 (25)</td>
<td>-8 [-36,+8]</td>
<td>0.02</td>
</tr>
<tr>
<td>CIMT cm</td>
<td>0.043 (0.01)</td>
<td>0.036 (0.01)</td>
<td>-0.01 (0.01)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>cfPWV m/s</td>
<td>8.6 (3)</td>
<td>8.9 (2)</td>
<td>0.3 (2.2)</td>
<td>0.5</td>
</tr>
<tr>
<td>Alx %</td>
<td>22 (15)</td>
<td>22 (14)</td>
<td>1.1 (17)</td>
<td>0.99</td>
</tr>
<tr>
<td>Pulse pressure mmHg</td>
<td>59 (19)</td>
<td>58 (17)</td>
<td>-1.6 (21)</td>
<td>0.67</td>
</tr>
<tr>
<td>Systolic BP mmHg</td>
<td>131 (26)</td>
<td>134 (21)</td>
<td>2 (29)</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Change of LVMI was also associated with change of phosphate (B=0.95 (0.04, 1.86) $P=0.04$). Change of LVMI was also associated with baseline Troponin T (B=1.671 (0.3, 3.1) $P=0.021$) but no association was seen with change of Troponin (B=0.8 (-0.4, 1.9) $P=0.2$). No significant association was identified with NTproBNP and LVMI. On multifactorial analysis change of phosphate was the only factor which achieved significance (B=1.23 (0.01, 2.5) $P=0.05$). Of note, change in FGF23 or iPTH showed no significant association with change in CIMT or LVMI.

The change of FGF23 and phosphate are correlated ($P=0.001$). In view of the significant findings with CIMT and LVMI the effect of phosphate and FGF23 were investigated with respect to vascular calcification. Characteristics of patients according to change of FGF23 and phosphate are detailed in Table 24. Patients with an increase in FGF23 during the study potentially showed a non-significant increase in vascular calcification; larger studies.
Table 24: Baseline characteristics and results according to baseline FGF23 and Phosphate

<table>
<thead>
<tr>
<th></th>
<th>ΔFGF23 &lt;0 N=17</th>
<th>ΔFGF23 &gt;0 N=16</th>
<th>B (95% CI), P value</th>
<th>ΔPhosphate &lt;0 n=22</th>
<th>ΔPhosphate &gt;0 n=12</th>
<th>B (95% CI), P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysis duration (m)</td>
<td>55 [24,79]</td>
<td>22 [15,139]</td>
<td>-0.1 (-0.5,0.2) P=0.4</td>
<td>35 [18,89]</td>
<td>33 [17,76]</td>
<td>0.02 (-0.3,0.4) P=0.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (6%)</td>
<td>2 (13%)</td>
<td>P=0.5</td>
<td>1 (5%)</td>
<td>3 (25%)</td>
<td>P=0.08</td>
</tr>
<tr>
<td>Smoker</td>
<td>3 (18%)</td>
<td>4 (25%)</td>
<td>P=0.6</td>
<td>8 (36%)</td>
<td>1 (8%)</td>
<td>P=0.08</td>
</tr>
<tr>
<td>Vit D dose</td>
<td>2 [0.75,4]</td>
<td>2.7 [1.2,3.5]</td>
<td>-1.6 (-6.3,2.9) P=0.5</td>
<td>3 [1.5,4.5]</td>
<td>2 [0.8,2.8]</td>
<td>-2.6 (-7.2, 2.0) P=0.3</td>
</tr>
<tr>
<td>ΔCIMT</td>
<td>-0.01 [-0.01,-0.005]</td>
<td>-0.005 [-0.01,0]</td>
<td>0.04 (-0.001,0.01) P=0.1</td>
<td>-0.01 [-0.01,-0.005]</td>
<td>-0.005 [-0.01,0.004]</td>
<td>0.01(0.001,0.01) P=0.03</td>
</tr>
<tr>
<td>ΔPhosphate</td>
<td>-0.6 (0.7)</td>
<td>0.01 (0.5)</td>
<td><strong>0.5(0.04,0.9) P=0.03</strong></td>
<td>-0.6 (0.6)</td>
<td>0.3 (0.3)</td>
<td></td>
</tr>
<tr>
<td>ΔPTH</td>
<td>-335 [-681,-127]</td>
<td>-407 [-767,26]</td>
<td>-0.05 (-0.4,0.3) P=0.8</td>
<td>-379 [-702,-32]</td>
<td>-435 [-808,-237]</td>
<td>0.01(-0.3,0.3) P=0.96</td>
</tr>
<tr>
<td>ΔFGF23</td>
<td>-5059 [-23090,-791]</td>
<td>9944 [1818,16654]</td>
<td>-791 [-19836,1733]</td>
<td>2643 [-1383,16519]</td>
<td><strong>0.6 (0.08,1.1) P=0.03</strong></td>
<td></td>
</tr>
<tr>
<td>ΔCoronary calcification</td>
<td>2.5 [-34.5,-4]</td>
<td>136 [34,262]</td>
<td>0.1 (-5.5) P=0.97</td>
<td>30 [-10,317]</td>
<td>174 [-65,809]</td>
<td>-0.1 (-5.1,4.8) P=0.96</td>
</tr>
<tr>
<td>ΔAortic calcification</td>
<td>239 [-12,779]</td>
<td>384 [0,1880]</td>
<td>1.2 (-3.6) P=0.6</td>
<td>409 [0,1320]</td>
<td>21 [-4,331]</td>
<td>-3.4 (-8.2,1.3) P=0.2</td>
</tr>
<tr>
<td>ΔTotal calcification</td>
<td>238 [29,1327]</td>
<td>675 [0,2868]</td>
<td>2.2 (-2.6,7) P=0.4</td>
<td>545 [33,1481]</td>
<td>365 [-54,1196]</td>
<td>-2 (-7.3) P=0.4</td>
</tr>
<tr>
<td>ΔLVMI</td>
<td>-14 [-33,0]</td>
<td>-4 [-45,+11]</td>
<td>0.5 (-0.4,1.4) P=0.2</td>
<td>-22 [-38,6]</td>
<td>-2 [-27,12]</td>
<td><strong>0.9 (0.04,1.9) P=0.04</strong></td>
</tr>
<tr>
<td>ΔcfPWV</td>
<td>0.3 [-0.4,1.8]</td>
<td>0.2 [-1.8,1.7]</td>
<td>-0.2 (-1.7,1.3) P=0.8</td>
<td>0.3 [-0.8,1]</td>
<td>1.3 [-1.1,2.2]</td>
<td>1.2 (-0.2,2.8) P=0.1</td>
</tr>
<tr>
<td>ΔAIx</td>
<td>3 [-8.18]</td>
<td>-4 [-15,7]</td>
<td>-4 (-14.6) P=0.4</td>
<td>1 [-4.5,7]</td>
<td>-9 [-18,17]</td>
<td>-4 (-14.6) P=0.4</td>
</tr>
</tbody>
</table>
would be necessary to investigate further the strength of this relationship. Progression of coronary calcification was also seen in patients who had an overall increase in their serum phosphate during the study though this was not seen with aortic calcification. On unifactorial analysis baseline phosphate and FGF23 were associated with coronary calcification change (Phosphate: 4.6 (1.0, 8.3) \( P=0.02 \); FGF23: B=3.7 (0.5, 6.9) \( P=0.02 \)) though not with change of aortic calcification score (Phosphate B=0.9 (-3.2, 4.9) \( P=0.7 \); FGF23: B= 0.7 (-2.8, 4.1) \( P=0.7 \)). Higher baseline haemoglobin (Hb) and greatest reduction of Hb were associated with a greater progression of abdominal aortic calcification (baseline Hb: 0.2 (0.04, 0.3) \( P=0.01 \); change Hb: B= -0.2 (-2.6, -0.6) \( P=0.02 \)). On multifactorial analysis the change in haemoglobin remained significant (B= -0.01 (-0.2, 0) \( P=0.05 \)).

22 / 36 (61%) patients had a reduction of phosphate over the 12 months (mean reduction of phosphate 0.6mmol/L) and these patients had a significantly higher phosphate at baseline than the patients who did not (\( P=0.01 \)). 70% of patients who had a phosphate reduction at week 52 had a baseline phosphate >1.7mmol/L (mean baseline phosphate 2.1mmol/L (0.5); range 1.28-3.14mmol/L) compared to only 50% in patients who had no reduction (mean baseline phosphate 1.6mmol/L (0.6); range 0.93-2.67mmol/L). The mean change in phosphate in patients who did not have a reduction in phosphate over the 12 months was 0.3mmol/L. There was no significant difference in starting dose or change of vitamin D doses administered between those who had a reduction in phosphate and those who did not (see table 24).

A Kaplan Meier plot comparing patients who had a positive or negative change in phosphate during follow-up is shown in figure 29. A negative change in phosphate was associated with improved survival by cox regression analyses (HR=10.2 (1.1, 104.5) \( P=0.049 \)). Smoking (HR=13.7 (1.0, 177) \( P=0.045 \)), gender (HR=0.2 (0.03, 0.9) \( P=0.04 \)) and age (HR=1.1 (1.0, 1.2) \( P=0.012 \)) also were found to have a significant effect on survival within the model. Change in FGF23 showed no significant effect on survival in this study (HR=1.9 (0.3, 14.3) \( P=0.5 \)). Baseline total calcification was split into equal numbers and the survival curve is shown in figure 30. This suggests a survival advantage with a lower calcification score though this did not reach significance in this small study (HR 2 (0.1, 33) \( P=0.6 \)). Change of LVMI and CIMT showed no effect on survival in this study (data not shown).
Figure 29: Survival differences in patients who had a reduction in phosphate compared to an increase over 12 months.

Figure 30: Survival differences in patients with a total calcification score above or below 1500 using the agatston score (coronary and abdominal aorta).
Discussion

This post hoc analysis of a randomised trial has enabled us to investigate the regression of LVMI and CIMT measurements further with an increased sample size along with survival data. The reduction of phosphate within the 12 months of the study was associated with regression of LVMI, reduction of CIMT and improved survival though these associations were not seen with iPTH reduction. There are many studies detailing that an increased phosphate is associated with an increased mortality risk in CKD and dialysis patients.11, 57, 62, 64, 260, 299. There are also many studies comparing different phosphate binders and morbidity104, 106 but there is an evidence gap with respect to the reduction of elevated phosphate leading to improved patient outcomes. The current study suggests that the patients have an improved outcome when their phosphate is reduced. The patients whose phosphate reduced during the study had a higher baseline phosphate and therefore benefitted from the reduction. It is important to state that these results are a post-hoc analysis in a small number of patients and these findings, although very interesting, would need to be verified by a larger clinical trial. As we already routinely lower phosphate in CKD and dialysis patients such a trial would present an ethical dilemma.

Higher serum phosphate has been shown to be associated with LVH in the normal population,326 predialysis322 and dialysis patients327 and it follows that lowering phosphate may be beneficial. The mechanism for this could be related to the formation of vascular calcification, and hence stiffness, which has been shown to be associated with phosphate levels.61, 328 Another potential mechanism could be via FGF23 as this has also been associated with increased left ventricular mass.311 Faul et al have shown a klotho independent effect of FGF23 suggesting that high levels of FGF23 have a direct effect on LVH. However, in the current analysis phosphate reduction led to regression of LVH despite progression of calcification or vascular stiffness suggesting that LVMI reduction was related to some other factor such as FGF23. No association with FGF23 was found in this study though this may be linked to the limitations of small numbers or length of the trial.

Our results suggest that the patients with increased Troponin T suggesting on-going cardiac damage had less regression of LVH and this association has been shown in other
Other studies have also shown an association with NTproBNP and iPTH though we did not find associations in our population.

We have also shown an association with reduction of phosphate and reduced CIMT in this study. Higher serum phosphate has been linked to an increased CIMT in the general population and in the renal population. Again the mechanisms for this could be due to a direct action of phosphate leading to vascular changes. FGF23 has also been investigated with respect to CIMT but the study showed a negative association between FGF23 and CIMT, the authors hypothesising that FGF23 had a protective effect as the high concentrations seen in renal disease may stimulate other FGF receptors leading to modulation of lipid metabolism. Our study also found an association with change of CIMT and change of NTproBNP. A positive association between CIMT and NTproBNP has been shown in rheumatoid arthritis patients previously and the link is thought to be due to a simultaneous process of atherosclerotic development leading to the release of NTproBNP and the formation of CIMT.

Change of coronary calcification was associated with higher baseline serum phosphate and FGF23 levels though this association was not shown with aortic calcification measurements. Neither change of phosphate nor change of FGF23 reached significance for progression of calcification though there was a trend seen for increasing FGF23 levels to be associated with increasing calcification. FGF23 has been linked with calcification across various stages of CKD and has been associated with an increased mortality. Though there was progression of vascular calcification in this study this was lower than seen in other studies. Calcification changes may take time to occur and this small population may have had a positive outcome with longer follow-up. A study in the general population found that CIMT measurements predicted calcification scores measured an average of 3 years later. A survival benefit with sevelamer hydrochloride after 2 years has also been suggested in a post-hoc analysis of the DCOR study. We propose that the changes seen in this study may be part of a complex process and tight control of phosphate and iPTH may lead to attenuation of calcification progression.

Baseline haemoglobin and change of haemoglobin were found to be associated with abdominal aortic calcification in this study and this contradicts other literature. In this small study the patients who were above the clinical target for haemoglobin at the
beginning of the trial had a large reduction in haemoglobin in the trial. This reduction could have been due to erythropoietin resistance secondary to infections or underlying inflammation. Naturally these patients would then be expected to have more progression of their calcification though of note there was no association with CRP in this study.

There are many limitations in this study; firstly this is a post hoc analysis of a RCT and the results should only be used to generate hypotheses for larger trials. This trial was not powered to examine the effects of changes of phosphate and whilst these were interesting they need to be confirmed in other appropriately designed studies. The achievement of tight control of secondary hyperparathyroidism using standard therapies is difficult and in this trial intensive management was given to all patients. This is difficult to replicate outside of the trial setting and therefore the reproducibility is limited in clinical practice.

Further trials in this area would benefit from measurements of circulating klotho as the deficiency in this factor may be leading to FGF23 resistance and increasing levels. Klotho deficiency could mask the effects of FGF23 and further investigations within this area are important. The ability to control secondary hyperparathyroidism without increasing FGF23 with vitamin D needs to be investigated and a role for cinacalcet may become more evident in the future.

In summary, this post hoc analysis suggests that tight control of phosphate and iPTH, by whatever means, reduces LVMI and CIMT both of which predict cardiovascular death. The reduction in phosphate may also improve survival and phosphate seemed to have a greater impact than FGF23 in this study.
5.5 EXPERIMENTAL CHAPTER 5:

Variation in PTH concentration measured in laboratories across the North West region of the UK;
An assay variability study linked to clinical audit data

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\textsuperscript{2} Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia.
\textsuperscript{3} Renal Department and Manchester Institute of Nephrology and Transplantation, Central Manchester and Manchester Children’s Hospital Trust
**Study development**

This study was devised during the randomised controlled trial reported previously. The patients in the trial were still undergoing routine clinical measurements of their iPTH in their local laboratory but all trial samples were performed in a central laboratory. When the management was altered, or not, within the remit of the trial the clinicians involved in the overall care of the patient contacted me to question my practice. The difference in laboratories was then noticed and lead to further investigation of the variation seen. Following a literature search and further reading the idea to perform this study alongside the regional audit arose.
Abstract:

Intact parathyroid hormone (iPTH) measurements are used to guide therapy in renal patients but variability in results can be produced depending on the assay used. This study has investigated iPTH assay variation in North West England and paired data with regional audit data to determine clinical relevance of assay variability.

37 haemodialysis patients had blood taken (EDTA plasma and serum) and samples were processed at 17 laboratories that analyse iPTH for North West dialysis patients. Correction factors were calculated and applied to the iPTH assay results to enable direct comparisons. These correction factors were also applied to Regional Audit data to determine if iPTH assay variability explains any of the variation in unit performance in achieving PTH targets.

The iPTH results from the 37 patients were significantly different when either analysed by different assays and/or different laboratories \((P<0.001)\). There was up to 24 % difference between assays with the Abbott Architect method producing the highest iPTH results. Of the 37 patients between 49-65% would achieve the KDIGO iPTH target\(^{17}\) depending on assay used. When results were adjusted using correction factors 21% of patients would potentially have a change of management. Data from all haemodialysis units submitted for the regional audit were adjusted to the Roche assay and this led to small change in achievement of KDIGO iPTH targets\(^{17}\) in individual units when compared to each other.

The variation in achieving iPTH targets is probably due to a combination of iPTH assay variability and diversity in clinical management. Both need to be improved or standardised to improve patient care.
Introduction:

Chronic Kidney Disease (CKD) patients undergo regular intact parathyroid hormone (iPTH) measurements as part of their normal care. This measurement has been used to guide therapy in patients with CKD-mineral bone disorder (CKD-MBD) which is complex and can be difficult to treat.\textsuperscript{208} Recent publications have noted that there is a wide variation in iPTH results depending on the assay used\textsuperscript{325,337,338} and that these differences may be influencing the clinical management of patients. Target concentrations of iPTH have been published for nephrologists to work towards; originally these were set targets such as KDOQI\textsuperscript{143} where a range of 150-300pg/mL was suggested. Subsequent guidelines suggest the use of the upper limit of the normal range (ULN) of iPTH as provided by the local laboratory should be used to determine a target. UK Renal Association\textsuperscript{258} have previously advised 2-4x ULN though this has been revised recently\textsuperscript{339} and is now consistent with the KDIGO\textsuperscript{17} recommendation of 2-9 x ULN as a target range for dialysis patients. Such recommendations incorrectly assume all laboratories have local population defined reference ranges.

Some studies have shown that if the iPTH results are adjusted according to the assay method used then this could lead to a more uniform approach to CKD management.\textsuperscript{337,338} This ‘correction’ could reduce misclassification and therefore sub-optimal treatment of dialysis patients. Correction factors are not used routinely in clinical practice and in addition sample type, plasma or serum, can also influence the assay result.\textsuperscript{338,340} Almond et al\textsuperscript{337} have published recommended assay-specific target ranges which were calculated from a large renal population in Scotland and in this study we aim to compare our data to their recommendations. In practice clinical audit is used to monitor performance in CKD-MBD management and to compare this with that of other units. When auditing iPTH performance, sample type and the specific assay used by a given laboratory are rarely taken into consideration, but logic suggests that they should be.
In this study we aim to investigate the following:

- The iPTH assay variability in the North West of England and determine if this variability would affect clinical management
- If conversion factors could be established to align the different assay methods used in the North West and whether these would be consistent with other published conversion factors.
- If the variation in iPTH targets achieved in regional audit data could be attributed to the iPTH assay used and whether, if corrected, this would alter performance of individual renal units.

**Materials and Methods:**

Thirty seven maintenance haemodialysis patients from Salford Royal Hospital were selected at random and provided written informed consent for their blood samples to be used in this study. In the North West Region there are 17 laboratories that analyse iPTH for haemodialysis patients. Within these 17 biochemistry laboratories, 8 different methods using 5 different assays are used. The assays are summarised in Table 25 and include Roche modular e170, Roche Cobas e411 and 600 (Roche Diagnostics, West Sussex, UK), Siemens ADVIA Centaur and Siemens Immulite 2000 and 2500 (Siemens Healthcare Diagnostics, Surrey, UK) and Beckman Access DxI (Beckman Coulter UK Ltd, Buckinghamshire, UK). These laboratories provide biochemical services to the 24 (5 hubs and 19 satellite units) haemodialysis units whose data features in the North West Regional renal bone chemistry audit.

During a single working day all 37 patients had pre-dialysis blood taken in 5 different blood collection bottles (Becton Dickinson – Serum Separation Tube (SST) and ethylenediaminetetraacetic acid (EDTA), Sarstedt – 2 SST and 1 EDTA). No phenotypic data was collected for these patients. Prior to blood collection each laboratory stated the sample (e.g. EDTA or SST) and bottle type (e.g. BD, Sarstedt, Greiner) used routinely within their laboratory for iPTH measurement. BD tubes were used for laboratories using Greiner tubes. Within 3 hours of the blood draw samples were centrifuged (10 minutes at 1400g using a Hettich Rotina 420R centrifuge). All samples were divided into 0.5mL
aliquots, according to original sample bottle and make, which were then anonymised. All patients provided enough blood for at least 1 sample to be sent to all laboratories; where possible 2 samples per patient were sent. Samples were frozen at -80°C overnight and then distributed to all 17 laboratories on dry ice the following day via same day courier. These samples were stored frozen overnight at each centre and then analysed the following day (i.e. 48 hours after collection). One laboratory analysed the samples on day 6. This was in line with their standard operating procedure. All samples were defrosted on site and processed within one hour (normal practice). All iPTH results have been reported as pg/mL. Where laboratories reported iPTH as pmol/L a conversion factor of 0.106 was used.341

Table 25: Summary of the laboratories, their assays and the associated dialysis units which utilise them for iPTH assay measurements

<table>
<thead>
<tr>
<th>Laboratories</th>
<th>Assay used</th>
<th>Sample used</th>
<th>Laboratory provided ref range pg/mL</th>
<th>Associated dialysis units</th>
<th>Audit data available</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Roche e170</td>
<td>Serum</td>
<td>15-65</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Roche e170</td>
<td>EDTA</td>
<td>10.4-65.7</td>
<td>R &amp;S</td>
<td>No Data</td>
</tr>
<tr>
<td>3</td>
<td>Roche e170</td>
<td>EDTA</td>
<td>10-60</td>
<td>C</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Roche e170</td>
<td>Serum</td>
<td>15-65</td>
<td>N &amp;Q</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Roche e170</td>
<td>Serum</td>
<td>15-65</td>
<td>H &amp; L</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Roche e411</td>
<td>EDTA</td>
<td>15.2-65.7</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Roche e411</td>
<td>Serum</td>
<td>11-65</td>
<td>D &amp;P</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>Roche 6000</td>
<td>Serum</td>
<td>10.4-65.7</td>
<td>X &amp; Y</td>
<td>No Data</td>
</tr>
<tr>
<td>9</td>
<td>Advia Centaur</td>
<td>Serum</td>
<td>8-73</td>
<td>J</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Advia Centaur</td>
<td>Serum</td>
<td>15.2-65.7</td>
<td>T</td>
<td>No Data</td>
</tr>
<tr>
<td>11</td>
<td>Advia Centaur</td>
<td>EDTA</td>
<td>14.2-72.4</td>
<td>U &amp; V</td>
<td>No data</td>
</tr>
<tr>
<td>12</td>
<td>Beckman DXI</td>
<td>Serum</td>
<td>15-88</td>
<td>F</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>Beckman DXI</td>
<td>Serum</td>
<td>15.2-65.7</td>
<td>B</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>Immulite 2500</td>
<td>Serum</td>
<td>12.4-64.7</td>
<td>E</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>Immulite 2500</td>
<td>Serum</td>
<td>12-65</td>
<td>I &amp; K</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>Immulite 2000</td>
<td>Serum</td>
<td>12.4-64.7</td>
<td>A &amp;W</td>
<td>A only</td>
</tr>
<tr>
<td>17</td>
<td>Abbott Architect</td>
<td>Serum</td>
<td>10-70</td>
<td>O</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Almond et al337 published a thorough paper suggesting use of differing target ranges according to assay used devised from the correction factors developed from their data in Scotland (Table 26). We used our 37 patient iPTH results with a view to validate these
targets in a post hoc analysis. Number of patients achieving x2-4 and x2-9 times ULN targets were calculated using their laboratory provided normal ranges and the ranges suggested by Almond et al.\textsuperscript{337}

North West regional audit data was collected for November 2009; in this study we have only used submitted iPTH data for haemodialysis patients in the North West. The audit used the most recent iPTH within the last 6 months for the audit.

All biochemistry laboratories were approached for approval to access UK National External Quality Assessment Service (NEQAS Edinburgh) data. Average bias and variance for iPTH measurement over the last year was obtained for 15 of the 17 laboratories and for the remaining laboratories the national average UK NEQAS data for their respective methods were used for the month in which bloods were drawn.

Statistics:

The mean iPTH value produced for each centre was the mean of all averaged samples. Where only one sample was available then this result was used. The absolute difference shown is the mean absolute difference between the two results where two samples were available for analysis. Percentage difference is the absolute difference as a percentage of the mean. Where two readings were available coefficient of variance (CV) was calculated as standard deviation/mean%.

To assess inter-assay variability the Friedman test was used as this does not assume normal distribution. This was performed for all laboratories and also between laboratories using the same assay and method. To generate correction factors a graph was plotted of all results between pairs of laboratories. Outliers were excluded and a reference line was generated using SPSS 16.0 software. The gradient of the line was used to determine a correction factor for each laboratory combination. A Spearman rank correlation test was performed to further determine the correlation of iPTH results between all laboratories.

Using the 37 patients’ results the percentage of samples that achieved the KDIGO\textsuperscript{17} target were calculated for each laboratory. The data was analysed using Chi-Square to determine if there was a significant difference in achieving targets. All samples were then corrected
to laboratory 3 Roche e170 assay using the correction factor generated. This was chosen as it is thought to produce similar results to the, now unavailable, Nichols Allegro assay which was used to develop the KDOQI guidelines.\textsuperscript{143, 342} The number of patients who, after correction, changed classification according to targets was calculated and the significance tested using Chi-square. When corrected an upper limit of iPTH reference range was set at 65pg/mL (mean ULN of all Roche assays) to calculate achievement of targets.

For the post-hoc analysis using Almond \textit{et al} suggested target ranges significance was assessed using the Wilcoxon Rank Test.

Percentage bias using the all laboratory trimmed mean (ALTM) for the study patients was calculated using the equation: (result-ALTM)/ALTM x100. The percentage of regional audit data within UK Renal association guidelines\textsuperscript{258} as per audit protocol 2009 (2-4xULN) was determined for each dialysis unit. The correlation between the median iPTH for each dialysis unit and the bias from both this study and UKNEQAS was analysed using the Spearman rank correlation. The audit data was corrected to the Laboratory 3 Roche e170 assay and a Wilcoxon Signed Rank test was performed to assess difference. Median iPTH was plotted from both original audit data and post correction to see if this altered the clinical picture.

Table 26: Method-specific target values for parathyroid hormone (pmol/L) proposed for interim use in the management of patients with chronic kidney disease. Almond \textit{et al} Ann Clin Biochem 2012; 49(1): 63-67. Copyright 2012 Royal Society of Medicine Press, UK\textsuperscript{337}

<table>
<thead>
<tr>
<th>Method</th>
<th>Equivalent to multiples of the upper limit of normal (ULN)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 x ULN</td>
</tr>
<tr>
<td>Roche Elecys E170*</td>
<td>14</td>
</tr>
<tr>
<td>DiaSorin Liaon</td>
<td>12</td>
</tr>
<tr>
<td>Beckman Access DxI</td>
<td>13</td>
</tr>
<tr>
<td>Siemens ADVIA Centaur</td>
<td>15</td>
</tr>
<tr>
<td>Abbott Architect</td>
<td>16</td>
</tr>
<tr>
<td>Siemens Immulite 2000</td>
<td>22</td>
</tr>
</tbody>
</table>

The values are computes from regression analysis and have been rounded to the nearest whole number as appropriate. * Baseline immunoassay for comparative purposes.
**Results:**

Table 27 shows descriptive statistics of the iPTH measurements produced from each laboratory. Two laboratories did not provide a result for all 37 samples due to sample haemolysis. There were 36 CV results >10%; 21 of these could be attributed to four patients’ serum samples. No haemolysis or clots were noted in these samples by the laboratories and therefore these results have been included in all analyses except in the generation of correction factors. Only 4 laboratories (24%) had a CV <10% for all samples analysed and they all used a Roche assay (e170, e411 or 6000).

Despite a strong correlation between all laboratories ($P<0.001$ for all combinations) the iPTH results were statistically different across the region, $\chi^2(2)=248$, $P<0.001$. The clinical significance of this result is difficult to determine. Further Friedman analyses showed significant differences in individual patient results when the same assay was used in different laboratories; Roche e170 $\chi^2(2)=64$ $P<0.001$, Roche e411 $\chi^2(2)=28$ $P<0.001$, Advia Centaur $\chi^2(2)=29$ $P<0.001$, Beckman DXI $\chi^2(2)=16$ $P<0.001$, Immulite 2500 $\chi^2(2)=3$ $P=0.07$. Mean correction factors for each assay were generated and these are shown in Table 28. The Abbott Architect method consistently produced higher iPTH results by a factor of 1.3 compared to the Roche e170 assay. The average difference between all assays was 5% but the range of differences varied between 0 and 24%.

The percentage of the 37 patients reaching the KDIGO ‘target’ was examined for each laboratory. The results are summarised in Table 29 and show a 16% difference in the number of patients achieving the KDIGO target depending on where their samples were analysed. The highest variation (24%) was seen in patients who were above target. There is variability in iPTH reference ranges but many laboratories have similar ranges despite being locally derived. When we applied the same reference range across the region (ULN 65pg/mL) the variation in patients achieving target was reduced to 10% with only a 19% variation seen above target.
Table 27: Detailed results on 37 patient iPTH measurements per laboratory

<table>
<thead>
<tr>
<th>Biochemistry Dept (sample n=37 unless stated)</th>
<th>No of patients with 2 samples analysed</th>
<th>Mean iPTH pg/mL</th>
<th>Median (range) iPTH pg/mL</th>
<th>Mean absolute diff (range)</th>
<th>Mean % diff (range)</th>
<th>Mean CV% (range)</th>
<th>mean% deviation of results from mean for each pt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=35)</td>
<td>27</td>
<td>269</td>
<td>189 (8-792)</td>
<td>8 (0-57)</td>
<td>3 (0-13)</td>
<td>2.0 (0-9.4)</td>
<td>-2.7</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>243</td>
<td>180 (10-662)</td>
<td>12 (0-93)</td>
<td>4 (0-18)</td>
<td>3.1 (0-12.8)</td>
<td>-8.9</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>268</td>
<td>209 (9-712)</td>
<td>5 (0-25)</td>
<td>2 (0-7)</td>
<td>1.5 (0-4.7)</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>239</td>
<td>184 (9-706)</td>
<td>9.5 (0-20)</td>
<td>6 (0-15)</td>
<td>4.0 (0-10.4)</td>
<td>-10.6</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>250</td>
<td>194 (0-758)</td>
<td>12 (2-43)</td>
<td>6 (1-15)</td>
<td>4.0 (0.7-10.7)</td>
<td>-6.2</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>293</td>
<td>226 (8-785)</td>
<td>9 (0-37)</td>
<td>3 (0-9)</td>
<td>2.0 (0-6.3)</td>
<td>8.5</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>261</td>
<td>202 (9-784)</td>
<td>6 (0-20)</td>
<td>3 (0-13)</td>
<td>2.5 (0-9.5)</td>
<td>-2.2</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>295</td>
<td>209 (7-852)</td>
<td>5 (0-21)</td>
<td>2 (0-5)</td>
<td>1.2 (0-3.6)</td>
<td>6.7</td>
</tr>
<tr>
<td>9</td>
<td>37</td>
<td>290</td>
<td>218 (2-864)</td>
<td>19 (0-128)</td>
<td>7 (0-50)</td>
<td>3.6 (0-11.3)</td>
<td>1.2</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>279</td>
<td>206 (0-808)</td>
<td>15 (0-55)</td>
<td>6 (0-19)</td>
<td>4.5 (0.6-13.5)</td>
<td>-0.9</td>
</tr>
<tr>
<td>11</td>
<td>37</td>
<td>317</td>
<td>240 (1-861)</td>
<td>20 (0-99)</td>
<td>8 (0-71)</td>
<td>5.4 (0-50.4)</td>
<td>9.7</td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>246</td>
<td>194 (3-745)</td>
<td>16 (1-63)</td>
<td>9 (1-23)</td>
<td>6.3 (0.4-16.1)</td>
<td>-11.2</td>
</tr>
<tr>
<td>13 (n=36)</td>
<td>9</td>
<td>278</td>
<td>225 (20-781)</td>
<td>12 (0-47)</td>
<td>5 (0-14)</td>
<td>3.3 (0-9.7)</td>
<td>-3.6</td>
</tr>
<tr>
<td>14</td>
<td>28</td>
<td>287</td>
<td>200 (3-926)</td>
<td>14 (0-86)</td>
<td>5 (0-16)</td>
<td>3.6 (0-11.6)</td>
<td>-3.0</td>
</tr>
<tr>
<td>15</td>
<td>27</td>
<td>269</td>
<td>193 (3-993)</td>
<td>11 (0-53)</td>
<td>6 (0-18)</td>
<td>4.0 (0-12.7)</td>
<td>-6.5</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
<td>319</td>
<td>220 (4-1162)</td>
<td>15 (0-76)</td>
<td>7 (0-67)</td>
<td>4.7 (0-47)</td>
<td>8.8</td>
</tr>
<tr>
<td>17</td>
<td>27</td>
<td>336</td>
<td>245 (4-1029)</td>
<td>15 (0-54)</td>
<td>5 (1-14)</td>
<td>3.9 (0.7-10.1)</td>
<td>20.9</td>
</tr>
</tbody>
</table>
Table 28: Assay correction factors calculated for the North West Region using sample population

<table>
<thead>
<tr>
<th></th>
<th>Roche</th>
<th>Advia</th>
<th>Beckman</th>
<th>Immulite</th>
<th>Abbott</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche</td>
<td>1.14</td>
<td>1.0</td>
<td>1.14</td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td>Advia</td>
<td>0.88</td>
<td>0.87</td>
<td>1.0</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>Beckman</td>
<td>1.01</td>
<td>1.15</td>
<td></td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td>Immulite</td>
<td>0.88</td>
<td>1.0</td>
<td>0.88</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>Abbott</td>
<td>0.77</td>
<td>0.87</td>
<td>0.76</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>

Table 29: Number of patients from the sample population categorised according to published guidelines.

<table>
<thead>
<tr>
<th>KDIGO (2-9xULN)</th>
<th>low</th>
<th>in target</th>
<th>high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min n</td>
<td>9</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Max n</td>
<td>16</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>DIFF %</td>
<td>19%</td>
<td>16%</td>
<td>24%</td>
</tr>
</tbody>
</table>

To review assay variation, correlation and Bland Altman plots were obtained and are shown in figure 31. Figure 31 a & b shows the relationship between laboratory 2 (Roche e170) and laboratory 17 (Abbott Architect). It is clear to see that the Abbott Architect assay consistently reads higher than the Roche e170 assay despite being well correlated ($R^2=0.982, P<0.001$). Figure 31 c & d compares 2 laboratories (Roche e170) one of which uses EDTA, the other serum samples. The assays are in agreement and are well correlated ($R^2=0.971 P<0.001$) however, despite using the same assay, the EDTA plasma samples produce persistently higher iPTH results.

Using the correction factors previously generated all 37 patients’ results were corrected to Laboratory 3. When corrected, 21% patients would have changed classification according to KDIGO targets$^{17}$ and would have potentially undergone different clinical management ($P<0.001$). This again suggests that clinical decision making is currently influenced according to which laboratory a sample is analysed in. The variation in achieving KDIGO targets$^{17}$ is reduced from 16% to 8% after correction factors are applied. Patients who were classified as above target reduced from 24% to 13%.
Figure 31: Comparison of assays and sample types using correlation and Bland-Altman plots. Figure 1a and b compares the Roche e170 and the Abbott Architect assay. Figures 1c&d compare the Roche e170 assay using EDTA plasma and serum.

The comparisons of laboratory ranges to the suggested target ranges per assay as suggested by Almond et al are shown in Table 30. The results indicate that for all laboratories using the Immulite assay by applying the correction factor all patients would be classified as if their results were much lower than initially found in our study \((P<0.005)\). Variation was also seen within the classification after correction of the Beckman assay. The generation of correction factors relies on the specific assay being performed in the same way across the country using the same sample type allowing but for some variation in reagents and other minor assay changes. Our results highlight the difficulty in generating correction factors and their ability to be transferred between laboratories and different sample populations.
Table 30: Number of patients within each classification according to 2-4xULN and 2-9xULN; use of the proposed adjusted targets according to assay by Almond et al. compared to ranges generated from laboratory upper limit. As the Roche e170 assay was used as baseline in their study only laboratories using other assays are shown. Significance is at $=0.003$ using Bonferroni method.

<table>
<thead>
<tr>
<th>Lab</th>
<th>Assay /ULN</th>
<th>Laboratory normal range</th>
<th>Adjusted targets according to assay</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Advia</td>
<td>&lt;2 2-4x 2-9x &gt;9</td>
<td>&lt;2x 2-4x 2-9x &gt;9</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>Centaur</td>
<td>11 11 21 4</td>
<td>12 11 21 4</td>
<td>0.83</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>12 10 21 4</td>
<td>11 11 22 4</td>
<td>0.32</td>
</tr>
<tr>
<td>12</td>
<td>Beckman</td>
<td>16 12 21 0</td>
<td>10 13 24 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>13</td>
<td>DXi</td>
<td>9  13 23 4</td>
<td>9  10 23 4</td>
<td>0.83</td>
</tr>
<tr>
<td>14</td>
<td>Immulite</td>
<td>11 11 9 6</td>
<td>19 9 18 0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>12 12 21 4</td>
<td>23 6 13 0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>9  12 21 7</td>
<td>17 11 19 0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>17</td>
<td>Abbott</td>
<td>10 13 18 9</td>
<td>10 13 22 5</td>
<td>0.046</td>
</tr>
<tr>
<td>18</td>
<td>Architect</td>
<td>11 12 21 7</td>
<td>17 11 19 0</td>
<td></td>
</tr>
</tbody>
</table>

The North West regional audit gathered data from 1703 patients in 17 dialysis units which included 4 hubs and 13 satellite units. A further seven dialysis units did not submit audit data and were therefore excluded from this study. The percentage of haemodialysis patients achieving the UKRA iPTH target (2-4xULN in 2009) was variable across the region (17-44%, data not shown). We used the Spearman Rank correlation to determine if each dialysis units’ achievement of iPTH targets was related to the assay used taking into consideration the bias (percentage variation from the mean) and no association was seen (NEQAS $P=0.08$, study $P=0.7$). This suggests that although variable results are seen they are not solely due to different assays. To further investigate the relevance of iPTH audit data we used the correction factors generated and adjusted all results to Laboratory 3 (Roche e170 assay). This should remove a large proportion of the assay effect. In Figure 32 we show the median iPTH for each dialysis unit before and where required, after correction. The difference is statistically significant ($P=0.04$) though clinically it is less relevant with similar results across the region pre and post correction.
Figure 32: Median iPTH values from the North West Regional Audit. Variation of results when adjusted to Roche assay

Discussion:

This study investigated the variation in iPTH assay performance in laboratories across the North-West Region and aimed to determine if this variation was clinically important such that it would lead to variations in patient management or achievement of guideline iPTH targets.

Our study has confirmed the significant variability between iPTH assay methods and supports conclusions of previous studies. There were significant differences between laboratory results even when the same assay was used and this led to patients being classified differently in relation to international PTH guidelines. One of the aims of this study was to be able to use the data generated by our 37 patient sample group to enable better comparison of clinical audit data. To do this we required samples to be processed as close to clinical practice as possible. We also collected different sample types, plasma and serum, and the results confirm the importance of using appropriate samples for different...
methods. This also highlights the possible limitations of applicability of other study ‘correction factors’ when used in clinical practice.

Utilisation of conversion factors to harmonise the results from different assays has been investigated previously. Such studies have used the Roche Elecys assay as the standard as this had been shown to correlate well with the Nichols Allegro assay which was used to develop the KDOQI guidelines. We determined a conversion factor of 1.3 was needed between the Roche Elecsys and Abbott Architect methods, and this is consistent with other studies. Almond et al have published data from Scotland emphasising iPTH measurement variability in haemodialysis patients. Ours and Almonds’ study show significant variation in iPTH results depending on the assay used. However the suggested correction / relationship between assays does differ. The main difference between studies was seen with the Immulite assay. Almond et al used EDTA samples for all analyses whereas all the laboratories using this assay in the North West used SST samples. EDTA samples have previously been shown to produce higher iPTH results than serum samples processed in the same way and this may account for the difference. This highlights the difficulty in adjusting assays in different laboratories, where samples are analysed under different conditions. Unfortunately due to sample volume limitations our study did not have the ability to analyse results with both serum and EDTA in all assays.

The variation of iPTH results seen with different assays has clinically significant implications given the expense and difficulty in managing secondary hyperparathyroidism in end-stage renal disease patients. We found that if the results were corrected to one assay method then 21% of patients would have required a clinical management change based upon the corrected result. However the North West region audit data also emphasised the importance of the clinician in the management of CKD-MBD. The 2008 UK renal registry data showed a 17% difference in achievement of renal association iPTH targets between dialysis units. Given the significant variability in assay performance it is important to take this into consideration when reviewing audit data. This study has shown that although there are significant differences between assay results, in dialysis units that have a low percentage of patients within the iPTH target there is room for improvement at a clinical level compared to a unit with a high percentage of patients within the iPTH
target. The assay variability, though important, may not account for all of the variation observed in an audit.

One of the limitations of this study is that it was designed to be applicable to the regional audit performed in the North West. Given the previously published variation of iPTH results with differing sample types and the assay variability, the use of the conversion factors we generated may not be applicable to all populations. For some of the assays used in this study we only had one laboratory using the method so the applicability of this data to generate conversion factors is also limited. Most patients had two samples analysed at each laboratory but even this led to a wide variation in results produced in the same patient and the same laboratory using the same method. Gardham et al recently published data to suggest that iPTH would need to be measured 26 times to provide 95% confidence of an accurate iPTH level and this further highlights the problems we have in altering patients’ clinical management based on iPTH results.

There is still some confusion about which PTH ‘peptides’ are measured by each assay and whether peptide fragments such as PTH (7-84) are clinically significant. The PTH (7-84) fragment (and other carboxy terminal peptides) have been shown to have some effects that are antagonistic to PTH (1-84) and they accumulate as renal function declines. A third generation assay is available but this is not widely used. This assay has an antibody directed to PTH (1-4) as well as PTH (53-84) and does not cross-react with PTH (7-84). However, the studies that have examined the association between PTH measured by third generation assays and bone biopsy data have yielded conflicting results. The lack of consistency may be explained by skeletal resistance to PTH (1-84) that develops in patients with renal disease though variation in PTH ‘peptide’ levels may also have a role. Further assay development is on-going but this currently leaves us with uncertainty regarding clinical management on a day-to-day basis.

Overall our study highlights the need for development of better markers of bone turnover and in the future we may have a combination of laboratory markers to help guide our management. A very important aspect of current assay development is standardisation of all PTH assays using a recognised international material; once available, standardisation of assays should reduce inter and intra assay variation and will hopefully improve our clinical management.
Despite the variation recognised in iPTH assays there is increasing evidence regarding the association of low and high iPTH levels and increased mortality and it has been suggested that a narrower target for iPTH is required. These latter studies incorporated large numbers of patients and this may have attenuated the confounding impact of the variability of the iPTH assays onto the final outcome. Further bone biopsy studies will be needed in the future in order to improve the validity of iPTH assays and to identify accurate target ranges that are applicable at different levels of CKD.

In conclusion the iPTH assays used in the different laboratories in the North West region of the UK are significantly variable and this variability contributes to a misclassification of patients when considered according to current iPTH guidelines. However, when the regional audit data is also considered, it should not be overlooked that clinician management is also an important factor contributing to non-achievement of target iPTH values in their patients.
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6. FINAL CONCLUSIONS
The collection of clinical studies presented in this thesis has produced some important findings regarding CKD-MBD.

**Serum phosphate and mortality**

In the 1960’s phosphate infusions were used to treat hypercalcaemia and one study of six autopsies was performed after patients developed fatal complications. Interestingly the patients were found to have features of ectopic calcification.\(^\text{355}\) At the commencement of the studies in this PhD phosphate had been shown to be associated with an increased mortality risk in certain populations.\(^\text{57, 62}\) The link between phosphate and the development of the osteoblast-like transformation of vascular smooth muscle cells had also been established.\(^\text{1}\)

We investigated the association between serum phosphate and survival in large population of pre-dialysis CKD patients. In the first experimental chapter examining the association between serum phosphate and mortality in CKD patients, we found a significant increase risk of death for each 0.323mmol/L increase in serum phosphate even when phosphate was within the normal laboratory range. This may be explained by further studies which have shown an association between phosphate and cardiovascular disease and the association has also been shown within large general population studies.\(^\text{59, 60}\) Genetic polymorphisms have now been identified in patients with a higher serum phosphate and an eGFR >45ml/min/1.73m\(^2\) and these are located in areas coding for a kidney specific sodium-phosphate co-transporter, FGF23 and the calcium sensing receptor; further work is needed to validate and explore these findings.\(^\text{356}\)

In experimental chapter 4 ‘Does tight control of secondary hyperparathyroidism lead to cardiovascular or survival benefits in haemodialysis patients?’ we have shown that all patients (irrespective of their randomisation status) who had a reduction of their serum phosphate throughout the randomised trial had an improved survival after the study. These patients had a higher serum phosphate at the beginning of the study and therefore should have had a poorer prognosis, and this provides indirect evidence that lowering phosphate has a benefit. Although this was a post-hoc analysis and the study was not designed for this analysis, we believe that this is the first time this has been demonstrated. The phosphate reduction seen was associated with significant regression of LVMI and CIMT both of
which are associated with an increased mortality risk. Higher serum phosphate has been associated with an increased CIMT within the general population and dialysis populations which suggests it may have a significant role in the development of early atherosclerosis or medial calcification. A positive association between LVMI and phosphate has been shown and there are now studies suggesting a link between FGF23 and the development of LVH. This association is thought to be klotho independent as klotho is not expressed in cardiac myocytes.

The relatively recent discovery of the FGF23 – klotho axis has expanded our understanding of phosphate regulation but this has also been implicated in insulin resistance and oxidative stress. FGF23 has been associated with increased mortality independent of phosphate and this may be due to its’ potential impact on other processes such as LVH, vascular dysfunction and atherosclerosis. We found no association between FGF23 and calcification, LVH or survival in this small study though this may be explained by the small numbers involved.

It is unknown which of either phosphate, FGF23 or klotho is more important in explaining the increased morbidity and mortality seen in renal patients. Klotho deficiency has now been shown to occur early in the development of CKD, prior to the increase in FGF23 suggesting that the klotho deficiency leads to the early increases in FGF23 levels. This may be explained by the recently determined cross-talk between the renin – angiotensin – aldosterone system (RAAS) and the FGF23-klotho axis. Angiotensin II is now thought to down-regulate klotho which in turn leads to an increase in FGF23. FGF23 is known to inhibit 1 α-hydroxylase in the kidney thus leading to less active 1,25(OH)_{2} vitamin D which is known to lead to an up-regulation in renin. Klotho, independently of FGF23, has also been associated with protection against endothelial dysfunction, protection against vascular calcification and anti-aging effects. Further understanding and knowledge regarding the FGF23-klotho axis and the cross-talk with the RAAS may facilitate developments which could reduce the progression of CKD and its complications. The early measurement and management of abnormal FGF23 and klotho levels may have larger implications than just reduction of cardiovascular morbidity and mortality.
**CKD-MBD guidelines**

Currently klotho and FGF23 as treatment targets are not included in any published guidelines. This may change in the future if further randomised trials provide evidence to suggest a treatment benefit. The most recent guidelines for CKD-MBD that are widely followed are the KDIGO guidelines published in 2009\textsuperscript{17} and the UK Renal Association also updated their guidelines in 2010\textsuperscript{339} and advised the same targets. At the start of the research for this PhD the KDOQI guidelines\textsuperscript{143} were the contemporary ones and hence much of the research contained in this thesis refers to these, now old, guidelines. In the first experimental chapter, Serum phosphate and mortality in patients with chronic kidney disease, we showed that a lower phosphate, below the KDOQI target\textsuperscript{143}, was associated with improved survival compared to patients within or above target.\textsuperscript{152} This highlighted the fact that it had been studies in dialysis patients that had been used to develop guidelines in pre-dialysis patients\textsuperscript{143} which is flawed. There is a distinct lack of robust evidence within the area of bone chemistry to aid the development of guidance and this was highlighted by the KDIGO guidelines where future research was suggested throughout.\textsuperscript{17} The KDIGO guidelines also recommended adjusting treatment based on trends of change which seems inherently sensible.

One of the major changes seen within the KDIGO guidelines\textsuperscript{17} compared to KDOQI\textsuperscript{143} was the iPTH target in dialysis patients. KDOQI had previously suggested an iPTH target of 150-300pg/mL in dialysis patients based on a bone biopsy study which used the Nicholls Allegro iPTH assay which is no longer available.\textsuperscript{143} As the variability of iPTH assays became more widely appreciated the 2007 UK Renal association guidelines suggested aiming for a target adjusted according to the local reference range (2-4 x upper limit of normal).\textsuperscript{258} The KDIGO working group reviewed the data and suggested that there was a lack of evidence to support this and suggested a target of 2-9x upper limit of the local reference range for iPTH.\textsuperscript{17} This range is roughly equivalent to 130-585pg/mL as even though the iPTH target is locally derived 65pg/mL seems to be the most frequent upper limit quoted as seen in experimental chapter 5: Variation in iPTH concentration measured in laboratories across the North West region of the UK; an assay variability study linked to clinical audit data.
Since the release of the KDIGO guidelines\textsuperscript{17} an observational study has suggested that an iPTH within the KDOQI target of 150-300pg/mL\textsuperscript{143} is associated with an improved survival.\textsuperscript{299} The KDOQI guidelines\textsuperscript{143} were used to set the target for the iPTH, calcium and phosphate within the randomised controlled trial which formed the basis for experimental chapters 3 and 4 (‘A Randomised control trial to examine the effects of cinacalcet on bone and cardiovascular parameters in haemodialysis patients with uncontrolled secondary hyperparathyroidism’ and ‘Does tight control of secondary hyperparathyroidism lead to cardiovascular or survival benefits in haemodialysis patients?’). Within the trial we achieved equivalent lowering of iPTH and phosphate in both arms which is difficult to achieve within normal clinical practice and required intensive management. In experimental chapter 4 we showed a significant reduction of LVMI and CIMT when targeting the KDOQI guidelines\textsuperscript{143} for iPTH, phosphate and calcium, regardless of which treatment is used. Change in iPTH was not associated with the changes in CIMT and LVMI in this research. PTH has been associated with LVH in normotensive haemodialysis patients\textsuperscript{268} and rat studies have suggested a potential role of PTH in the development of uraemic cardiac injury.\textsuperscript{369}

In the trial we showed a similar percentage progression of vascular calcification compared to that seen in other randomised trials. There was a 20% overall progression of total calcification with only a 12% annual progression of coronary calcification. Unlike the ADVANCE study\textsuperscript{111} our study did not exclude patients whom had a coronary calcification score <30 and these patients seem less likely to progress and therefore may behave differently to the rest of the population.\textsuperscript{370} This may have lowered the overall percentage progression of coronary calcification in our study and is therefore not easily compared to the ADVANCE study (Coronary artery calcification percentage progression 24% and 31% in each arm).

Intact PTH control could be important in the progression of calcification with over-suppression being equally problematic as to excess levels. This is relevant to the interpretation of major trials comparing sevelamer hydrochloride and calcium carbonate. In the Treat to Goal study there was a 25% progression of coronary calcification over 1 year within the calcium carbonate arm compared to only 6% progression in the sevelamer arm.\textsuperscript{103} However 57% of the calcium carbonate treated arm had an iPTH result lower than the target of 150-300pg/mL compared to only 30% in the sevelamer arm. In comparison,
Quinbi et al\textsuperscript{104} compared sevelamer to calcium acetate in over 200 dialysis patients with the addition of lipid lowering medications in the calcium acetate arm to provide equivalent lipid reduction between the two arms (CARE-2 study). This study showed no benefit in the sevelamer arm compared to patients receiving calcium binders with 28\% and 32\% progression of coronary artery calcification seen at 12 months though the overall study median iPTH was much higher (about 400pg/mL)\textsuperscript{104}. A study in rats has shown an association between an increased iPTH and vascular calcification\textsuperscript{55} and this has now also been confirmed in human studies.\textsuperscript{371-373} Noordzij et al\textsuperscript{372} found a relationship between iPTH concentrations >300pg/mL, compared to iPTH \( \leq 300\text{pg/mL} \), and progression of vascular calcification (OR 4.36 (95\% CI 1.35)) whereas Jean et al\textsuperscript{373} suggest iPTH values greater than 190pg/mL were associated with increased progression of calcification. These values are much lower than the target suggested by the KDIGO working group but as no randomised trial has been published suggesting definitively that aiming for a lower target affects cardiovascular outcome then I personally will continue to follow the KDIGO\textsuperscript{17} guidelines at this time.

\textit{Intact PTH assays}

Experimental chapter 5, ‘Variation in PTH concentration measured in laboratories across the North West region of the UK; an assay variability study linked to clinical audit data’, highlights the variability of iPTH assays used in different laboratories and found that the variation in assays could lead to a change in clinical management in a significant number of patients. This variation in assays could account for the lack of and /or conflicting evidence sometimes seen in studies involving iPTH.\textsuperscript{10, 62, 63, 374} Many of these studies have used a variety of iPTH assays at several centres within a large population which may explain some of the differences.\textsuperscript{10, 62, 63, 374} As discussed, the iPTH assays available measure different PTH peptides alongside the active 1, 84 PTH peptide. The bioactivity of the other peptides is currently unknown but the 7,84 PTH peptide has been shown to have antagonistic properties.\textsuperscript{347} Further understanding of PTH peptides and their actions, along with knowledge regarding which peptides are measured by the available assays, will greatly increase our understanding of CKD-MBD and its management. Any research assessing the association of iPTH and bone or cardiovascular outcomes will be hindered by this lack of information. The iPTH variability study within this thesis showed that variation in achievement of these targets was partly due to differences in local clinical management.
Calculated assay correction factors did reduce some of the variation seen between dialysis units in the regional audit however the median iPTH in each of the units varied widely between 130pg/mL to over 400pg/mL.

**Clinical management**

The variation of clinical management of bone chemistry is reflected by the results seen in regional audits but it can also be seen within departments with many nephrologists having personal preferences for particular phosphate binders, vitamin D analogues and whether, if necessary or able, to use cinacalcet or proceed with parathyroidectomy in patients with advanced hyperparathyroidism. However, the question is how does one unit achieve a higher proportion of patients attaining CKD-MBD targets and how can this management be replicated in other units?

Achievement of iPTH targets cannot be achieved without also improving phosphate and calcium control. I personally believe there to be an important role for the maintenance of adequate 25(OH) vitamin D levels in achieving targets and providing the patient the best chance of survival. As previously discussed low levels of 25(OH) vitamin D are associated with an increased risk of infection, hypertension and diabetes in the general population which are significant complications for renal patients. Replacement could alleviate some of the complications associated with low levels of 1,25(OH)₂ vitamin D such as activation of the RAAS system though this has not been shown in experimental studies as yet. Randomised controlled trials are needed in this area to further explore this potential benefit.

Several studies now suggest that cinacalcet may have beneficial effects on vascular calcification, and these have been published throughout the duration of this research. Cinacalcet was the main focus of the randomised controlled trial which was reported within this thesis but no significant difference in vascular calcification was seen between arms. This may be explained by achievement of equivalent iPTH and phosphate control in both arms of the trial by diligent clinical management, although the trial was too small to draw definitive conclusions. There was a trend towards further regression of LVH in the cinacalcet arm compared to standard therapy alone but this did not reach significance. CaSR are known to be present on cardiac myocytes suggesting a potential
mechanism for this finding and calcimimetics have been shown to interfere with cardiac remodelling in rats.

An alternative explanation as to why this trial was negative is the length of the study. The effect of calcimimetics on calcification may take many years to lead to a significant change. The ADVANCE study’s primary outcome of change in coronary calcification measured by agatston score was negative but potential benefit of cinacalcet was shown in all sites where measurements were performed. The EVOLVE trial, which is an outcome driven study, is investigating whether cinacalcet and low dose vitamin D compared to variable dose vitamin D will impact on cardiovascular outcomes. This study has now finished and the final results are eagerly awaited.

To improve the cardiovascular outcomes in renal patients we should not just focus on CKD-MBD parameters. In experimental chapter 2: factors associated with vascular stiffness: cross-sectional analysis from the Chronic Renal Insufficiency Standards Implementation Study, we showed that increased vascular stiffness was associated with higher blood pressure measurements and blood pressure is an important determinant of cardiovascular outcome in renal and general populations. The SHARP study has also confirmed that renal patients should have their LDL actively lowered using simvastatin and ezetimibe to impart a cardiovascular benefit. Low bicarbonate levels are often seen in CKD patients and there is now increasing evidence that low levels are also associated with increased cardiovascular risk.

In conclusion, the pathophysiology of the poor outcome of renal patients is multi-factorial and many factors need to be adjusted. Intervening early in the development of CKD may slow down the development of bone and cardiovascular complications and there are many exciting new potential targets upon which to focus further research.
Limitations to the methodology and study design in general

- The CRISIS study is a single-centre study so therefore the findings of the study may not be applicable outside of the Greater Manchester area. Only 2% of CRISIS patients are not Caucasian again limiting its applicability. The study is labour intensive and currently only follows patients until renal replacement commencement or death. Expansion would require more nursing support to allow this study to expand out of the single centre or to continue to monitor patients once started on renal replacement therapy.

- The randomised controlled trial using cinacalcet included only a small number of patients due to difficult recruitment. The power calculation was developed prior to the publication of any other studies examining vascular calcification with cinacalcet and therefore the trial was underpowered.

- The number of investigations the patients underwent within the randomised trial may have led to recruitment of the more able patient. Patients with cardiovascular disease which had required intervention such as coronary stents and coronary artery bypass operations were excluded to enable the scans to be interpreted therefore limiting the applicability of these findings to patients with severe coronary disease.

- The applicability of the findings of the randomised trial is limited as the education and intensive management of each patient in the first 12 weeks of the trial which led to the reduction of iPTH and phosphate within the trial setting would be very difficult to replicate within the realms of current normal clinical practice.

- Aiming for a tight range for iPTH can be difficult and some patients then develop a low iPTH which is equally problematic. This led to a number of patients’ iPTH concentrations becoming over-suppressed during the trial which may have further weakened the study.

- Within the randomised study bone status was assessed using bone mineral density measures. These are difficult to interpret in renal patients and do not provide
accurate information as to bone turnover and strength. The inclusion of bone biopsy would have strengthened this study.

- In the iPTh assay variability study not all biochemistry laboratories received 2 samples for each of the 37 haemodialysis patients. Unfortunately only one laboratory used the Abbott Architect iPTh assay within the North West which therefore did not allow comparison with any of the laboratories using this method.

**Strengths of the study design and methodology**

- The CRISIS study is a large prospective single centre study designed to identify factors associated with CKD progression, development of cardiovascular events and survival. This allowed us to investigate whether serum phosphate within or outside the normal range was associated with increased mortality in non-dialysis CKD patients. Investigation of the association of vascular stiffness with a wide range of phenotypic parameters was also possible.

- The randomised controlled trial, though small, was mainly aimed to generate further hypotheses. This allowed us to investigate potential factors across the spectrum of CKD-MBD which may be affected by cinacalcet.

- The intensive management of secondary hyperparathyroidism that was provided for patients in the randomised controlled trial would be difficult to replicate in a larger study. The equivalent management in the active and control arms enabled further evaluation of LVMI and CIMT regression in a pooled analysis of all patients that gave more insights into potential cardiovascular benefits. The importance of phosphate reduction was also highlighted further.

- The iPTh assay variability study replicated clinical practice and allowed consideration of clinical audit data in the light of assay variability. Other studies investigating iPTh assay variability have tended to follow controlled assay methods which are not reflective of the variation seen across clinical practice.
7. SUGGESTIONS FOR FUTURE WORK
Prevention of cardiovascular co-morbidities in early CKD

There is substantial evidence that shows that by the time patients reach renal replacement therapy they have significant co-morbidity. There are a number of potential targets that change early in CKD that may slow renal progression and/or improve cardiovascular and bone health.

- I have shown that serum phosphate is associated with an increased risk of death in pre-dialysis patients, particularly in the earlier stages of CKD. An intervention study aiming for a serum phosphate <1.3mmol/L in the active treatment arm and determining its effect on cardiovascular outcomes would be the next logical study. To achieve this level of phosphate control a combination of dietary modification and non-calcium phosphate binders would be required to ensure that oral calcium load did not confound the results. This would require a multicentre study with a large population and/or a long follow-up to achieve adequate power.

- Another potential modifiable factor is 25(OH) vitamin D which has been shown to decrease as CKD progresses. The majority of stored and circulating 25(OH) vitamin D is converted to active 1,25(OH)₂ vitamin D within the cell and exerts its action via an autocrine/paracrine pathway. It is unlikely these pathways can be adequately replaced with systemic calcitriol or other vitamin D analogues without leading to hypercalcaemia due to the endocrine actions of the hormone. An intervention study aiming for higher 25(OH) vitamin D estimations of >75nmol/L compared to no active replacement in a pre-dialysis CKD stage 3-4 population would be interesting. The outcome could be a surrogate such as changes in calcification, biomarkers or cardiac morphology but hard end-points such as survival, renal disease progression and hospitalisation would be better. The media spotlight in this area may lead to a high patient drop-out and the seasonal variation, which naturally occurs, would lead to a requirement of large patient numbers to be adequately powered. The follow-up would also need to be long enough to show an effect and the previous points made could make this a difficult trial to complete. The administration of active vitamin D analogues also stimulates secretion of FGF23 which is another potential modifiable factor.
FGF23 has been associated with the development of LVH and vascular calcification and has been associated with increased mortality.\(^{53, 311, 324, 358}\) FGF23 may be lowered with the use of sevelamer\(^{383}\) and further studies investigating dietary restriction and/or other phosphate binders are on-going. Cinacalcet use has also been associated with a reduction in FGF23\(^{310}\) though this has not been confirmed by other studies. Studies in CKD populations investigating the effects of lowering FGF23 are ongoing.

Klotho is also an interesting potential modifiable factor. Studies replacing soluble klotho and/or forcing over-expression by genetic modulation show potential for reducing renal fibrosis and progression of renal disease.\(^{27}\) Unfortunately soluble klotho is not available as an investigative medicinal product for use in clinical trials as yet and no medication has been shown to increase its levels.

To definitively investigate these factors large multi-centre trials would be required to recruit enough patients to enable a positive outcome but these are obviously extremely expensive.

**Correlation of histopathological findings to study findings**

Bone biopsies are the gold standard method for diagnosing the bone abnormalities found in renal patients and the only true way of determining the activity of the bone in any individual. If designing a similar study again I would aim to include bone biopsies as this would provide more information as to the bone activity and would be more useful in determining bone health.

A study to measure the progression of vascular calcification measurements and changes in bone biopsies in CKD patients over time would provide interesting data and if paired with biochemical and biomarker analysis and clinical cardiovascular markers would lead to further hypotheses as to potential targets for future therapies.
Application of findings from this thesis

Further, larger, randomised controlled trials comparing different targets of phosphate and iPTH are required to help determine the correct target for these parameters. In view of the findings in this thesis outcomes of LVMI, vascular calcification and CIMT could be considered important end-points although ideally a study with cardiovascular end-points rather than surrogates would be more beneficial. The effect of cinacalcet on LVMI also warrants further investigation.

Overall the outcomes of the studies within this PhD suggest that aiming for a low normal phosphate in CKD patients may improve outcome. Aiming for tight control of iPTH (roughly 150-300pg/mL) and lowering the phosphate could improve outcomes in dialysis patients.


Ref Type: Abstract


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Ref Type: Report


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APPENDIX 1: PROTOCOL, CONSENT FORM AND QUESTIONNAIRE

A randomised controlled trial to examine the effects of calcimimetic therapy on bone and cardiovascular health in end-stage renal disease.

PROTOCOL SUMMARY:

Secondary hyperparathyroidism is a known complication of end-stage renal disease (ESRD) and is associated with renal bone disease, cardiac structural abnormalities, and increased vascular stiffness and calcification. Cinacalcet is a new calcimimetic agent licensed for use in secondary hyperparathyroidism in ESRD. This is a new type of agent which targets the calcium-sensing receptor and lowers calcium, phosphate and parathyroid hormone (PTH) into the target range (as stated in the UK Renal Association guidelines). It is used concomitantly with the standard treatment of secondary hyperparathyroidism i.e. vitamin D analogues and phosphate binders. Its intended site of action is the parathyroid gland calcium-sensing receptor; however calcium sensing receptors are widely distributed throughout the body. There has been one small study looking at the effects on bone mineral density in 14 patients while taking cinacalcet; this showed an improvement over the 6 month period compatible with the observed fall in PTH. (1) There have been no studies examining the effects of Cinacalcet on cardiac structure and vascular parameters.

This project is a randomised control study of 40 patients with secondary hyperparathyroidism and chronic kidney disease (CKD) stage 5 (glomerular filtration rate <15ml/min) who are on dialysis. The patients will be randomised to receive cinacalcet plus standard treatment compared to standard treatment alone. Due to cost and lack of outcome data, this new treatment it is not routinely available for all patients at this time.

We plan to record comprehensive cardiovascular and bone investigations at baseline and after 12 months. These will include non-invasive cardiovascular tests such as pulse wave analysis (PWA), pulse wave velocity (PWV), carotid intima-media thickness (CIMT) measurements, cardiac MR scan and CT scan for vascular calcification. Bone health will be studied with quantitative CT, Dual energy x-ray absorptiometry (DXA) and peripheral quantitative CT of the forearm to determine measures of bone density, size and biomechanical properties. Serum will be collected at baseline and after 12 months to
measure serological markers of vascular calcification. These comprehensive investigations will give us an overall picture of changes in the organs of interest.

The patients will also complete a short interview which will enable us to determine their past medical history, co-morbidity and medication history. The questionnaire will also be used to calculate the New York Heart Association (NYHA) and Canadian Cardiovascular Society (CCS) scores for heart failure and angina respectively.

This study will give us data on the effects of cinacalcet on the heart, vessels and bones in our ESRD population compared to standard therapy. This will be novel important information as the life expectancy of patients with ESRD is significantly reduced with premature cardiovascular disease accounting for about half the deaths (2).

**Aims of the study**

The aims of this project are therefore to:

a) Determine the effects of cinacalcet compared to standard treatment alone on cardiovascular structure and function.

b) Determine the effects of cinacalcet compared to standard treatment alone on markers for bone health

**BACKGROUND**

Secondary hyperparathyroidism is a known complication of CKD. Raised parathyroid hormone (PTH) is thought to be a causative factor in the pathogenesis of renal bone disease and cardiovascular risk factors such as hypertension, deranged lipid profile and glucose intolerance (3). Secondary hyperparathyroidism has also been linked to left ventricular hypertrophy, interstitial fibrosis and arteriolar wall thickening of the heart (4). These abnormalities are common in CKD and 85% of people on dialysis are found to have some structural change of their heart (5).

Disruption of mineral metabolism occurs with moderate renal impairment and is most severe in ESRD. It is thought to lead to medial arterial calcification and vascular stiffening, which increases myocardial oxygen demand, sub-endocardial ischaemia, and vascular after-load, with a propensity to develop LVH (6). Coronary artery calcification is also
highly prevalent and has even been demonstrated in adolescents and young adults with ESRD (7). An inverse relationship is known to exist in some populations between bone mineral density and vascular calcification and has been postulated to exist in uraemia, possibly accounting for some of the excess cardiovascular risk known to exist in this population (8, 9). The vascular calcification in uraemia is also thought to be different to ‘classical’ atherosclerosis; calcifying the medial layer of the vessel. This is thought to be an active process mediated by a variety of serological markers including pyrophosphate and osteopontin (10). The two types of calcification do occur simultaneously though plaque formation in renal patients does differ in composition, not size, when compared to non-uraemic controls (11).

We propose to examine the effects of calcimimetic therapy, compared to standard therapy, on bone and cardiovascular health in a group of ESRD patients, with measures of vascular calcification, aortic stiffness, left ventricular mass, serological markers and bone mineral density and their relationship to each other as well as other clinical and laboratory parameters and vascular events.

This is an open-label randomised control study of patients with CKD stage 5 and who are on dialysis. These patients will be identified as having the clinical indications for the new calcimimetic agent, Cinacalcet. This will incorporate patients with secondary hyperparathyroidism whose calcium, phosphate and PTH are difficult to control with the standard methods available currently (i.e. phosphate binders and vitamin D analogues). Cinacalcet is known to lower phosphate, calcium and PTH towards target levels when the dose is titrated accordingly. It has also been demonstrated in a small pilot study of 14 patients to increase BMD at the proximal femur as measured by DEXA at 26 weeks. (12)

**Assessment of arterial stiffness using PWV and PWA measurements**

PWA and PWV are well validated methods of measuring vascular stiffness. They have been shown to have predictive power for all cause and cardiovascular mortality (13). PWA and PWV will be performed according to recommended procedures and published protocols (14).
Cardiac magnetic resonance scan

Cardiac magnetic resonance scans will be used to examine left ventricular mass index, chamber volumes, ejection fraction and aortic flow. The MR scan is non-invasive and does not expose the patient to any ionising radiation. It will provide us with electrocardiographic gated images of the whole cardiac cycle. From the scans we acquire at baseline and at 12 months we will be able to identify small changes in cardiac structure and function that would not be identifiable on echocardiography.

CT scan of coronary arteries and aorta

Arterial calcification is a common complication of chronic kidney disease. The extent of arterial calcification has been found to be highly predictive of future cardiovascular disease and mortality beyond established risk factors (15, 16). The calcification found has been shown to be negatively associated with bone mineral density suggesting that they could be part of the same process. This is true for patients with and without kidney disease (8, 17). The CT will be analysed using ‘smartscore’ software to generate a calcification score for the coronary arteries and aorta.

Bone mineral density

Renal osteodystrophy is a major complication of chronic kidney disease leading to decreased bone strength. This is thought to be due to problems with bone turnover, bone density and bone architecture. In this study, we will perform a number of investigations of bone density, size and biomechanical parameters. Dual-energy X-ray absorptiometry (DXA) is widely used to assess bone mineral density. Many studies have found a relationship between bone density and vascular calcification. DXA of the spine, however, does present problems in uraemic patients due to the increased frequency of aortic calcifications which then lead to an artefactually high measurement of bone mineral density. Quantitative CT (QCT) of the spine obviously circumvents this problem, allowing uncontaminated regions of interest to be studied. QCT also gives us information about the relationship between cortical and trabecular bone which will give us an indication of the rate of bone turnover. High bone turnover is associated with thickened, sclerotic trabeculae while low turnover is associated with abnormally thin trabeculae (18). Further quantitative
information will be obtained with a peripheral quantitative CT of the forearm. From this additional test we will also be able to assess biomechanical parameters and cross-sectional muscle area to allow further investigation of the muscle/bone unit (19).

**PLAN OF INVESTIGATION**

(See appendix 1)

**Ethical approval**

This is currently being sought at our local ethics committee

**Recruitment of patients**

48 patients in total will be recruited. 24 patients will receive Cinacalcet and standard therapy; the other 24 will receive standard therapy alone. These patients will be identified as suitable by a search of Hope hospital renal database. We have identified over 60 patients who have a PTH level >300pg/mL, who have a corrected calcium ≥ 2.1mmol/L. These patients will be assessed for the further inclusion and exclusion criteria listed below. A screening interview will be arranged to coincide with a routine clinic or a dialysis session. Past medical history, a record of all medications, height, weight and blood pressure will be recorded on all patients. The data collection form (see appendix 4) will be filled out with data including the NYHA and CCS score for heart failure and angina respectively. Blood tests indicated as part of routine clinical follow up will be taken and the results recorded. An extra 4 teaspoonfuls of blood will be taken and frozen at -80°C for subsequent measurement of serological markers of vascular calcification at the end of the study.

During the study subjects will be dialysed to maintain their fluid and electrolyte needs. Haemodialysis patients will have their URR maintained at >65% and peritoneal dialysis patients weekly Kt/v >1.7 as per the UK Renal Association standards (20).
Inclusion criteria:
- Age: ≥18, ≤75
- Corrected calcium ≥ 2.1mmol/L
- PTH >300 pg/mL
- Established on dialysis for > 90 days

Exclusion criteria:
- Atrial fibrillation
- Any contraindications to MR scan or to cooperate with scan
- Any factors which will influence CT e.g. artificial heart valves, previous sternotomy wires, stents
- Contraindication to cinacalcet e.g. pregnant, breast feeding, known reaction
- Moderate to severe liver disease (ALT > x3 normal range)
- Have a poor record of compliance with medication
- Have participated in a study involving an investigational drug during the 30 days prior to the 1st visit.
- Be involved in any other research study which exposes the patient to radiation above that of normal clinical practice.

Investigations

All scans involved in the study will take place at baseline and at 12 months post allocation. The PWA, PWV and CIMT will take place in either dialysis unit or clinic setting (see appendix 6). Patients will attend the Central Manchester and Manchester Children’s Hospital (CMMC) site for the following over one morning or afternoon: CT scan of coronary arteries and aorta and QCT in the radiology department; cardiac magnetic resonance scan in the Welcome clinical research facility; bone studies in the University of Manchester Clinical radiology department. Patients will have a member of the research team to accompany them for these appointments. These scans will equate to a total dose of 6.81 mSv over a 12 month period compared to the average background radiation exposure in the UK of 2.2 mSv per annum. This equates to a theoretical risk of 1 in 3000 of fatal cancer induction. We feel this is justified since ESRD patients have an annual mortality of
20%, mainly due to cardiovascular disease and this study is investigating a treatment which may improve cardiovascular parameters.

After baseline scans the patients will be randomised according to the procedure shown below.

**Medication**

The patients will either have standard treatment plus cinacalcet or standard treatment alone. This is an open-label study.

There is a 12 week titration phase in which patients will have doses adjusted of standard and cinacalcet treatment adjusted (See appendix 2). During this time patients are required to have at least fortnightly blood tests which are recommended by the manufacturer for patients taking Cinacalcet. This is to allow for the titration of cinacalcet as required and other intensive treatment adjustment. After this time patients will continue to have 2-monthly blood tests for measures of corrected calcium, phosphate and parathyroid hormone and medication still may be adjusted accordingly.

**CINACALCET SUPPLY:**

This will be supplied from the hospital for the duration of the study. All other medications will be supplied by the GP.

At the end of the study it will be decided if the patients who are taking cinacalcet need to continue with this medication. If a beneficial effect is shown then the patients in the control arm will be given the option of the medication if indicated.
Serious Adverse Events

These are defined as adverse events that:

- Result in death
- Are life-threatening
- Require in-patient hospitalisation or prolongation of existing hospitalisation
- Result in persistent or significant disability or in-capacity
- Results in congenital anomaly or birth defect
- Are important medical events in the opinion of the responsible investigator (i.e. any event not immediately life-threatening and does not result in death or hospitalisation but which may jeopardise the participant or may require intervention to prevent one or other outcomes listed above)

Any serious adverse event (expected or unexpected) that in the opinion of the reporting investigator could be due to the study medication is to be reported within 24 hours of learning of the event. This needs to be reported a member of the data monitoring committee for the report to be reviewed urgently. This information will also then be sent to the ethics committee who have approved the study.

Serious adverse events that are not thought to be due to the study medication will be recorded at each follow-up visit and reviewed by the data monitoring committee.

Adverse Events

These are reported using the appropriate form for all medical events (expected or unexpected)

The data monitoring committee will review all adverse events at 6-monthly intervals.
Randomisation procedure

Patients will be randomised but stratified according to the PWV measurement taken at recruitment and their diabetic status. This is to ensure adequate distribution in view of the smaller numbers involved in this trial.

Colour coded envelopes will be prepared by an independent person. All colours of envelopes will have an equal number of each arm available.

Colours used will be as below:

If patients have PWV ≥ 11m/sec and NO diabetes: RED envelopes
If patient has PWV ≥ 11m/sec and has diabetes: BLUE envelopes
If patient has PWV < 11m/sec and NO diabetes: GREEN envelopes
If patient has PWV <11m/sec and has diabetes: YELLOW envelopes

Outline of patient visits

Screening / recruitment visit:

The patient will already have reviewed the patient information leaflet. The exclusion and exclusion criteria will be checked with the patient and most recently available blood tests. If there are no potential problems identified then the patient will be invited to participate in the study. The study will be re-explained to the patient and any questions answered. Patients are discouraged from participating if they are unwilling to have the blood tests required during the initial phase of the study for safety reasons. Patients will be asked to provide their written, informed consent for the study.

1st Study visit

This can be performed at the same time as the screening/ recruitment visit if patient wishes. During this visit the questionnaire needs to be completed with the patient to cover past medical history, medications and the NYHA and CCS score for heart failure and angina.
respectively. They will also complete a safety questionnaire for the MR scan. The patient will then have the arterial stiffness studies and the recording of the carotid-intima media thickness.

Blood samples will be taken at this time.

Patients can be randomised at this visit and provided with required medication. However the patients will be asked not to start their medication until after the CMMC visit.

**CMMC visit**

For this the patients will be sent an appointment through the post. Transport costs will be reimbursed at standard rates. A member of the research team will be present throughout the appointments to help the patients. They will attend for the MR scan, CT scan and bone studies on the same day.

**Titration phase**

Patients require at least fortnightly blood tests throughout this time. The aim of this phase is to improve control of the hyperparathyroidism and is according to manufacturers guidelines for the use of Cinacalcet. After each blood test the corrected calcium, phosphate and PTH are used to adjust the medication according to the algorithm in Appendix 2.

During this phase any side effects of the medication needs to be documented as an adverse event.

**Routine Clinic visits**

Patients will be seen 3 monthly. The dialysis patients have regular blood tests monthly normally and these will be reviewed. At these visits any adverse events will be recorded appropriately.
12 month visit

This visit is to occur 12 months post CMMC visit. Blood tests will be taken and arterial stiffness measurements and carotid intima-media thickness will be recorded. The questionnaire will be completed as before, including the MR safety questionnaire. Patients will be informed as to whether their medication will be altered depending on the clinical indication at that time after discussion with their clinician.

Subject withdrawal

Patients can withdraw their consent from the study at any time. If they are intolerant of cinacalcet but are still willing to take part they will continue in the study. If a patient in the standard treatment arm develops an urgent indication for Cinacalcet (e.g. calciphylaxis) they will be started on the medication. They will also continue in the study if happy to do so.

Data recording

Data will be recorded on the attached data collection form (see appendix 3). The study number will be allotted at time of randomisation.

Primary end-point

Absolute change in calcification score between the two arms.

Secondary End-points

The data will be analysed to compare each arm and monitor progression within each arm of bone measurements, arterial stiffness parameters, carotid intima-media thickness and abnormalities of cardiac structure and function. Mortality and cardiovascular endpoints will also be analysed
Statistical considerations

We expect a mortality rate of about 20% per annum in our dialysis population. Analysis will be with SPSS version 12.0 for windows. We will perform a t test to test for statistical significance.

Power calculation:
This is a new medication and a unique study so we do not have any previous results to relate to. Using the primary outcome the following calculation has been done. Our sample size is 20 per arm and we are expecting a 33% loss per annum leaving 16 per arm. This gives us 80% power at the 5% significance level to detect a difference of 1 standard deviation in the percentage change of calcification score.
Study number: srhths1
Patient identification number for this trial:

CONSENT FORM

Title of project: A RCT to examine the effects of Cinacalcet on bone and cardiovascular health in ESRD

Name of Researcher: Please initial box

1. I confirm that I have read and understood the information sheet (version…….) for the above study and have the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of my medical notes may be looked at members of the research team who are not necessarily involved in my medical care. I give permission for these individuals to have access to my records.

4. I agree for my GP and any other relevant medical practitioners involved in my care to be informed of my participation in this study.

5. I agree to take part in the above study.

_________________________  ______________________  ______________________
Name of Patient          Date                    Signature

_________________________  ______________________  ______________________
Name of person taking consent (if different from researcher)  Date                    Signature

_________________________  ______________________  ______________________
Researcher                Date                    Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes
Eligibility screening form:

Before entering trial, all of the following selection criteria must be marked.

Study number:
Patient identification number:

Inclusion criteria:

1. On dialysis for >90 days  
2. Corrected calcium ≥ 2.2 mmol/L  
3. Parathyroid hormone > 300 pg/mL  
4. Age ≥ 18 ≤ 70  

Exclusion criteria:

1. Atrial fibrillation  
2. Contraindication to magnetic resonance scan (see questionnaire)  
3. Inability to cooperate with MR or CT scan  
4. Factors which would influence CT scan  
   e.g. artificial heart valve, previous sternotomy, wires, coronary stents  
5. Contraindication to Cinacalcet  
   e.g. pregnancy, breast feeding, known reaction  
6. Moderate to severe liver disease (ALT > x3 normal range)  
7. Have a poor record of compliance with medication  
8. Have participated in any other research involving an investigational drug during the last 30 days prior to this visit  
9. Is involved in any other research study which exposes the patient to radiation above that of normal clinical practice.

Will the patient participate in the trial?  YES □  Date of consent: __/__/____  
   NO □

Investigators signature: ___________________________ Date _____________
# Appendix 4 - Datasheet for initial interview

<table>
<thead>
<tr>
<th>Patient initials:</th>
<th>Patient no:</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASELINE VISIT</td>
<td></td>
</tr>
<tr>
<td>Demographics</td>
<td>dd / mm / yyyy</td>
</tr>
<tr>
<td>Date of birth</td>
<td>__ / __ / ____</td>
</tr>
<tr>
<td>Gender</td>
<td>Male       Female</td>
</tr>
<tr>
<td>Weight</td>
<td>Kg</td>
</tr>
<tr>
<td>Height</td>
<td>M</td>
</tr>
<tr>
<td>Race (see procedures)</td>
<td></td>
</tr>
<tr>
<td>Past medical history</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>If yes - Date</td>
</tr>
<tr>
<td>Previous MI</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Treated for angina</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Coronary artery bypass graft (CABG)</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Percutaneous transarterial coronary angioplasty (PTCA)</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Stroke</td>
<td>Yes / No</td>
</tr>
<tr>
<td>TIA</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Non-coronary arterial surgery or angioplasty</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
</tr>
<tr>
<td>Form of dialysis</td>
<td>HD / CAPD / APD</td>
</tr>
<tr>
<td>Date started on dialysis</td>
<td></td>
</tr>
<tr>
<td>Previous transplant</td>
<td></td>
</tr>
<tr>
<td>Date of transplant</td>
<td></td>
</tr>
<tr>
<td>Date transplant failed</td>
<td></td>
</tr>
<tr>
<td>Age at first presentation of renal disease</td>
<td></td>
</tr>
<tr>
<td>Cause of renal disease (see procedures)</td>
<td></td>
</tr>
<tr>
<td>Cardiac risk factors</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Treated?</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Yes / No   Type?</td>
</tr>
<tr>
<td>If yes age at diagnosis</td>
<td></td>
</tr>
<tr>
<td>On insulin?</td>
<td>Yes / No   Since?</td>
</tr>
<tr>
<td>Current smoker</td>
<td>Yes / No   How many/day?</td>
</tr>
<tr>
<td>If no: Previous smoker</td>
<td>Yes / No   How many/day?</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>Yes / No   Units/week?</td>
</tr>
</tbody>
</table>

Completed by ……………………………. Date ………………………..
Datasheet for initial interview

Patient initials: | Patient no: | Heart

symptoms

Please circle the number (1 – 4) most applicable to you

1  Ordinary physical activity does not cause me to be unduly short of breath, or to get angina, palpitations or fatigue.

2  I am comfortable at rest, but ordinary physical activity causes me some breathlessness, or angina or palpitations or fatigue.

3  I am comfortable at rest, but less than ordinary physical activity causes me to feel breathless, get angina, palpitations or to feel fatigue.

4  I am unable to carry on any physical activity without discomfort. Symptoms of breathlessness or angina may be present at rest. If any physical activity is undertaken, my discomfort is increased.

Angina

Please circle the number (0 – 4) most applicable to you

0  I do not experience any angina

1  Ordinary physical activity does not cause my angina, such as walking and climbing stairs. I may experience angina with strenuous or rapid or prolonged exertion at work or recreation.

2  My angina causes slight limitation of ordinary activity. For example I may experience angina walking or climbing stairs rapidly, walking uphill, walking or stair climbing after meals, or in the cold, or under emotional stress or during the first few hours after awakening. I may get angina walking more than two blocks on the level and climbing more than one flight of stairs at a normal pace and in normal conditions.

3  I have marked limitation of ordinary physical activity. I may get angina walking one or two blocks on the level and climbing one flight of stairs at normal pace.

4  I experience an inability to carry on any physical activity without discomfort – my angina may be present at rest.

Date completed …………………………………
# Datasheet for initial interview

<table>
<thead>
<tr>
<th>Patient initials:</th>
<th>Patient no:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medication history</strong></td>
<td>Duration (months)</td>
</tr>
<tr>
<td>Calcium based phosphate binder</td>
<td>Yes / No</td>
</tr>
<tr>
<td>If yes: prescribed elemental calcium dose/day (see procedures)</td>
<td></td>
</tr>
<tr>
<td>Active vitamin d analogue</td>
<td>Yes / No</td>
</tr>
<tr>
<td>All other medication</td>
<td>Dose</td>
</tr>
</tbody>
</table>

| | |
| Ca dialysate | No: | Ca mmol/L |
| Latest Kt/v or URR | |
| | |

Completed by ................................................. Date.................................................
Magnetic Resonance scan safety questionnaire

1. Do you have a pacemaker or artificial heart valve? YES/NO
2. Do you have a hydrocephalus shunt? YES/NO
3. If so, is it a programmable shunt? YES/NO
4. Have you had any operations on your head? YES/NO
5. Have you had any surgery to your head or body within the last two months? YES/NO
6. Do you have any joint replacements or metal implants? YES/NO
7. Have you EVER had metal in your eyes or worked with metal at high speed, e.g. in a machine shop? YES/NO
8. Do you have any shrapnel from a war injury? YES/NO
9. Do you wear a false limb, calliper or brace? YES/NO
10. Do you have dentures, a dental plate or a hearing aid? YES/NO
11. Have you suffered from epilepsy or blackouts? YES/NO
12. Do you have any ear implants, e.g. cochlear? YES/NO

TO BE ANSWERED BY WOMEN OF CHILD BEARING AGE

a. Do you have any intrauterine contraceptive device or coil? YES/NO
b. Could you be pregnant? YES/NO

If the patient has answered yes to any questions above then discuss with physician involved in the study or phone the Wellcome MR department on 0161
APPENDIX 2: Response to MHRA regarding serious breach of GCP guidance.

<table>
<thead>
<tr>
<th>Your Name: Dr Stephen Waldek</th>
<th>Your Organisation: Salford Royal NHS Foundation Trust</th>
</tr>
</thead>
<tbody>
<tr>
<td>Your Contact Details: Executive Medical Director and Acting R&amp;D Director, R&amp;D Directorate, Clinical Sciences Building, Salford Royal NHS Foundation Trust, Stott Lane, Salford, M6 8HD</td>
<td>Date Breach Identified by Sponsor: 8th May, 2009</td>
</tr>
</tbody>
</table>

Details of Individual or Organisation committing breach: Research Team and Salford Royal NHS Foundation Trust

Details of related study (e.g. study title, EudraCT No) if applicable:

2005-0003871-19

Please give details of the breach. Where possible, please include your rationale (e.g. patient safety / data integrity issue and relevant legislation if known).

Non-compliance of Pharmacovigilance requirements that could have compromised patient safety should an SAE or SUSAR resulted from the medicinal product (Cinacalcet) concerned.

This clinical trial has been active since 2006. Salford Royal NHS Foundation is the Research Governance Sponsor. The site and master files have been monitored routinely by the Sponsor since its inception. However, the Pharmacy file has never been audited until this year. Previous audits by various R&D staff that have subsequently left the organisation did not review the pharmacy file. During routine monitoring undertaken by the current R&D Lead and R&D Support Officer, the pharmacy file was requested for review. They were expecting to find this in the in-patient pharmacy. This is where all Medicinal Products are supplied and where we can ensure that record keeping and regulatory standards are met. However, they were informed by the Inpatient pharmacy that they had no record of this trial. Upon further investigation, they learnt that the outpatient pharmacy had been employed to administer the Cinacalcet and other standard therapies. The outpatient pharmacy staff were of the impression that this was compassionate use supply of a non-formulary (at that time) drug (Cinacalcet) to support a post-marketing surveillance study of a licensed product being used for its licensed indication. Therefore many of the pharmacovigilance requirements (drug accountability and temperature monitoring of areas storing the drug had been bypassed by the outpatient pharmacy. Had the Pharmacy file been monitored previously this problem would have been identified and action taken immediately to resolve the situation. Neither the current R&D Lead nor the Support Officer had been involved in previous monitoring visits else this would have been identified sooner.

The Chief Investigator (Dr Philip Kalra) believed that all of the prescriptions were marked with the trial code 'C16 calc' and the term 'trial drug' was included on the
prescriptions. However, there was no consistency in the prescriptions indicating that this was indeed a clinical trial of a medicinal product.

We can report that there have been no SUSARS or SAEs reported to the Sponsor that have been related to the trial drug (Cinacalcet) and these have been verified by the Trial’s Independent Data Monitoring Committee. Annual safety reports have been submitted routinely to both Competent Authority and Ethics Committees at appropriate times throughout the trial.

All other documentation in the trial site file is present and in accordance with ICH GCP. The use of Cinacalcet in this study has always been within licensed indication. Patient recruitment ended 10 months ago. The last patient randomised to Cinacalcet received their final dose of Cinacalcet about 4 weeks ago, and hence they are now ‘washed out’ from this product. Only 2 patients remain under follow up, both receiving conventional therapy; the last trial follow up of the final patient is in 6 weeks.

A GCP inspection by the MHRA in November 2007 recommended that the In patient Pharmacy Clinical Trials Unit adopted a clear Green Light Procedure which has been in place and is working well. However, the inception of this trial predates this recommendation and therefore may have contributed towards the “misunderstanding” by Out Patient Pharmacy. There has also been a significant improvement in communication between Pharmacy and the R&D Dept such that all parties are now aware of every single Clinical Trial being undertaken within the Trust.
Please give details of action taken:

As an organisation we are clear as to how this breach of ICH GCP occurred and truly believe that mechanisms have been in place since November 2007 that would prevent this from occurring again.

However, the following action has been taken by the Sponsor:

1. A letter will be sent out from the Executive Medical Director to all Clinicians in the Trust reminding them of the need to use In patient Pharmacy Clinical Trials Facilities for all Clinical Trials of Medicinal Products.
2. In patient pharmacy clinical trials facility to request monitoring visits from Sponsors of all non-commercial clinical trials every 8 weeks or justification as to why these are not required.
3. Lead Pharmacists in Clinical Areas utilising Out Patient Pharmacy to undertake GCP training so that they are aware of Pharmacovigilence requirements and can cascade this down to other Pharmacy colleagues.
4. Green Light Procedures for CTIMPS to be covered in Local Induction Programme for all new Pharmacists employed by Salford Royal NHS Foundation Trust whether or not they are involved in a clinical trial of a medicinal product. These will be signed off by all.
5. Salford Royal NHS Foundation Trust to provide a Generic Prescription template with key information that can be tailored to individual clinical trials whilst retaining information such as identifying that this drug is being used in a clinical trial.
6. Representatives from Pharmacy to be involved in all feasibility meetings for new Clinical Trials being conducted at Salford Royal NHS Foundation Trust.
7. Temperature monitoring is now undertaken routinely in out patient pharmacy.
8. Regular Meetings (once a month) have been taking place prior to this incident between In Patient Pharmacy Clinical Trials Facility and R&D Dept to ensure that effective communication between departments. These are working very well.
9. R&D office to generate a Standard Operating Procedure that can be provided to all staff undertaking Clinical Trials of Medicinal Products that reinforces the requirement for CTIMPs to be stored and dispensed by In Patient Pharmacy Clinical Trials Facility.
10. Sponsor to increase frequency of monitoring visits for trials they are sponsoring to ensure that oversights such as the above are not repeated.