The University of Nottingham

BODY COMPOSITION AND FUNCTION IN CHRONIC KIDNEY DISEASE

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Abstract

Chronic kidney disease (CKD) is a significant public health issue. The uraemic milieu is associated with profound alterations in body composition and function. Therapeutic interventions to preserve renal function and to provide adequate homeostasis to improve outcomes in all stages of chronic kidney disease may promote other unwanted functional adversities which with careful attention to individualised treatment may be modifiable.

The aim of this thesis is to clearly document these disorders of body composition and function and investigate whether commonly practiced interventions can indeed have additional deleterious impact. Our work involved subjects with different levels of CKD and included:

- Antihypertensive therapy and falls in older persons with CKD 3/4.
- Assessment of dynamic bone function in ERF subjects treated with haemodialysis and consequences of phosphate binder medication.
- Distinguishing the dominant cardiac functional abnormalities in ERF subjects treated with haemodialysis and determination of the effects of haemodialysis on carnitine depletion and its functional consequences (skeletal and myocardial).

Key results included:

- Antihypertensive therapy in older subjects with CKD was associated with a reduction in muscle mass over time and reduced overall function but no significant falls risk was noted.
- Commonly utilised measurements to determine bone turnover in ERF subjects treated with haemodialysis do not appear to correlate with dynamic collagen formation rates.
- Dobutamine-atropine stress with non-invasive assessment of cardiac parameters can be used to identify the dominant functional abnormalities that predispose to intradialytic hypotension in ERF subjects.
- Skeletal muscle total carnitine decreases over the first 12 months of dialysis. Change in muscle total carnitine correlated weakly with exercise capacity. Carnitine replacement did not confer any measurable cardiovascular benefit over the first 12 months of dialysis.

Body composition is highly variable over time in CKD. This is seen both in subjects receiving haemodialysis and in pre-dialysis patients. The interplay of these common alterations with the effects of treatments is potentially underestimated but should always be considered in the individualisation of patient care.

Declaration

Except where acknowledged, I declare that this thesis is entirely my own work and is based upon research carried out in the School of Graduate Entry Medicine and Health, University of Nottingham and Department of Renal Medicine, Derby Hospitals NHS Foundation Trust between May 2006 and August 2008.

Paul Owen

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Publications and abstracts arising from this thesis

Peer reviewed publications

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2. McIntyre CW, Harrison LE, Eldehni MT, Jefferies HJ, Szeto CC, John SG, Sigrist MK, Burton JO, Hothi D, Korsheed S, Owen PJ, Lai KB, Li PK. Circulating endotoxemia: a novel factor in systemic inflammation and cardiovascular disease in chronic kidney disease. Clin J Am Soc Nephrol. 2011 Jan;6(1):133-41.

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4. Owen PJ, Priestman WS, McIntyre CW. Bone collagen formation rates do not correlate with measured iPTH levels in chronic male haemodialysis patients. Haemodialysis International 2011. Under review.

Oral presentations

Body composition and functional consequences of goal directed antihypertensive therapy in older people with chronic kidney disease. British Geriatric Society 2009

Poster presentations

Osteotropic Factors and Skeletal Function in Chronic Haemodialysis Patients. PJ Owen, CW McIntrye: J Am Soc Nephrol 17; 2006

Bone collagen formation rates in haemodialysis patients do not correlate with circulating parathyroid hormone levels. PJ Owen, WS Priestman, CW McIntyre: J Am Soc Nephrol: 2007; Nov

Intravenous L-Carnitine supplementation confers no cardiovascular benefit in incident haemodialysis patients. PJ Owen, F Stephens, L Chesterton, WS Priestman, P Greenhaff, CW McIntyre: J Am Soc Nephrol: 2007; Nov

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Circulating Parathyroid hormone levels do not correlate with measured bone collagen formation rate in prevalent male haemodialysis patients. PJ Owen, CW McIntyre. ERA: 2008; May

Baroreflex sensitivity is not reduced by antihypertensive therapy in older people with chronic kidney disease. SG John, PJ Owen, CW McIntyre. ERA: 2008; May

Total muscle carnitine content, exercise capacity and cardiovascular response to intravenous L-carnitine supplementation in incident

haemodialysis patients. PJ Owen, F Stephens, CW McIntyre. ERA: 2008; May

Body composition in older people with chronic kidney disease: response to antihypertensive therapy. PJ Owen, SG John, CW McIntyre. BRS/RA: 2008; May

Removal of carnitine by dialysate in haemodialysis and peritoneal dialysis patients. F Stephens, PJ Owen, CW McIntyre. BRS/RA: 2008; May

1 INTRODUCTION

INTRODUCTION

1.1 Chronic Kidney Disease

Chronic kidney disease (CKD) is a global public health issue. It is defined as the presence of markers of kidney damage for ≥ 3 months, characterised by structural or functional abnormalities of the kidney, with or without a decrease in glomerular filtration rate (GFR). It is manifest by either pathological abnormalities in the composition of blood or urine, or abnormalities in imaging tests. It can also be defined as the presence of a GFR<60ml/min/1.73m^2 for ≥ 3 months, with or without other signs of kidney damage (1).

The current international classification system of the five different stages of CKD, developed by the US National Kidney Foundation in their 'Kidney Disease Outcomes Quality Initiative (K/DOQI) is shown below.

Stage	Description	GFR (ml/min/1.73m^2)
1	Kidney damage with normal or high GFR	≥90
2	Kidney damage and mild decrease in GFR	60 to 89
3	Moderate decrease in GFR	30 to 59
4	Severe decrease in GFR	15 to 29
5	Kidney Failure/Established Renal Failure (ERF)	<15 or on dialysis

Table1. Classification of CKD

A survey of blood samples from patients in the South East of England taken in 2000-2001 found the prevalence of diagnosed CKD to be 5554 per million population (2). Only a minority of subjects with CKD 1 and 2 will go on to develop Established Renal Failure (CKD 5). Of those with CKD 4 only one third will survive to progress to CKD 5 and potentially require dialysis. The number of patients being accepted onto renal replacement programmes is however increasing. Some models suggest acceptance rates may not reach steady states for another 25 years (3).

Patients with CKD 5 consume a disproportionate share of health care resources. In the UK, renal replacement therapies account for over 2% of the total NHS budget. Despite the resources committed and the substantial improvements in the qualities of dialysis therapies, these patients still have significant mortality and morbidity. Survival probabilities for dialysis patients at 1, 2, 5 and 10 years are approximately 80%, 67%, 40% and 18% respectively (4). Timely, cost-effective intervention, to address known risk factors for progression in the earlier stages of CKD is therefore paramount and has been the focus of the development of national treatment and referral guidance to specialist services over recent years.

1.2 Body composition in chronic kidney disease

The uraemic milieu is associated with alterations in body composition and function that result in a wide ranging set of clinical consequences. These sequelae tend to be more significant with an increasing degree of renal impairment.

1.2.1 Muscle

CKD is associated with both muscle wasting and reduced functional capacity (5, 6). The aetiology of these has not been fully elucidated. The potential mechanisms involved are multifaceted and complex. These include decreased physical activity, alterations in substrate delivery and muscle perfusion, poor nutritional status and the presence of a catabolic state, mediated by factors such as metabolic acidosis, insulin resistance and pro-inflammatory cytokines.

In terms of structure, studies of patients with CKD 5 have demonstrated significant atrophy of myofibres, across all fibre types (particularly type II fibres), in both locomotor (7) and non-locomotor muscles (8). Although disuse atrophy in these subjects is probably a factor this evidence supports the contributory role of direct effects of uraemia.

Reduced muscle protein synthesis has been demonstrated in subjects with CKD as compared to non-uraemic controls (9). No data are available on whether or not this is proportional to the severity of uraemia. Metabolic acidosis is known to cause accelerated protein degradation in children (10), adults (11) and subjects with CKD (12, 13). Metabolic acidosis is an inevitable consequence of renal failure (14). The

correction of metabolic acidosis has been shown in clinical trials to decrease protein breakdown, highlighting why maintenance of a normal serum bicarbonate level should be part of routine clinical care. The mechanism by which metabolic acidosis causes muscle wasting is by activation of the Ubiquitin-proteasome system and caspase-3 (15, 16). In addition, acidosis modifies hormonal responses, contributing to insulin resistance and increased glucocorticoid production. Both of these processes are involved in the activation of protein degradation (17, 18).

CKD is associated with chronic low grade inflammation. Subjects with CKD exhibit elevated circulating levels of interleukin-6 (IL-6) (19, 20). IL-6 can negatively affect insulin and insulin-like-growth-factor I (IGFI) receptor signalling promoting catabolism and muscle atrophy. The pro-inflammatory cytokine Tumor necrosis factor-α (TNF-α), is also commonly elevated in CKD patients. Activation of TNF-α receptors can potentially promote muscle protein wasting by a number of actions. TNF-α signalling can activate caspase-3 a factor involved in breakdown of myofibrillar proteins (15). TNF-α can also stimulate the activation of the transcription factor NF-kB, thereby increasing transcription of components of the ubiquitin system (21).

Activation of the renin-angiotensin system plays a major role in the progression of renal disease (22). It has also been shown to promote catabolism and skeletal muscle atrophy (23) through its actions on IGFI. This phenomenon may in part be responsible for the reduction in muscle mass associated with congestive cardiac failure in which the renin-angiotensin system is also commonly activated.

There are a myriad of somatic systems that can regulate the anabolic/catabolic balance in skeletal muscle. Renal impairment, its consequences and in some instances its associated therapeutic interventions, can interact with and alter many of these systems critical for the maintenance of muscle mass (24).

1.2.2 Consequences of Dialysis on muscle

Dialysis processes in their own right can also directly contribute to muscle wasting and dysfunction. There is compelling evidence that haemodialysis and the associated depletion of circulating amino acids contribute to net skeletal muscle protein loss (25). Elevated levels of circulating pro-inflammatory cytokines such as IL-6 may increase further in response to haemodialysis (26).

In prevalent ERF patients treated with haemodialysis, the characteristics of cardiovascular disease and progressive impairment of exercise capacity have been linked with reduced serum L-carnitine and secondary depletion of skeletal muscle carnitine. Carnitine is a naturally occurring compound found in meat and dairy products. In health it is also produced in the liver and kidney. The majority is stored within skeletal and cardiac muscle where it has a major role in translocation of fatty acids into mitochondria for β-oxidation and eventually ATP production. Two main mechanisms account for carnitine deficiency in haemodialysis patients. Firstly, the failing kidneys are unable to synthesise carnitine and the body must rely on dietary sources to maintain carnitine stores. The second and more severe is that during dialysis, carnitine is readily filtered and not replaced, causing a rapid fall in plasma carnitine levels.

A recovery in this fall in plasma carnitine is observed several hours post haemodialysis due to a leaching of carnitine from skeletal and perhaps cardiac muscle. This results in a gradual deficiency in muscle stores over time. Improvements in the incidence of skeletal muscle carnitine levels and function have been reported in patients receiving L-carnitine supplementation. Interestingly, relating to myocardial function, L-carnitine supplementation has also been shown to ameliorate cardiac arrhythmias (27) and left ventricular ejection fraction (28) in prevalent haemodialysis patients. More importantly there is also some published evidence that supplementation is associated with a significant reduction in the occurrence of intradialytic hypotension (29, 30) offering a potential solution to this driver of long-term myocardial damage within the haemodialysis population.

Published investigations have been limited by a lack of any data on the natural history of carnitine depletion, by size and scope of techniques applied (particularly with respect to myocardial function) and paucity of data on intramuscular carnitine levels.

1.2.3 Fat

There is considerably less literature regarding changes in fat mass and function in the uraemic setting when compared to the evidence base relating to muscle. In patients with ERF treated by haemodialysis fat mass correlates with increasing age (31). Low baseline percentage body fat and fat loss over time have been independently associated with increased mortality (32).

Much of the available data regarding body fat in CKD was generated through work on the Malnutrition-Inflammation-Atherosclerosis (MIA) complex. These factors often coexist in both pre-dialysis and dialysis dependent subjects with CKD (33, 34, 35). The presence of these factors often predicts poor outcome (33, 34, 36). It is becoming increasingly evident that far from being an inert energy storage facility, fat contributes to the elevated levels of chronic inflammation prevalent in the CKD population via secretion of adipokines (including leptin and adiponectin) and cytokines (TNFα and IL-6) (37).

1.2.4 Bone

Although often considered an inert organ, in health, the skeleton is metabolically active and is constantly remodelling. Its metabolic functions are chiefly as a store for calcium, phosphate and carbonate. Structurally it is critical for locomotion, respiration and protection of internal organs.

Normal bone turnover is preserved by continued remodelling. Old or damaged bone is removed by osteoclastic mediated resorption. This is followed by new bone production mediated by osteoblasts. This coupled bone formation and resorption occurs in discrete compartmentalised units termed Bone structural units (BSU). The total number of BSUs on the bone surface as well as the activity of the osteoblasts and osteoclasts within each, dictate the rate of bone turnover.

The interaction between osteoclasts and osteoblasts is governed by the receptor activator NFkB ligand (RANK-L). This is a transmembrane

protein that is expressed predominantly on the surface of cells from the osteoblast lineage. It interacts with a receptor on osteoclast precursors called RANK. This RANK-L/RANK interaction causes activation, migration and differentiation of the osteoclast lineage to begin the process of resorption. Osteoprotegerin (OPG) is a decoy receptor, also expressed by osteoblasts. It blocks the interaction of RANK-L/RANK, thereby inhibiting osteoclast differentiation and activity (38). This RANKL/RANK/OPG relationship is mediated by various cytokines and systemic factors such as IL-6, IL-1, TNFα, thyroid hormone, PTH and Vitamin D, thus impacting on bone metabolism and remodelling (39).

With progressive renal dysfunction CKD-mineral bone disorder (CKD-MBD) ensues. This is almost universal when patients have ERF and tends to progress as the state of renal failure is prolonged with dialysis therapies. This systemic disorder of mineral and bone metabolism is manifest by a combination of:

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

Renal osteodystrophy is an alteration of bone morphology in patients with CKD. It is one measure of the skeletal component of CKD-MBD that is quantifiable by histomorphometry of bone biopsy (40). It consists of a spectrum of disorders. Patients often have evidence of more than one

specific disorder. For the purpose of simplification it can be divided into four main categories:

1) Osteitis Fibrosa Cystica (OFC)

2) Osteomalacia

3) Adynamic Bone Disease

4) Mixed Osteodystrophy

The exact pathophysiological processes involved in OFC are not yet fully elucidated. However, there are several key mechanisms which play a role. With deteriorating GFR there is an associated reduction in the filtered phosphate load leading to phosphate retention. This in turn results in reduced calcium levels causing an increase in parathyroid hormone (PTH). This is mediated via the calcium sensing receptor which is highly expressed in the parathyroid glands, permitting variations in the serum calcium concentration to be sensed by the parathyroid glands leading to the desired changes in PTH secretion. There is also an increasing failure of the kidney to synthesise 1.25 Dihydroxyvitamin D3 (calcitriol). In part this may be due to a reduction in functioning renal mass. Phosphate retention may adversely affect calcitriol production by influencing the regulation of the enzyme responsible for its production. In addition, experimental studies suggest that the conversion of calcidiol to calcitriol can be diminished by substances retained in renal failure, including uraemic toxins and uric acid (41). These may also contribute to decreased responsiveness to calcitriol by modulating calcitriol receptor synthesis and receptor function. Decreased calcitriol activity leads to reduced intestinal calcium absorption, hypocalcaemia and consequently

an increase in PTH. With reduced levels of calcitriol there is also a reduction in the normal inhibitory action it would have on the parathyroid glands to limit the production of PTH. The subsequent action of PTH on the skeleton causes a release of calcium (and phosphate), thereby restoring plasma calcium levels towards normal. Initially the inhibitory effect of PTH on proximal phosphate reabsorption can lead to a reduction in the fraction of filtered phosphate reabsorbed from the normal proportion of 80-95% to 15%. However, once this limit is attained, phosphate reabsorption cannot be lowered any further. Another factor that appears to contribute to the genesis of secondary hyperparathyroidism in CKD is the development of skeletal resistance to the calcaemic action of PTH. This resistance appears to be due in part to down regulation of PTH receptors, induced by elevated circulating levels of PTH. Calcitriol deficiency and hyperphosphataemia are also thought to play a role. Continued PTH-induced release of phosphate from bone exacerbates hyperphosphataemia, results in bone disease and contributes toward metastatic calcification. The characteristic histological findings at bone biopsy in such patients are of increased number and activity of osteoblasts, expansion of osteoid surfaces and numerous osteoclasts and resorptive surfaces. Distinct tetracycline labels will indicate accelerated bone formation in the absence of a mineralisation defect.

Osteomalacia is characterised by a reduction in bone turnover, the appearance of osteoclasts and osteoblasts and an increase in the amount of unmineralised bone. It can occur as a result of Vitamin D

deficiency. A large number of cases over previous decades now appear to have resulted secondary to aluminium intoxication from treatment with aluminium containing phosphate binder medication, used to prevent hyperphosphataemia in patients with CKD. The use of these medications has now fallen dramatically as has the incidence of this condition in CKD patients.

In adynamic bone disease, bone turnover is markedly reduced. It is characterised histologically by thin osteoid seams that display no active mineralisation, inactive appearing osteoblasts, and decreases in osteoclast number and bone resorptive surfaces. It is the most common form of bone disease found in those patients receiving haemodialysis. Several risk factors have been identified: increasing age, diabetes and increased calcium load. The principle mechanism is thought to be excess suppression of hyperparathyroidism with phosphate binding medication and Vitamin D analogues. Adynamic bone (associated with a low PTH level) with reduced functional status impairs the ability of the skeleton to buffer exogenous calcium load and increases the risk of metastatic calcification. A study of haemodialysis patients in 2004 undergoing assessment of vascular calcification by ultrasound and bone formation rate by histomorphometric biopsy demonstrated that those with lowest bone formation rates and decreased osteoblastic surface had the greatest degree of vascular calcification (42).

1.2.5 Therapeutic stratergies in CKD-MBD

Hyperphosphataemia

The K/DOQI clinical practice guidelines for bone metabolism and disease in CKD (43) recommend target serum phosphate concentrations of:

- 0.87-1.49 mmol/L in patients with a GFR in the range 15-59 mL/min/1.73m^2
- 1.13-1.78 mmol/L in patients with a GFR ≤15 mL/min/1.73m^2

A large percentage (60-70%) of dietary phosphate is absorbed by the gut and in health would normally be excreted by the kidneys. There are two general modalities used to attempt to reverse the hyperphosphataemia of CKD. These are restriction of dietary phosphate intake and administration of agents that bind ingested phosphate in the gut and prevent its absorption.

Phosphate restriction is accomplished by limiting protein intake. This may also have some benefits in earlier phases of CKD as there is evidence to suggest that a low protein diet may reduce their rate of progression of renal impairment. Such approaches need careful monitoring however. Malnutrition is a relatively common finding in patients with advancing renal failure. The aetiology of this is complex but includes reduced appetite as a consequence of increasing uraemia and altered metabolism. A large proportion of dialysed patients have either overt or borderline malnutrition. In a study of nearly 54,000 maintenance haemodialysis patients, low daily protein intake or decrease in its magnitude over time has been shown to be associated with an

increased risk of death (44). Low albumin and other markers of malnutrition, independent of markers of chronic inflammation, are also associated with increased rates of vascular calcification (45).

Ideally, as kidney function deteriorates the net quantity of phosphate absorbed from the GI tract should be proportionally reduced to match the decrease in kidney function (46). Given the limitations to dietary restriction outlined above, oral phosphate binding medications are commonly utilised to minimise phosphate absorption.

Currently oral binders based on calcium (predominantly carbonate or acetate) are the most widely used therapeutic options to cope with hyperphosphataemia in CKD. Calcium is limited by toxicity in a significant proportion of patients (47).

Sevelamer is a mineral free phosphate binder. This drug is a cationic polymer that binds phosphate through ion exchange. It has been shown to be effective in both short and long term studies (48, 49). The reduction in elemental calcium load seen with the use of this agent may be important in reducing the effect of cardiovascular and metastatic calcification (50). Studies in haemodialysis patients have shown that the management of hyperphosphataemia in these subjects with calcium salts is associated with a significantly greater progression of coronary artery calcification score than those treated with sevelamer (51, 52). In subjects new to haemodialysis baseline coronary artery calcification score is a significant predictor of all cause mortality. Treatment with sevelamer was associated with a significant survival benefit as compared to the use of calcium salts (53). A study using a murine

model of CKD-induced vascular calcification has suggested that Sevelamer Carbonate may actually be capable of a reduction in established vascular calcification, reverse CKD-induced trabecular osteopenia and increase bone formation rates (54).

Lanthanum carbonate has significant phosphate binding properties. Multiple studies have shown it to be effective at lowering phosphate levels. In a large multi-centre European study its effect at lowering phosphate was shown to be comparable to that of calcium carbonate but the incidence of hypercalcaemia was markedly lower, 0.4 versus 20.2% (55). In addition, lanthanum carbonate has also been shown to improve the histomorphometric bone biopsy findings when compared to calcium carbonate and may therefore be useful as a means to normalise bone turnover in dialysis patients (56). No significant clinical adverse effects have yet been reported with lanthanum.

Hyperparathyroidism

The medical management of secondary hyperparathyroidism in patients with CKD principally involves control of hyperphosphataemia and suppression of PTH production using active vitamin D analogues and calcimimetics.

The contribution of calcitriol deficiency to the development of secondary hyperparathyroidism constitutes the rationale for the use of active vitamin D analogues. The ability of all available vitamin D analogues to induce hyperphosphataemia and hypercalcaemia and promote vascular calcification in high doses, obviously impacts on their usefulness. Up to

one half of patients with severe hyperparathyroidism show little or no decline in plasma PTH levels with calcitriol. Limiting factors include altered calcium sensitivity of parathyroid cells, larger functioning parathyroid mass and ensuing hypercalcaemia and hyperphosphataemia.

Calcimimetic agents, which increase the sensitivity of the calcium sensing receptor in the parathyroid glands to calcium, are an additional option for reducing PTH levels in more resistant patients. Cinacalcet is currently the only available calcimimetic. It has been shown in large efficacy studies to improve the likelihood of achieving K/DOQI defined clinical targets for patients with CKD related bone disease (57).

1.3 Cardiovascular risk and myocardial dysfunction

The leading cause of mortality and morbidity in subjects with CKD is cardiovascular disease (CVD). Indeed the risk of death due to CVD is much higher than the risk of ultimately requiring renal replacement therapy (58, 59). Subjects with CKD have been shown to have a higher prevalence and incidence of ischemic heart disease (IHD) and heart failure (60, 61). They are also at higher risk of death after an acute myocardial infarction (62, 63). This is very clearly shown in several epidemiological studies researching morbidity and mortality in patients with CKD. In the Cardiovascular Health Study which was carried out in a population over 65 years of age, 26% of subjects with CKD had IHD and 55% had hypertension. This was in contrast to patients without CKD,

3% had IHD and 36% were hypertensive. Furthermore, it was shown that CKD subjects had a CVD event rate of 102 per 1000 patient year in contrast to subjects without CKD (event rate of only 44 per 1000 patient year (64). In a cross sectional study of 175 patients, 27% of patients with creatinine clearance (CrCl) >50 mL/min and 31% of patients with CrCl 25-49 mL/min had left ventricular hypertrophy (LVH). The prevalence of LVH increased to 45% when CrCl dropped below 25mL/min (65). This is in contrast to the 20% prevalence of LVH in an aged-matched general population (66).

Cardiovascular risk is clearly present in the early stages of CKD. In part this is attributable to the presence of more traditional risk factors such as hypertension and diabetes. However, there is an increasing body of evidence to show that CKD alone is an independent risk factor for cardiovascular disease.

Vascular calcification is recognised as a common and functionally important finding in patients with CKD. Patients receiving haemodialysis who have vascular calcification have a 40 fold increase in their relative risk of cardiovascular death compared to those who do not (67). Vascular calcification is rapidly progressive in both the majority of patients receiving dialysis and those with CKD stage 4 (51, 45). Disorders of bone and mineral metabolism have been shown to be associated with cardiovascular mortality. Progressive calcification of the arterial tree is one mechanism by which this occurs. Vascular calcification or ossification, as it might be more accurately termed, is an active process with phenotypic transformation of vascular smooth

muscle cells to an osteoblastic phenotype. This results in active production and mineralisation of bony matrix. This process is modulated by many factors. These include phosphorus, vitamin D and circulating modulators of calcification. Fetuin and matrix gla protein have been reported to be involved in this ossification (68). Elevated serum levels of osteoprotegerin have been described as being associated with both prevalent and progressive aortic calcification in haemodialysis patients (69). Vitamin D deficiency, a common finding in patients with ERF, has been shown to be independently associated with abnormal conduit and capacitive functions of large arteries in stable haemodialysis patients (70).

Patients with ERF are subject to an epidemic of cardiovascular disease with massively elevated rates of cardiovascular morbidity and mortality (71). Amongst the ERF population treated with dialysis, CVD accounts for up to 45% of deaths (72). Within the haemodialysis population, the prevalence of factors such as LVH, coronary artery disease and vascular calcification amongst others, predispose to reduced coronary flow reserve, resulting in myocardial hypoperfusion. This can lead to segmental ischaemia, inducing segmental left ventricular dysfunction (73), a process known as myocardial stunning (74). This recurrent injury is critically dependant on intradialytic hypotension (IDH) and ultrafiltration rate. IDH has been reported in about 30% of dialysis sessions (75, 76, 77). It is associated with high rates of morbidity and mortality (78). The pathophysiology of IDH is multifactorial but involves inadequate compensatory mechanisms to hypovolaemia induced by

ultrafiltration. These include a decrease in venous capacitance, increase in total peripheral resistance and increased cardiac output (75). Failure of any of these mechanisms predisposes patients to IDH. The resultant myocardial stunning is cumulative and leads to irreversible left ventricular dysfunction in ischaemic heart disease. This pattern of injury is common in both adults and children receiving haemodialysis and is associated with a marked increase in mortality. Modifications in the standard dialysis prescription to improve the haemodynamic response have been shown, in the short-term, to ameliorate this cardiac injury.

1.4 Hypertension and blood pressure guidelines

Given the burden of cardiovascular risk within the CKD population, aggressive treatment to reduce risk factors is an essential tenet in the management of CKD at all stages. Effective management of hypertension is a cornerstone of therapy.

The treatment of hypertension provides benefit by both slowing the rate of progression of CKD and reducing cardiovascular risk. The optimal level of blood pressure control is less clear. The Modification of Diet in Renal Disease (MDRD) Study Group demonstrated that the level of proteinuria at baseline significantly modulated the effect of blood pressure lowering such that a lower target blood pressure of ≤125/75mmHg compared to ≤140/90mmHg was associated with a slower rate of GFR decline among patients with >1g/day proteinuria (79). Subsequent data analysis from this study demonstrated significant

correlation between rate of GFR decline and achieved blood pressure prompting suggested target blood pressure levels of <130/80mmHg for subjects with <1g/day of proteinuria and <125/75mmHg for those with >1g/day of proteinuria (80). More recently, the ESCAPE trial group published results from their study of intensified blood pressure control among children with CKD. This demonstrated that randomisation to a lower target blood pressure was associated with a significantly reduced risk of reaching ERF or doubling of serum creatinine (81). A meta-analysis of 11 randomised control trials comparing the efficacy of antihypertensive regimens with or without ACE inhibitors for patients with predominantly non-diabetic renal disease, including data from 1860 subjects, reported the lowest risk for kidney disease progression to be at levels of systolic blood pressure between 110 and 129mmHg with urine protein excretion >1g/day. A potential increase in risk of progression was noted at systolic blood pressure of <110mmHg (82). The results of the Irbesartan Diabetic Nephropathy trial again demonstrated that progressive lowering of systolic blood pressure to 120mmHg was associated with improved patient and renal survival. However, below this threshold, all-cause mortality increased (83). The ACCORD Study reported no additional benefit with respect to cardiovascular end-points among patients with type II diabetes randomised to strict blood pressure control (<120mmHg systolic) or standard control (<140mmHg) but increased treatment related adverse events and demonstrated a greater decline in GFR in the lower target blood pressure group (84). Similarly, secondary analysis of data from the ONTARGET trial demonstrated a

higher cardiovascular mortality in subjects who achieved a systolic blood pressure of <120mmHg than those achieving 120-129mmHg (85). Thus there are potential dangers with excessive blood pressure lowering and caution must be exercised particularly in subjects with evidence of autonomic neuropathy or postural hypotension. Autonomic dysfunction is relatively common in subjects receiving dialysis and those with significant CKD. Short term regulation of blood pressure is largely controlled by appropriate autonomic activity through the baroreflex arc. Activity of this system or baroreflex sensitivity (BRS) is well recognised as a composite marker of the overall integrity of the autonomic nervous system (86). Alterations in BRS are associated with an increased incidence of cardiac arrythmias, falls propensity, intradialytic haemodynamic instability, cardiovascular events and all cause mortality (87, 88, 89).

CKD is prevalent in older people. Greater than 25% of individuals aged above 70 years have CKD 3 or 4. Only a small proportion of these will ever progress to CKD 5. Treatment is directed towards reducing cardiovascular risk factors, in particular hypertension. Advancing age is associated with changes in body composition. These include hydration status, alteration in lean body mass, reduction in muscle function and skeletal changes. As already discussed, these are also features prevalent in the CKD population. With aggressive reduction of blood pressure in these subjects there would be an expected reduction in renal and cardiovascular risk. However, in a group with already well documented cardiovascular dysregulation (90, 91), aggressive reduction

in blood pressure may potentially increase falls risk, resulting in further mortality and morbidity.

Current Renal association guidelines recommend that in patients with CKD, systolic blood pressure should be lowered to <140mmHg with a target range of 120-139mmHg with a diastolic blood pressure <90mmHg. For those subjects with diabetes mellitus or urinary protein losses of >1g/day, systolic blood pressure should be lowered to <130mmHg with a target range of 120-129mmHg and diastolic blood pressure <80mmHg. However, given the evidence from recent data relating to the potential dangers of excessive blood pressure reduction and the potential for exacerbating other features within the CKD population that might predispose to further mortality/morbidity, the emphasis is that antihypertensive treatment should be individualised.

2 HYPOTHESIS, AIMS AND PLAN OF INVESTIGATION

HYPOTHESIS, AIMS & PLAN OF INVESTIGATION

This thesis has been planned to test the following hypothesis:

Chronic kidney disease (CKD) is associated with profound alterations in body composition. These have far reaching consequences at cellular and systemic level and can adversely effect overall patient function across the entire range of CKD. We hypothesise that therapeutic interventions commonly utilised in this population may compound these functional anomalies.

Research questions

To test this hypothesis, the following research questions will be addressed:

1. Does goal directed antihypertensive therapy in older subjects with predialysis CKD result in reduced functional status, significant disturbances in body composition and increased falls risk?

2. Do serum intact parathyroid hormone assays reflect dynamic measurements of bone collagen formation rates in prevalent male haemodialysis patients?

3. What is the effect of calcium containing and non-calcium containing phosphate binder medication on dynamic

measurements of bone collagen formation and expression of osteotropic factors in prevalent male haemodialysis patients?

4. Does non-invasive measurement of cardiovascular parameters in prevalent haemodialysis patients undergoing pharmacological stress permit identification of the dominant functional abnormalities that might predispose to intradialytic hypotension?

5. How does haemodialysis impact on skeletal muscle total carnitine depletion over the first 12 months of treatment in incident haemodialysis patients?

6. Is this carnitine deficiency associated with a reduction in exercise tolerance?

7. Does this carnitine deficiency result in myocardial dysfunction?

8. Does carnitine supplementation impact on exercise tolerance or cardiac functional status?

Plan of investigation

4 separate investigational studies were designed with the aim of answering the above questions.

- Body composition, cardiovascular and functional consequences of chronic kidney disease in older people.
- Bone collagen formation rates and expression of osteotropic factors in male CKD 5 subjects undergoing haemodialysis

- Myocardial contractile function and intradialytic hypotension
- Prospective study of the determinants of secondary carnitine depletion and the skeletal and cardiac functional effects of L-carnitine supplementation in incident chronic haemodialysis patients

3 METHODS

METHODS

Methodology is described in detail in this chapter and then referred back to for reference in the relevant results sections. Certain methods and techniques were used in more than one study.

3.1 Measurement of Body Composition

BIA

Bioelectrical impedance analysis was performed using the InBody S20 (*Biospace Ltd*, Korea) mutlichannel, multisegmental analyser.

This provided data on intracellular/extracellular fluid ratios, skeletal muscle, bone and fat mass for the whole body, and also segmentally for trunk and each limb.

Initially we requested that subjects remove all metal jewelry as this has the potential to affect impedance measurement. Subjects were then required to lie in a 30° supine position (as recommended by the manufacturer). After cleaning the skin to ensure good contact, adhesive pads provided by the manufacturer were attached to the palmar aspect of the terminal phalanx of the middle fingers and thumbs on both hands, and to the lateral malleolus and 2^{nd} metatarso-phalangeal joints of both feet. The placement of the adhesive pads was standardised in each subject to ensure good reproducibility of the measurements. Electrodes in the form of crocodile clips were then connected to the BIA unit via the adhesive pads. Demographics such as age, gender, height and weight were entered as requested prior to measurement. Race specific option

was also available and could have been used for subjects of Afro-Caribbean and Asian origin. During the measurement period, subjects were requested to lie still. An undetectable alternating current was then passed through the subject's body. The analyser then transformed the electrical data mathematically to specific body composition outputs.

Once subjects had been connected to the electrodes the technique was fully automatic. Intra-observer variability was therefore reduced as a consequence. The manufacturers claim a measurement error of 1% with reproducibility of 99%. It has been successfully validated against the 'gold-standard' of deuterium dilution for total body water (TBW) assessment (92), and has been used as the reference in published research on TBW equation (93). It has also been validated against DEXA for total and regional body composition in healthy subjects (94) and peritoneal dialysis patients (95).

DEXA

DEXA was performed using available equipment in the University of Nottingham at Derby (Lunar Prodigy; GE Healthcare, Slough, UK). Scans were performed by Mrs Margaret Baker, clinical assistant, who had received appropriate radiation protection training to operate the scanner.

Prior to scanning subjects were asked to empty their bladder. They were asked to remove heavy clothing and wear an examination gown. Full body DEXA was then performed with the subject lying supine and specific body composition measurements generated by the scanners

inbuilt software. Body composition data were then manually downloaded by the investigator.

Servicing and calibration are managed by the University. DEXA is a clinical procedure, and thus has excellent reproducibility.

Computed Tomography

Muscle and fat Cross-sectional area were simultaneously assessed by a multi- slice spiral CT scan. Studies were performed using a clinical scanner at Royal Derby Hospital (GE Medical Systems Lightspeed 16 multi-slice spiral CT scanner). Scans were performed by trained radiographers from the department of Radiology. Scans were taken through a standardised section of the right thigh, 20cm above the tibial plateau, 5 cm in length, imaged in 2.5mm slices, with care taken to ensure that none of the slices overlapped. This did not require either intravenous or oral contrast.

Muscle and fat CSA were then measured by the investigator using GE Medical Systems Advantage Workstation software with appropriate windowing for tissue density (fat and non-contractile connective tissue -200-0 Hounsfield Units, muscle 0-200 Hounsfield units.) CSA was measured at the top, middle and bottom slices of this cylinder of thigh and the mean of these three values then adjusted for height .This technique has been used previously in our group (96).

This technique also permits assessment of vascular calcification within the segment of superficial femoral artery found within the scanned field.

3.2 Functional assessments

TUG test

Simple assessment of patients' functional status was undertaken. We used the "Timed get-Up and Go" (TUG) test (97). This involved the patient rising from a chair (of a standard height), walking 3 metres, turning and returning to sitting. The movement was explained and demonstrated and the patient habituated to the procedure. Patients were then timed whilst performing this procedure once. This technique has been extensively used in falls research, and is well validated against more detailed assessments.

Shuttle walking test

Subjects were required to attend one week prior to commencing and at 6 and 12 months after initiation of haemodialysis therapy, at the same time of day on each visit, to perform an incremental exercise tolerance test to volitional exhaustion. The exercise test was a standardised incremental shuttle walking test originally designed to provoke a symptom limited maximal performance in chronic obstructive pulmonary disease patients in order to provide objective measurement of disability (98). The test required the patients to walk back and forth along a flat 10 metre course, set out in a quiet corridor of the Royal Derby Hospital Medical School, at a gradually increasing speed, paced by audio signals (bleeps), until they were exhausted and/or could no longer keep up with the bleeps. Total distance walked was taken as a measure of aerobic exercise capacity,

as performance on the test relates strongly to VO_2max, the traditional indicator of aerobic capacity (99).

Isometric handgrip strength

This assessment was carried out on the same visit as the incremental shuttle walking test. Isometric handgrip strength was measured using a dynamometer devised by Bassey et al. (100). Patients were asked to squeeze a gripping bar with their dominant hand as hard as possible for ~3 s. Each patient made three attempts with a rest of at least 1 min between attempts. The highest recording was taken as the measurement of handgrip strength. Verbal encouragement was given to each patient throughout.

3.3 Tissue sampling

Bone biopsy

To assess fractional synthetic rate of collagen within bone in our male chronic haemodialysis population we performed bone biopsy. This was carried out by the investigator following appropriate training. Subjects were fasted from 8 p.m. the day before the study to reduce any effect of dietary protein on collagen formation rate. Approximately 120 minutes prior to the expected bone biopsy, basal blood samples and a skin biopsy from the groin crease for basal 13-Carbon levels (following infiltration of local anaesthetic) were taken. Following this 0.75g of [^{13}C] Proline (99 Atoms %) and 3 g of unlabelled Proline were administered

over 5 min through an intravenous cannula. Blood samples were taken (using a second cannula) at 5 min intervals for 15 minutes and then every 15 minutes until the bone biopsy was taken.

At 120 minutes a bone biopsy from beside the tibial tuberosity was taken. This was performed under local anaesthetic (lidocaine 1%). A small 1cm incision was made in the skin over the shin, and then the biopsy taken using a standard bone biopsy tool (Bone Tenmo, Allegiance Medical, UK). The skin was then closed with sutures. These were removed one week later.

The second bone biopsy was carried out according to the above outline. The only difference being that 0.75g of [^{15}N] proline (99Atoms %) and 3g of unlabelled proline was administered in place of [^{13}C] proline as an alternative marker.

The bone, skin, and blood samples were coded and then frozen at -80 degrees and stored until analysis for collagen and gene expression was carried out by the investigator in conjunction with laboratory staff located within the School of Graduate Entry Medicine, Derby.

Muscle biopsy

Muscle biopsies were performed one week prior to starting haemodialysis and at 6 and 12 months post initiation of therapy by the investigator having had appropriate training in the technique described by Bergstrom (101). Subjects who were anticoagulated, had abnormal clotting times or thrombocytopenia were excluded. Subjects were

requested to lie supine. The vastus lateralis biopsy site, at a point 25cm proximal from the tibial tuberosity and 5cm lateral from the midline of the femoral course was prepared with chlorhexidine solution. Skin and subcutaneous tissues were infiltrated with 5-10ml of 1% lidocaine solution. After ensuring sufficient analgesia, a stab wound was made in the lateral thigh at the biopsy site, directly through the overlying skin, subcutaneous fat and fascia lata. A 6mm Bergstrom biopsy needle was then inserted through the fascia and advanced to ensure that the cutting chamber window was lying fully within muscle. An assistant then applied suction to the biopsy needle to improve biopsy yield. The biopsy was then taken. Samples were snap frozen in liquid nitrogen. The process was repeated up to a maximum of three passes to obtain sufficient muscle for analysis. The wound was sutured and a dry dressing applied. Sutures were removed by the investigator 7 days later. Muscle samples were processed and analysed by Dr Francis Stephens and laboratory staff from the Centre for Integrated Systems Biology, University of Nottingham, for relative carnitine content. Results were analysed by the investigator.

3.4 Haemodynamic data collection

Finometer

Continuous measurement of haemodynamic variables during haemodialysis sessions was performed using the Finometer.

The Finometer (Finapres Medical Systems, Arnhem, Netherlands) is a tool for blood pressure and haemodynamic monitoring. The advantage

of the Finometer is that it is accurate and robust and provides continuous measurement of multiple cardiovascular variables in one device. As a result, it is a versatile, non-invasive monitoring system that measures multiple haemodynamic variables on a beat-to-beat basis. The accompanying BeatScope® software allows online monitoring, control, storage and offline review of all the data on a PC.

The Finometer is particularly useful due to its non-invasive nature and ability to provide continuous readings over a period of several hours. The Finometer works by continuous pulse-wave analysis at the digital artery and utilises the finger-clamp method, in which changes in digital arterial diameter are detected by means of an infrared photoplethysmograph (102) and opposed by an ultra-fast pressure servo controller that changes pressure in an inflatable air bladder, both mounted in a finger cuff. This generates an arterial waveform that is measured on a beat-to-beat basis and is used to reconstruct a central aortic waveform (103). This allows calculation of a full range of haemodynamic variables on a continuous basis; these include pulse rate (HR), blood pressure (BP), stroke volume (SV), cardiac output (CO) and peripheral resistance (TPR). All data are subsequently downloaded to the PC based analysis program, allowing averaging of results over defined time periods. This technology provides unprecedented resolution of changes in the critical cardiovascular variables. Previous work has validated the Finometer against invasive haemodynamic measurements in normal individuals, unstable intensive care patients and in cardiac surgery patients, a proportion of whom had vascular calcification (103,

104, 105). This has shown the Finometer to be accurate in tracking relative change. Data are therefore presented as percentage change from baseline except for BP, which is calibrated against brachial readings using a return to flow method and absolute values can therefore be used (106).

3.5 Haematological and Biochemical parameters.

All blood specimens were sampled by the investigator. All routine haematological and biochemical assessments were performed by staff within the department of Haematology and Biochemistry at Royal Derby Hospital unless otherwise stated.

4 CHANGES IN BODY COMPOSITION AND FUNCTIONAL CONSEQUENCES OF GOAL DIRECTED ANTIHYPERTENSIVE TREATMENT IN OLDER SUBJECTS WITH CKD

4.1 Introduction

Significant CKD affects approximately 5% of the UK population. The prevalence of CKD rises dramatically with age. Based on the results of the National Health and Nutrition Examination Survey 1999-2004, more than one third of those aged 70 years or more have CKD 3 or higher (107). Older patients are less likely than younger subjects with similar levels of eGFR to progress to ERF and require renal replacement therapy (108, 109). The majority of patients in this age group will maintain relatively stable reduced renal function or die from competing mortality, in particular cardiovascular disease (108-11).

CKD is associated with a wide ranging set of clinical consequences. There is an increasing evidence base between these sequelae and drivers for increasing cardiovascular pathology such as vascular calcification (45,112-3); malnutrition, inflammation and atherosclerosis complex (MIA) (34,114); alteration in adipokine profiles (115) and increasing blood pressure dysregulation (45,112). Disturbances in skeletal metabolism and function with increased fracture potential are common (116,117). CKD is associated with both muscle wasting (5, 6) and reduced functional capacity (118) that seem to progress with the degree of renal impairment. This has previously been demonstrated in a study by McIntyre et al (2006), in which subjects with dialysis treated CKD 5 exhibited more functionally significant muscle wasting than patients with CKD 4 (96).

The process of aging itself is associated with a well defined set of changes in body composition. Throughout life human muscle undergoes constant changes. However after the age of 50 years, muscle mass decreases at an annual rate of around 1-2%. Muscle strength declines by 1.5% between ages 50 and 60 and by 3% thereafter (119). Degenerative bone disease and osteoporosis are increasingly common with advancing age and associated with increasing mobility problems and fracture risk.

The major management challenges in older CKD subjects relate to reduction in cardiovascular risk factors. Crucially, the cornerstone of management is aggressive treatment of hypertension. Blood pressure control has been shown to be problematic for persons aged ≥ 70 years and in individuals with CKD. Prevalence rates of hypertension in older subjects range between 60 to 80% (120). Several large studies looking at blood pressure reduction, specifically in older subjects, have demonstrated an associated reduction in death rate from stroke and cardiovascular disease with improved blood pressure control (121, 122). It has been demonstrated in several large trials that blood pressure lowering has a more significant effect on renal progression in subjects with significant proteinuria. In this setting the use of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists have more relevance. However, most trials investigating the potential benefits of these drugs did not include subjects above the age of 70 (123). This is an important issue given that older subjects are more likely to have adverse side effects from therapy, such as hyperkalaemia or

acute kidney injury. Age-related declines in baroreflex sensitivity to hypotensive stimuli, manifest by failure to increase heart rate when blood pressure falls and reduced basal renin and aldosterone levels potentially promoting the development of volume depletion are some examples of factors that could be aggravated by antihypertensive medications in this group of subjects with an already well recognised characteristic cardiovascular dysregulation.

The National Service Framework for Older People reported that hip fractures associated with falls resulted in an annual NHS financial cost of £1.7 billion. There is a paucity of evidence relating to the potential effects of goal directed antihypertensive treatment in this group in relation to body composition, function and falls risk.

This investigational study was carried out jointly with Dr Stephen John who will use data relating to measurement of cardiovascular parameters, not documented here, at the same investigational study visits, as part of another thesis. The body composition, functional assessment, falls data and blood pressure response are presented here.

4.2 Aims

We hypothesised that pursuit of an optimal antihypertensive strategy in the older patient with CKD may result in an increased propensity to falls and be associated with clinically important changes in body composition, cardiovascular function and overall functional status.

The principle aims of this study were:

- To observe effects on body composition and functional assessment from baseline following antihypertensive withdrawal and at 4 weeks and 12 months following blood pressure (BP) optimisation in a group of hypertensive subjects aged >70 years with CKD 3, CKD 4 and aged matched non CKD controls.
- To document falls rate in this group of subjects over a 12 month period following blood pressure optimisation.

4.3 Methods

4.3.1 Subjects

We recruited volunteers from outpatient clinics at the Royal Derby Hospital and from GP practices in the Southern Derbyshire and Derby City regions who were hypertensive (BP> 130/80), aged ≥70 years with either CKD 3, CKD 4 or normal renal function (eGFR>60mls/min for purposes of this study). Patients were excluded from the study if they met any of the following criteria:

- Use of more than 3 drugs for blood pressure control at recruitment
- Likely to develop CKD 5 within one year
- Poor mobility precluding completion of assessment
- Diabetes
- Abbreviated mental test score <8
- Recent acute illness (within 3 months)
- Ischaemic heart disease requiring beta-blockade
- Involvement in another clinical trial within 3 months

- Attending day hospital or attending falls reduction services
- Residents in nursing or residential homes
- Renovascular disease precluding ACE-inhibitor or Angiotensin Receptor blocker usage
- BP > 160/90 prior to antihypertensive withdrawal
- Malignant hypertension
- Severe peripheral vascular disease or significant valvular heart disease
- Heart failure (NYHA III/IV) or other cause to prevent diuretic withdrawal
- Atrial fibrillation or other significant arrhythmia
- Currently taking antihypertensive medication for which MHRA approval (CTA) has not been granted
- Obstructive uropathy

The study was approved by the Southern Derbyshire Local Research Ethics Committee with clinical trial approval (CTA) from the MHRA. Before taking part in the study, all subjects gave their written informed consent to participate and were aware that they were free to withdraw at any point.

4.3.2 Experimental Protocol

Figure1 summarises the study schedule. After identifying potential participants and providing them with information about the study they were invited for a recruitment visit one week later. At this stage informed consent was obtained. An appropriate medical history was taken and clinical examination performed which included BP measurement. Providing that subjects met the above criteria they were given a plan to reduce and stop antihypertensive medication during a 4 week washout period. They were given an appropriately calibrated, serviced and approved oscillometric device to perform daily home BP measurements. They were reviewed at two weeks to ensure that they had not developed any significant problems. If systolic blood pressure (SBP) was greater than 180mmHg or diastolic blood pressure (DBP) greater than 110mmHg they were withdrawn from the study and antihypertensive medications were restarted. Following the washout period subjects attended for their first investigational visit. BP was measured at rest. Subjects provided a urine sample for quatification of urinary protein to creatinine ratio. Blood samples were obtained for measurement of FBC, U&E, bone profile, albumin, CRP, PTH and Vit D. Body composition was assessed using whole body dual energy x-ray absorptiometry (DEXA), whole body bioelectrical impedance analysis (BIA) and thigh fat and muscle surface area were assessed using multi-slice spiral CT scanning. Finally a "timed up and go" (TUG) test was performed as a marker of functional state.

Subjects were then given a plan to restart antihypertensive therapy with daily home BP monitoring aiming for a target blood pressure ≤130/80.

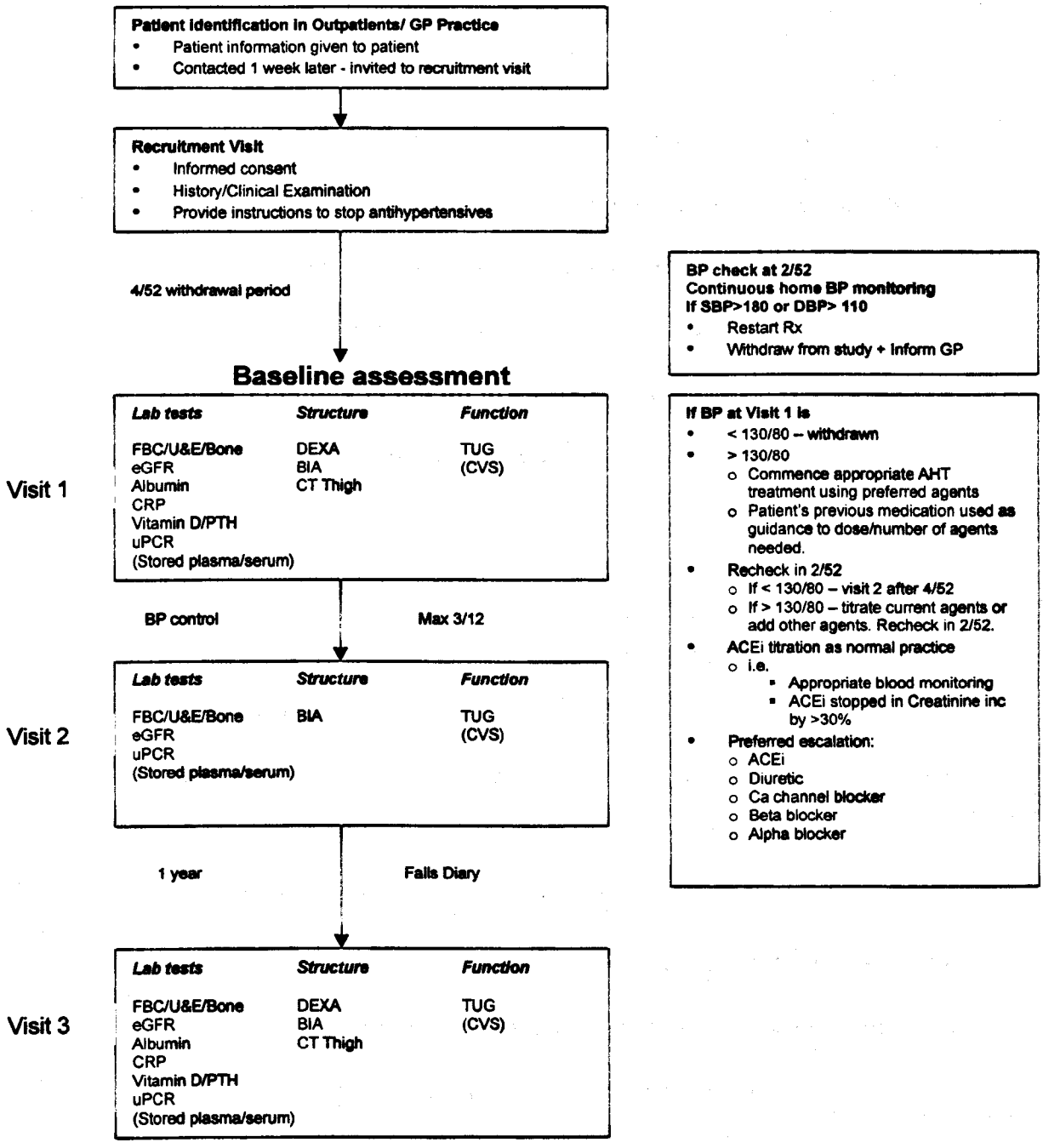


Figure 1. Study plan

They were reviewed every 14 days to assess response and medications adjusted accordingly. Having achieved target BP subjects continued their antihypertensive regimen for 4 weeks before returning for their second investigational study visit. The tests undertaken at this point are listed in Figure 1.

Subsequently subjects' medications were left unchanged. Their GP was instructed to make any particular adjustments as necessary based on clinical need. Subjects were asked to complete and return a falls diary each month for the next 12 months.

After 12 months subjects were invited back to repeat the series of tests carried out at their initial investigational visit.

4.3.3 Blood Pressure

The study involved both removal of antihypertensives and later reintroduction to best practice levels of blood pressure control (≤130/80).

1. **Measurement**

a. Measurement was performed with an appropriately calibrated, serviced and approved oscillometric device.

b. At recruitment visit BP was be measured in both arms. The arm with the higher reading was used for all subsequent measurements.

c. At all visits 3 measurements were performed, at least 2 minutes apart. The mean value was used for further analysis.

d. Postural BP was also recorded at all visits. A single set of measurements were taken at sitting (0), 1, and 3 minutes (124). If there was a postural drop of > 20/10mmHg, antihypertensive agents were reduced and the patient's GP informed.

e. Other aspects of measurement were performed to current best practice guidance.

2. Washout

a. BP increased during this period. The risk to the patient from this was low. Subjects monitored their BP daily at home, and had their BP checked at hospital after 2 weeks. If SBP was > 180 or DBP > 110, their antihypertensives were restarted and were withdrawn from the study and their GP informed.

b. The rise in BP during washout and the speed that agents were changed related to the number of antihypertensive agents that a patient was originally taking.

c. Patients taking 4 or more agents have difficult to manage hypertension, and it was felt would have a higher risk of developing severe uncontrolled hypertension on withdrawal.

d. We therefore limited this study to patients taking a maximum of 3 antihypertensive agents.

e. Antihypertensive agents were stopped singularly, with a period of 48 hours between agents. Beta-blockers were stopped gradually, by halving the dose, where practical, every 5 days.

3. Reintroduction

a. Patients were taking at least one antihypertensive agent prior to washout.

b. Reintroduction commenced at visit 1.

c. We returned BP to a level of control at least equivalent to baseline. Ideally, this will be ≤130/80.

d. If BP became <110/60 after an increase in treatment, the increase was reversed.

e. We used the known list of medications that individual subjects were taking at recruitment to guide reintroduction.

f. Similar to withdrawal, patients on multiple agents had them reintroduced at 48 hour intervals.

g. We aimed to use agents using best practice guidelines (125, 126).

h. BP was monitored at a hospital visit every 2 weeks during reintroduction.

i. A period of one month on stable medication was required before visit 2.

j. We checked U+E at initiation, one week and one month after dose titration of ACEi/ARB, where patients were naïve to these agents.

k. If we needed to introduce an ACEi, our preferred agent was ramipril.

4.3.4 Body composition

Whole body DEXA, BIA and thigh computed tomography was performed following antihypertensive withdrawal and at 12 months post blood pressure optimisation (visit 1 and visit 3). BIA assessment of body composition was also repeated at visit 2 post blood pressure optimisation.

Detailed descriptions of the specific methods used are found in chapter 3.

4.3.5 *Functional assessment*

To assess general function a timed get up and go test (TUG) was performed at each investigational visit. A detailed description of this test is found in chapter 3 (97).

4.3.6 *Falls Diary*

In keeping with the 2005 consensus statement on falls research (127), the definition of a fall in this study was "an unexpected event in which the participants come to rest on the ground, floor, or lower level".

Any history of falls over the sixth months prior to recruitment was documented at the initial assessment. To capture all falls subjects were asked to complete a prospective daily falls diary once established at target blood pressure. These were collected on a monthly basis for a twelve month period. Participants were given the diaries and asked to enter events that included a slip or trip resulting in a fall as well as other falls events as recommended in the consensus statement.

4.3.7 *Laboratory Tests*

Analysis of plasma and serum for FBC and routine biochemistry was carried out by staff within the Haematology and Biochemistry Laboratories at Royal Derby Hospital.

4.3.8 Statistical analysis

Data were analysed using GraphPad Prism 4.02 (GraphPad Software Inc, CA). Data were checked for normal distribution and unless otherwise stated were expressed as mean +/- standard deviation. Unpaired student's t-test was used to compare data between groups and paired t-test to compare data over time. Pearson's correlation was used to compare assessment methods and Bland-Altmann plots generated.

4.4 Results

Patients

60 subjects attended for an assessment visit. All of these were initially entered into the antihypertensive withdrawal phase. Unfortunately 6 subjects had to be withdrawn from the study as their blood pressure recordings during this phase exceeded our previously stated limits for safety.

	Non-CKD (n=25)	CKD (n=29)	P value
Age (years)	76.1	75.7	0.74
Male: Female ratio	1.25: 1	1.25: 1	0.95
Number of antihypertensive drugs	1.72	1.76	0.85
Percentage with history of fall within last 6 months	22%	17%	0.06
eGFR mls/min	87.7	42.9	<0.0001
Urine Protein: Creatinine Ratio (mg/mg)	0.12	0.31	0.03
Haemoglobin (g/dl)	13.9	12.7	0.008
SBP (mmHg)	152.8	153.0	0.97
DBP (mmHg)	80.8	79.6	0.65
Weight (Kg)	75.8	73.9	0.62
BMI (Kg/m^2)	27.2	26.7	0.61

Table 2. Basic demographic data for subjects following withdrawal of antihypertensive agents

Table 2 demonstrates demographic features and some baseline parameters of the remaining 54 subjects at their first investigational visit following withdrawal of antihypertensive therapy. Data are expressed as mean values unless otherwise stated.

There were no significant differences between groups in terms of age, male to female ratio, previous antihypertensive load, previous falls history, blood pressure recordings, weight and BMI. As would be expected the CKD group demonstrated higher urinary protein loss and mildly reduced haemoglobin.

All 54 subjects subsequently went on to have antihypertensive therapy reintroduced to achieve target blood pressure and repeat assessment at visit 2. Unfortunately during the 12 month period before visit 3 one non-CKD subject died. Two CKD subjects were unable to attend for their final investigational visit due to spousal ill health but they did return a complete set of falls diaries.

Blood Pressure

At assessment, mean average recorded SBP and DBP for the 54 subjects who successfully completed the withdrawal phase was 142mmHg and 75mmHg respectively with a mean number of 1.7 antihypertensive agents. On withdrawal of these drugs, values rose to 153mmHg and 80mmHg for SBP and DBP.

Drug Class	Non-CKD (% use)	CKD (%use)	P value
ACEi / ARB	50	67	0.227
Alpha Blocker	21	19	0.835
Beta Blocker	33	19	0.226
Calcium Channel Antagonist	38	41	0.813
Diuretic	42	33	0.539

Table 3. Percentage use drug class by group

		Assessment Visit	V1 (Withdrawal)	V2 (Treated to target)	V3 (12 months)
All participants	SBP mmHg (standard deviation)	142 (12)	153 (19)	129 (14)	131 (15)
	DBP mmHg (standard deviation)	75 (8.6)	80 (10)	69 (8)	69 (7)
	Number of AHT agents	1.7	0	1.8	1.7
CKD	SBP mmHg (standard deviation)	139 (13)	153 (21)	130 (16)	129 (16)
	DBP mmHg (standard deviation)	74 (9)	80 (10)	69 (9)	68 (7)
	Number of AHT agents	1.8	0	1.8	1.7
Non-CKD	SBP mmHg (standard deviation)	146 (10)	153 (16)	129 (12)	134 (13)
	DBP mmHg (standard deviation)	76 (8)	81 (10)	69 (7)	71 (6)
	Number of AHT agents	1.7	0	1.9	1.6

Table 4. Mean SBP, DBP and number of antihypertensive agents across study period

Table3 illustrates the percentage use of specific drug class at reintroduction. There were no significant differences in drug class usage between groups.

Following careful reintroduction of medications to achieve target BP of ≤130/80 or at least equivalent of initial control, mean SBP and DBP recordings were both significantly lower than at the initial assessment visit. Values were 129mmHg ($p<0.0001$) and 69mmHg ($p=0.0002$) respectively. The antihypertensive drug load was marginally higher at a mean of 1.8 drugs (range 1-3) but this was not statistically significant. This data is summarised in Table 4 along with a breakdown of response based on grouping according to renal status.

There were no adverse events reported during the antihypertensive reintroduction phase or over the 4 week period once established at optimal blood pressure.

Over the next twelve months no routine checks of BP or adjustments to medication were initiated by the study group. Participants remained under the care of their general practitioner and had any appropriate health checks and medication reviews as per normal practice. Blood pressure readings taken at visit 3, 12 months after visit 2, showed that the initial improvement to overall blood pressure control during the initial stages of the study appeared to have been maintained (mean SBP 131mmHg, mean DBP 69mmHg). There was no significant difference between recordings at these two time points.

Renal Parameters

		V1	V2	V3
CKD	Number of subjects	29	29	27
	Creatinine (SD) µmol/L	144 (57)	150 (61)	155 (57)
	eGFR (SD) mls/min	43 (14)	42 (14)	41 (15)
	uPCR (SD) mg/mg	0.31 (0.41)	0.30 (0.47)	0.26 (0.56)
Non-CKD	Number of subjects	25	25	24
	Creatinine (SD) µmol/L	72 (19)	73 (17)	80 (20)
	eGFR (SD) mls/min	88 (17)	86 (15)	80 (14) *
	uPCR (SD) mg/mg	0.12 (0.1)	0.11 (0.1)	0.11 (0.1)

* reduction eGFR V2 to V3 p=0.01

Table 5. Creatinine, eGFR and uPCR expressed as mean over study period

Values for serum creatinine, eGFR and uPCR for the study period are shown in table 5. Over the course of the study there were no significant changes in these parameters within the CKD group. However, within the Non-CKD group serum creatinine increased resulting in a significant decline in eGFR (p=0.01) over the 12 month between blood pressure optimisation and visit 3. Urinary protein losses were unchanged. The relative reduction in eGFR over this time period would be in excess of that expected due to renal senescence.

Muscle, Bone and Fat Mass

BMI did not change significantly within or between groups over the study period. Table 6 demonstrates measured Muscle, Fat and Bone mass over the study period by both BIA and DEXA.

Within the CKD group muscle mass decreased significantly between V1 and V3 as measured by both BIA and DEXA ($p=0.007$ and 0.02 respectively). This decline could equate to either a mean percentage reduction of 3% or 0.4% depending on the technique used (BIA/DEXA respectively). BIA assessment of muscle mass also demonstrated an average reduction of 2% within the Non-CKD group between V1 and V3 ($p=0.015$). This was not confirmed by DEXA which demonstrated no significant change.

There were no significant changes in body fat mass demonstrated by either technique over the study period.

Bone mass as measured by BIA reduced in both groups between V1 and V3. This was not confirmed by DEXA measurements. These suggested no significant change over time. However, dividing the total group by sex, both males and females demonstrated a small reduction in bone mass between V2 and V3. With BIA in men, bone mass reduced by a mean of 122g and in females by 78g, with DEXA male bone mass reduced by 110g and 28g in females. This only reached significance in the BIA measurement for males ($p=0.02$).

There were no significant differences between measurements of muscle, fat or bone mass between V1 and V2 on BIA measurements.

MUSCLE MASS		V1	V2	V3
CKD	BIA grams (SD)	46374 (10194)	45714 (9798)	44806* (10199)
	DEXA grams (SD)	44240 (8641)		44058♦ (8558)
Non-CKD	BIA grams (SD)	46452 (10037)	45106 (9226)	45420° (9622)
	DEXA grams (SD)	44760 (9666)		45213 (8925)
FAT MASS		V1	V2	V3
CKD	BIA grams (SD)	24466 (7868)	23928 (7503)	25200 (9121)
	DEXA grams (SD)	25530 (7762)		25358 (8632)
Non-CKD	BIA grams (SD)	26448 (9368)	26244 (9508)	27395 (9241)
	DEXA	26622 (8880)		26381 (8832)
BONE MASS		V1	V2	V3
CKD	BIA grams (SD)	2894 (576)	2855 (561)	2787 (572)
	DEXA grams (SD)	2699 (653)		2722 (675)
Non-CKD	BIA grams (SD)	2905 (568)	2838 (486)	2845 (536)
	DEXA grams (SD)	2600 (603)		2475 (746)

* P=0.007, ♦ P=0.02, ° P=0.015

Table 6. Muscle, Fat and Bone mass expressed as mean value in grams (SD) by BIA / DEXA over study period

Right thigh muscle cross-sectional area measurements corrected for height to give an approximation of right thigh muscle mass (96) demonstrated no significant change between V1 and V3 as did regional DEXA. When compared to regional DEXA measurement of right lower

limb CT tended to give consistently lower estimates ($r=0.7$, $r^2=0.5$, $p<0.0001$).

Assessment of right thigh fat mass using CT cross-sectional area with height correction showed no significant change from V1 to V3 again in agreement with DEXA assessment. In the assessment of fat mass CT gave consistently higher estimates than DEXA ($r=0.78$, $r^2=0.6$, $p<0.0001$).

Comparison of DEXA and BIA assessment of body composition

Direct correlation of measurements of muscle, bone and fat mass, taken at baseline, between DEXA and BIA derived data are illustrated in Figure 2 (r^2 values of 0.88, 0.68 and 0.82 for muscle, bone and fat respectively). Compared with DEXA, BIA demonstrated wide limits of agreement.

In assessment of muscle and bone mass, BIA tended to provide higher values than DEXA. This difference appeared to increase with increasing subject weight (Figure 3).

With regard to assessment of fat mass, BIA did not appear to demonstrate any specific systematic bias through the range of weights for the study population (Figure 3).

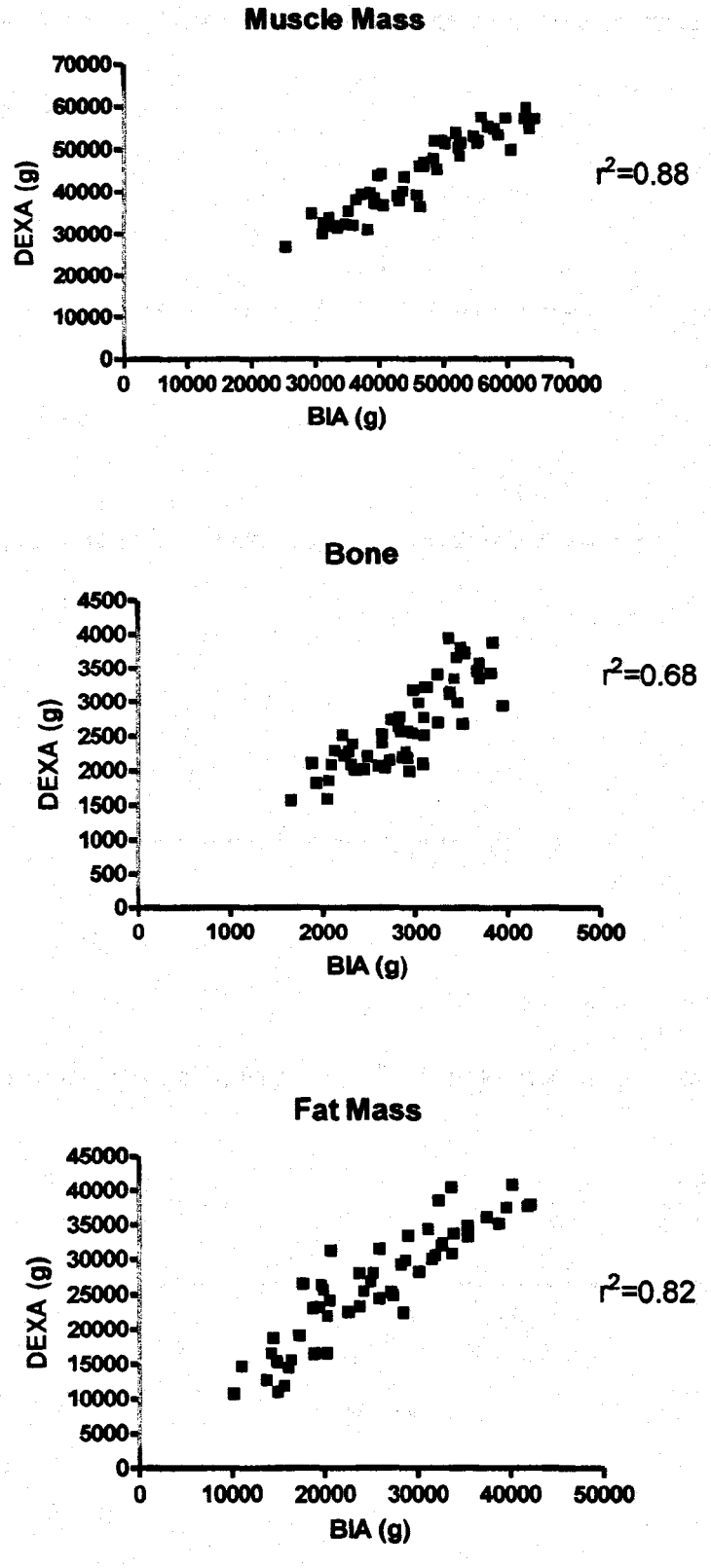
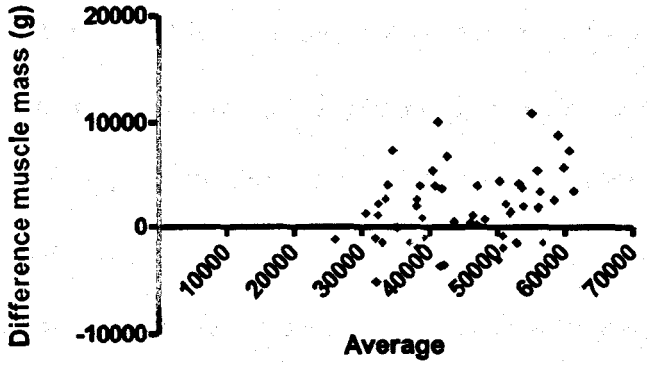
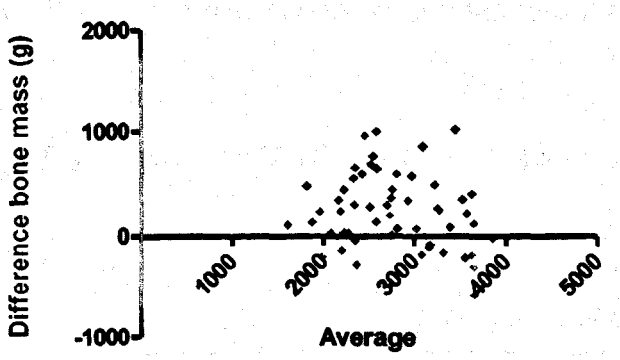


Figure 2. Correlation between BIA and DEXA measured Muscle, Bone and Fat mass

Bland-Altman BIA versus DEXA measured muscle mass



Bland-Altman BIA versus DEXA measured bone mass



Bland-Altman BIA versus DEXA measured fat mass

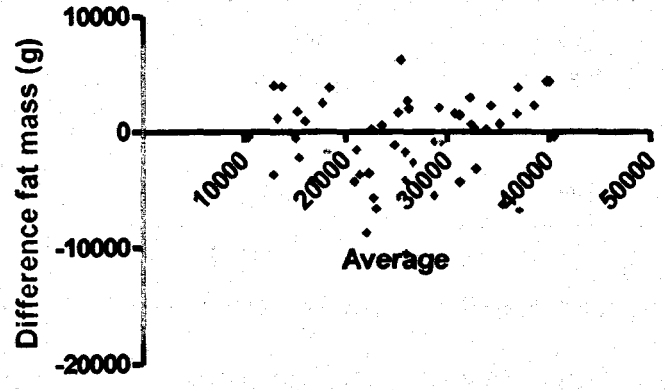


Figure 3. Comparison of BIA and DEXA measured muscle, bone and fat mass

Body Water

Taking the study group as a whole, mean total body water (TBW) fell between V1 and V2 (p=0.04) by a mean of 0.7L. There was further decline over the following 12 month period by a mean 0.3L (p=0.02). Unsurprisingly, given the pharmacological actions of AHT medication used, ECF volume fell by a mean of 0.3L at visit 2 following antihypertensive reintroduction (p=0.03). There was further reduction in ECF by a mean of 0.1L over the following 12 months (p=0.03).

Analysis of subjects by group (CKD/non-CKD) demonstrated no significant changes in body water at any stage during the study.

Functional Assessment 'TUG' test and Falls

	TUG seconds (Standard deviation)		
	V1 (Withdrawal)	V2 (Treated to target)	V3 (12 months)
All participants	9.2 (4.2) n=54	8.9 (3.5) n=54	10.0 (5.8)* n=51
CKD	9.9 (5.5) n=29	9.5 (4.5) n=29	11.3 (7.7)♦ n=27
Non-CKD	8.3 (1.3) n=25	8.2 (1.3) n=25	8.6 (1.5) n=24

Change in TUG overtime * p=0.009, ♦ p=0.03

Table 7. Timed Up and Go Results (TUG) over study period

For the group as a whole performance within the TUG test deteriorated slightly overtime (p=0.009). Although there was a large range of times (6-33 seconds V1, 5-28 seconds V2 and 5-45 seconds V3) only 2 subjects had times above 20 at any point to suggest more severe functional

problems. The proportion of subjects scoring in the range consistent with independent functional status (<10seconds) fell from around 89% at the start of the study to 82% at V3. Looking at the CKD group alone TUG performance deteriorated (p=0.03). However, taken separately there was no significant change in performance in the non-CKD control group.

These results demonstrate that both the non-CKD and CKD subjects have good functional status. This is reflected by data obtained from monthly falls diaries. In total only 27 episodes were reported involving 13 subjects (6 CKD, 7 non-CKD). Range was 1-6. Documentation suggested a 'mechanical' or 'accidental' cause in all instances. There was no association between TUG performance, BP or measures of body composition.

4.5 Discussion

This is the first description of both acute and chronic effects of goal directed antihypertensive therapy on body composition and functionality and long-term falls risk in a population aged ≥70 years with and without CKD.

With careful monitoring we were able to achieve the consensus target BP with a mean of 129/69mmHg across the entire study group. This was achieved without promoting unwanted adverse symptoms or drug side effects. This improvement in BP control appeared to be lasting as it was still apparent at follow up visits 12 months later.

As might be hoped, this optimisation of BP control was associated with stability in renal function in the CKD group. Mean eGFR fell by 1ml/min between V2 and V3 (p=ns) and urinary protein losses actually showed a minimal decline over the study period. This reinforces the importance of adequate antihypertensive intervention in the CKD population. Given that most of the major trials related to the use of ACE-I and ARB drugs did not include older subjects (123) these results provide extra evidence that in carefully selected older subjects with CKD, where these medications might be indicated, it is potentially safe to utilise them.

Within the non-CKD group, it was observed that eGFR did decline significantly over the 12 month period following BP optimisation from a mean of 86ml/min to 80ml/min (p=0.01). Urinary protein losses were unchanged. This reduction is above that which might be expected due to renal senescence (~1-2ml/min/year). A potential explanation for this increased decline in eGFR is renal hypoperfusion due to reduced BP. A similar acceleration in decline of renal function has been observed in the ACCORD study in those subjects randomised to strict BP control (<120mmHg systolic) (84). This finding adds weight to the evidence that BP targets in the absence of CKD and significant proteinuria should be higher.

Body composition is important in the assessment of nutritional status, disease risk, physical fitness and effectiveness of interventions (128). DEXA is used extensively for the assessment of body composition and is considered valid and reliable (129). Its application is limited by requirements for expensive equipment, trained technicians and

dedicated facilities. BIA on the other hand is a relatively simple, inexpensive non-invasive and portable means of assessing body composition giving it a broad application. The correlations between measurements of muscle, fat and bone mass obtained in the present study are comparable to those from other studies (130).

With respect to BIA obtained data, mean muscle mass was observed to decline in both CKD and non-CKD subjects over the 12 month period following BP optimisation. The mean reduction was 3% and 2% respectively. The difference between groups was not significant. The reduction in the non-CKD group would be at a level with that expected due to age related deterioration over time (119). The decrease in the CKD group might be expected to be higher as a result of other factors resulting in muscle loss more specific to the uraemic setting.

Equal numbers of CKD and non-CKD subjects were treated with ACE-I. Interestingly, those receiving ACE-I showed a reduction in muscle mass at 12 months by ~1%. Those not treated with ACE-I actually had an increase in total muscle mass ~2%. These values did not reach statistical significance but are the opposite of what might be expected given the current evidence base for ACE-I reducing sarcopenia (131).

In contrast to muscle, literature relating to fat mass in subjects with CKD is relatively sparse. The realisation that fat is more than an inert storage organ and may mediate inflammation via the effect of adipokines will hopefully increase the evidence base. We did not demonstrate any significant change in fat mass over the study period in either group irrespective of assessment method.

Most of an individuals bone mass is achieved by early adulthood. After that time, bone mass gradually declines throughout the rest of life. There is a normal rate of decline in bone mass with age in both men and women. For women, in addition to age, the menopause transition itself causes an extra degree of bone loss. This bone loss is greatest in the first three to six years after menopause. Women can lose up to 20% of the total bone mass during this time. Since women generally have a lower bone mass to begin with in comparison with men, the ultimate result is a higher risk of fracture in postmenopausal women as compared to men of the same age. The present study demonstrated a significant decline in overall bone mass within male subjects.

In terms of function, the present study has shown no deterioration over 12 months in the non-CKD group but a significant reduction within the CKD group. This may in part be related to the increased reduction in muscle mass in this group. However, this did not relate to falls risk. Falls are common amongst older people and a major public health concern in terms of morbidity, mortality and cost. The prevalence has been estimated as 28-35% in community dwelling older people aged 65 years and up to 42% in those aged over 75years (132). Falls occurred in approximately one quarter of our subjects over a twelve month period. Relatively this is actually quite low. This highlights one of the main problems with this study. Due to the strict criteria of entry we have excluded subjects who might be more prone to falling and therefore cannot really apply our findings to a broader population. However, we

have demonstrated that, in a carefully selected population of older subjects with CKD, the aggressive targeting of optimal blood pressure control to preserve renal function does not result in significant problems with falls in this group.

5 BONE COLLAGEN FORMATION RATES AND EXPRESSION OF OSTEOTROPIC FACTORS IN PATIENTS WITH CKD 5

5.1 Introduction

With progressive CKD characteristic disturbances in bone and mineral metabolism are common. These underlying biochemical disturbances in calcium, phosphate, vitamin D and PTH homeostasis associated with renal bone disease are apparent early. Bone turnover is predominantly under the influence of PTH. Uraemia is associated with a state of skeletal resistance. Secondary hyperparathyroidism is an almost inevitable consequence of untreated chronic kidney disease. Due to high circulating levels of PTH, both osteoblast and osteoclast surfaces are augmented and ultimately bone volume may be reduced (133).

Synthesis of 1,25 dihydroxyvitamin D (calcitriol) is impaired in subjects with CKD. This occurs at least in part due to loss of renal parenchyma and in part due to suppression of 25-hydroxyvitamin D-1α-hydroxylase by retained phosphate and Fibroblast growth factor 23 (FGF23) (134). As a result, reduced levels of calcitriol develop from CKD3 onwards, leading to a reduction in calcium absorption (135). The effects of low levels of calcitriol and reduced extracellular ionised calcium on the parathyroid gland lead to increased production of PTH. This will, initially, result in normalisation of calcium levels. Over time skeletal resistance to the calcaemic effect of PTH develops. Subsequent abnormalities in mineralization, bone morphology and electrolyte homeostasis, due to the inability of the skeleton to adequately buffer the internal milieu, predispose to bone pain and increased fracture potential, in addition to increasing drivers for vascular calcification.

To avoid renal bone disease and provide adequate skeletal activity to buffer the internal milieu, therapeutic interventions are directed towards ensuring normalised bone turnover. PTH levels are used as a surrogate biomarker to predict bone turnover in CKD patients.

5.1.1 Diagnostic Dilemmas

To date, the gold standard for the accurate diagnosis of renal bone disease remains histomorphometric and histochemical examination of a bone biopsy specimen.

Bone is typically obtained from the iliac crest after the administration of time-spaced tetracycline markers to determine the rate of new bone formation (136). The typical labelling schedule involves two three day periods of tetracycline labelling separated by 21 days. The main drawback with this technique is its invasive nature. This has limited its use primarily as a research tool. Another problem is that it does not reliably provide information about acute changes in bone collagen dynamics and its response to physiological/pathophysiological stimuli.

Measurements of the concentration of PTH in serum or plasma are widely used for initial diagnostic assessment, monitoring of progression and response to treatment of patients with renal bone disease. The different methods and assays available for measuring PTH in biological samples create a possible source of confusion for clinicians (137). First generation immunometric PTH assays (intact/iPTH assays), were thought to detect predominantly full-length, biologically active 1-84 PTH. It is now known that most of these assays also detect one or more

amino-terminally truncated peptides distinct from 1-84 PTH in addition (138, 139, 140). These assays therefore overestimate the concentration of 1-84 PTH.

Current clinical guidelines for the treatment of patients with CKD are largely based on the recommendations published in 2003 by the Kidney/Dialysis Outcomes Quality Initiative (K/DOQI). Two thirds of these recommendations are based on clinical opinion rather than direct experimental data.

Most of the data used to derive target levels for PTH at different stages of CKD originates from clinical studies of the relationship between plasma PTH levels and bone histology in patients on dialysis during the 1980s and early 1990s. These reflect the impact of therapeutic strategies, diagnostic tests and the demographics of the dialysis population during this period (137). Aluminium containing phosphate binders, which were used predominantly at this stage, are now rarely utilised. They have largely been replaced by calcium salts which can directly affect PTH levels. A study of patients on chronic maintenance dialysis in 1995 showed that bone turnover could not be predicted by serum PTH measurements in 30% of haemodialysis and 51.3% of CAPD patients (141). The authors proposed that to precisely assess bone turnover in patients with serum PTH levels between 65 and 450pg/mL, bone biopsy is required.

A separate issue with current guidelines is that there is little direct experimental data for patients with CKD not yet requiring dialysis. A study of 84 patients with ERF not yet started on dialysis, comparing the

histomorphometric and histochemical analysis of bone biopsy specimens to different plasma biochemical markers, showed that iPTH levels did not correlate significantly with any bone histomorphometric or histodynamic parameter (142).

5.1.2 Novel Investigational Technique

Professor Rennie's group developed a technique to study the direct, dynamic measurement of human bone collagen synthesis rate within hours. It depends on measuring the incorporation of a stable (non-radioactive) labelled collagen precursor ([^{13}C] or [^{15}N] proline) into hydroxyproline in bone. (The techniques are described fully by Babraj et al 2002 (143)). The technique utilises an initial skin biopsy to determine basal $^{13}C/^{15}N$ levels, which have previously been shown to be similar to those of bone collagen in non uraemic subjects (143), and therefore only requires a single bone biopsy of around 150mg after administration of tracer. The technique requires only 50 mg of bone and should leave adequate sample for RNA extraction and real time Polymerase Chain Reaction (PCR) of biologically relevant genes.

5.2 Aims

Utilising this new technique our primary aims were to investigate:

1. Bone collagen synthesis rate (CSR) in a cross section of prevalent male haemodialysis patients, having washed off from both vitamin D and oral phosphate binding therapies to determine the level of PTH that best replicates bone formation in healthy individuals.
2. The direct effects on bone CSR of phosphate normalisation with either calcium (Calcichew) or non-calcium containing phosphate binders (Sevelamer), with equal control of serum phosphorous.

Our secondary aim was to observe the effect of phosphate normalisation on expression of biologically relevant gene markers of bone turnover.

5.3 Methods

5.3.1 Subjects

We recruited male chronic haemodialysis patients from the haemodialysis program at Royal Derby Hospital. The study was approved by the Southern Derbyshire Local Research Ethics Committee in accordance with the Declaration of Helsinki. Before taking part in the study, all patients gave their written informed consent to take part in the study and were aware that they were free to withdraw from at any point. Patients were excluded if they were less than 16 years or greater than 80 years of age, serum PTH level was greater than 1000ng/L, serum phosphate was greater than 2.5mmol/L or serum corrected calcium was greater than 2.6 mmol/L.

5.3.2 Experimental Protocol

Patients were washed out from previous treatment for a four week period. This includes both vitamin D and oral phosphate binders. Patients were removed from the study and restarted on their previous treatment if their serum phosphate level exceeded 3 mmol/L. At the end of the washout period initial bone biopsy with proline incorporation was performed and blood sampled for 1-84 PTH allowing for comparison of results with age matched non-CKD control data from previous studies carried out by Professor Rennie's group (143,144).

For the second phase of study patients were allocated to receive either Calcium Carbonate or Sevelamer by the investigator to achieve the same degree of phosphate control (1.2-1.8 mmol/L). Doses were titrated over 4 weeks to achieve serum phosphorous level of 1.2-1.8 mmol/L and then maintained at this level for a further 8 weeks before undergoing a second bone biopsy to determine bone collagen formation rate, in concert with repeat assay of serum PTH. Vitamin D was not utilised to avoid confounding effects of vitamin D on osteoblastic function. At the end of the study patients were returned to their original pre study medication.

5.3.3 Protocol for Bone Biopsy

Subjects were biopsied on a non-dialysis day having been fasted from 8 p.m. the day before the study to reduce any effect of dietary protein on CFR. A detailed description of the biopsy protocol can be found in Chapter 3.

5.3.4 Sample Analysis

Collagen synthesis measured as incorporation of tracer proline into bone protein hydroxyproline

• Sequential extraction of collagen from bone.

50 mg of bone was ground to a fine powder under liquid N_2, and subjected to differential extraction with 0.15M NaCl-0.05 M Tris – 0.2 M EDTA (pH 7.5), 0.5 M acetic acid, and pepsin-0.5 M acetic acid and then precipitated with 5 M NaCl Purified collagen was collected by centrifugation at 50,000 rpm for 45 min, dissolved in 0.5 M acetic acid and stored at –20°C until analysis.

• Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS)

Isolated collagen was hydrolysed (6 M HCl, 120°C, 15 h), and the free amino acids purified using cation exchange chromatography (Dowex 50WX8-200, Sigma Ltd, Poole, UK). The proline and hydroxyproline were derivatized as their N-acetyl-n-propyl esters (NAP) and analysed by capillary GC-C-IRMS (Delta- plus XL, Thermofinnigan, Hemel Hempstead, UK)

• Gas Chromatographic-Mass Spectrometric (GC-MS) Analysis.

Plasma and tissue free proline, were extracted as previously described. Proline was prepared as the tert-butyldimethylsilyl (t-BDMS) derivative before analysis (MD800 GC-MS [Thermofinnigan, Hemel Hempstead, UK]).

Measurement of transcription and translation of osteoblast gene products

• RNA preparation and cDNA synthesis

Total RNA was extracted from bone for RT-PCR, the feasibility of which has been successfully proven by a number of recent studies (145). Bone (50-75 mg) was powdered under liquid N_2 and transferred to Qiagen RNeasy spin columns, as per the manufacturer's instructions, with Proteinase K/ DNase digestion steps. RNA concentration and purity were determined by measuring the fluorescence ratio at 260 and 280 nM. Total RNA was reverse-transcribed into cDNA into 50 ml using PE Applied Biosystems Taqman Reverse transcription reagents as per manufacturer's instructions. 1ml, i.e ~1-4 μg of input RNA were used in subsequent Taqman analysis.

• Taqman quantitative real-time PCR analysis

Sequence-specific primers and probes for Taqman quantitative PCR analysis of mRNA expression will be designed for our RNA targets using the Perkin Elmer Applied Biosystems Primer Express software, according to the manufacturer's protocol. Each assay is designed such that the probe spans an intron/exon boundary to minimize the possibility of co-amplifying genomic DNA. PCR (1 x (50°C, 2 min, 95°C, 10 min), 40 x (92°C, 15 sec, 60°C, 1 min)) is performed in the presence of 0.6 x Taqman Universal PCR Master Mix (PE Applied Biosystems), forward and reverse primers and a sequence-specific fluorescent probe. To ensure specificity of amplification, all primer sequences include a

minimum of 2 unique nucleotides at the 3' end. Optimal probe/ primer concentrations will be determined for each assay. A panel of cDNA clones, representative of each gene of interest, will be used to confirm specificity of amplification. Real-time PCR will be performed using an ABI Prism 7700 Sequence Detector ("Taqman"), in which fluorescence output, measured as cycle threshold (Ct), is proportional to input cDNA concentration. Ct values in the range 35-40 units will be interpreted as being at the limit of Taqman detection and therefore will not be quantitated. Input [cDNA] will be normalised to bone GAPDH ribosomal RNA using PE Applied Biosystems Ribosomal RNA control reagents.

Parathyroid Hormone Assays

Intact / 1-84 Parathyroid Hormone was measured using the DPC Immulite 2000 PTH assay by staff within the department of biochemistry at Royal Derby hospital. Reportable range 3.0-2500 ng/L. Normal reference range 11-67 ng/L (Serum).

5.4 RESULTS

Patients

We initially recruited 9 male patients from the chronic haemodialysis programme at Royal Derby Hospital. Demographic data is shown in Table 8 below.

Subject	Age (years)	HD Vintage (months)	Cause of ERF	PTH (ng/L)	cCalcium (mmol/L)	Phosphate (mmol/L)
1	66	49	Renovascular disease	107	2.50	2.50
2	48	12	Diabetic nephropathy	524	2.24	1.88
3	69	24	APKD	31	2.59	1.37
4	68	10	Diabetic nephropathy	53	2.50	1.44
5	77	42	APKD	156	2.31	2.04
6	68	19	APKD	383	2.34	1.51
7	63	28	Diabetic nephropathy	139	2.33	1.36
8	75	69	Obstructive uropathy	69	2.50	1.90
9	63	9	Amyloidosis	311	2.32	1.91

Table 8. Demographic data at recruitment visit

Collagen Synthesis Rate measured as Fractional Synthetic Rate (FSR) following washout period

None of the 9 subjects recruited developed a serum phosphate greater than 3mmol/L during the washout phase and so were eligible to remain within the study and proceeded to initial bone biopsy. FSR for collagen were determined. These data are presented alongside measured iPTH level recorded for each subject at time of biopsy in Table 9. There were no significant differences in serum phosphate or calcium levels between patients (2.1±0.29, 2.29±0.13 respectively). FSR (%/hr) was higher in haemodialysis patients than matched non uraemic controls (0.069±0.015 versus 0.054±0.006, p=0.013) (143,144). Circulating iPTH levels did not correlate with bone collagen FSR (r= -0.44, p=0.23).

Subject	FSR (%/hr)	iPTH (ng/L)
1	0.101	49
2	0.073	201
3	0.070	52
4	0.063	428
5	0.057	136
6	0.064	386
7	0.063	44
8	0.076	224
9	0.050	636

Table 9. FSR collagen versus iPTH level in prevalent male haemodialysis patients following 4 week washout of vitamin D and phosphate binder medication.

Phosphate normalisation with calcichew/sevelamer and collagen synthesis rate

Unfortunately two patients withdrew from the study following the washout period and initial biopsy. Of those remaining 4 were allocated to receive sevelamer and 3 to receive calcichew to achieve target phosphate control. Target phosphate level (1.2-1.8 mmol/L) was achieved and maintained in all subjects. Table 10 shows mean phosphate and mean corrected calcium over an 8 week period, phosphate binder dosage and iPTH at the time of repeat bone biopsy.

Sevelamer				
Subject	Serum Phosphate (mmol/L)	Binder Dose (g)	Serum cCalcium (mmol/L)	iPTH (ng/L)
3	1.55±0.22	4.00	2.52±0.17	57
4	1.54±0.25	7.20	2.50±0.13	487
7	1.34±0.21	3.20	2.41±0.06	66
9	1.31±0.24	2.40	2.34±0.05	705
Calcichew				
Subject	Serum Phosphate (mmol/L)	Binder Dose (g)	Serum cCalcium (mmol/L)	iPTH (ng/L)
5	1.33±0.23	6.25	2.47±0.21	202
6	1.69±0.12	8.75	2.35±0.12	217
8	1.34±0.15	5.00	2.21±0.24	354

Table 10. Mean serum phosphate, cCalcium, iPTH and binder dose by subject

Following 8 weeks of phosphate optimisation we attempted to reassess bone collagen formation rate using ^{15}N labelled proline to avoid any effect of remnant ^{13}C given at the time of the initial biopsy on determination of fractional synthetic rate.

Unfortunately data obtained using ^{15}N labelled proline demonstrated higher levels within skin at baseline than within bone 120 minutes following dose of labelled proline. This meant that we were unable to calculate a synthesis rate. We repeated the analysis two further occasions and obtained very similar results. At this point, given the invasive nature of the process we felt that it was not appropriate to continue recruitment until we could explain this anomaly and the study was closed.

Transcription and translation of osteoblast gene products

We were successful in demonstrating expression of RANKL and OPG in 4 subjects at baseline using the described techniques. However due to the problems with attempts to determine collagen formation rate and small samples obtained at bone biopsy we had insufficient bone to perform repeat testing post phosphate optimisation. We were therefore unable to determine the effect of calcium and non-calcium containing phosphate binding medication on gene expression of these osteotropic factors.

5.5 DISCUSSION

The inability of the skeleton to adequately buffer the internal milieu and maintain its essential role in homeostasis has far reaching consequences in subjects with CKD. Given the interdependence of calcium, phosphate, vitamin D and PTH status, no single intervention is usually sufficient to completely restore abnormalities in their metabolism in the setting of progressive uraemia. PTH is the major determinant of bone formation and turnover in patients with CKD (146). Accordingly, measurements of PTH levels in serum or plasma have been used for many years as a non-invasive biochemical method for diagnosis and monitoring progression and response to therapeutic intervention.

The initial data obtained from the study for basal collagen synthesis rate in our subject group suggests that there is no correlation between measured iPTH levels and bone turnover as measured by FSR. This would be in agreement with previous published data (141, 142). Measured iPTH at the time of bone biopsy ranged from 44ng/L to 636ng/L. Interestingly, the highest recorded level for iPTH (636ng/L) was associated with the lowest measured FSR (0.05%/hr). Characteristic histomorphometric findings at bone biopsy in the setting of an elevation in PTH of this level would typically be consistent with increased bone turnover.

Disappointingly, the raw data obtained following phosphate normalisation utilising ^{15}N labelled proline did not allow us to derive FSR in our subjects. The initial levels of ^{15}N detected in baseline skin samples was far in excess of those obtained from bone two hours after flooding dose

of tracer. A potential reason for this is that collagen kinetics within skin in the uraemic setting might differ from those in non-uraemic subjects and may be associated with greater recycling of proline. It is unclear at this time why this only appears to have been an issue relating to ^{15}N detection. However, in this setting it did mean that skin could not provide a surrogate reflection of collagen synthesis within bone and therefore could not be used to estimate a basal rate within bone. The investigational technique would therefore require revision before it is utilised again in the uraemic setting. One simple solution would be to replace the initial skin biopsy with bone biopsy. This obviously increases the invasive nature of the procedure and would lose some of the intended benefit. The difficulties experienced with the technique had necessitated repeat analysis of samples and as a direct consequence of this we had insufficient bone to perform PCR and establish expression of gene markers of bone turnover in many cases. In the instances where we were left with (theoretically) sufficient bone mass, it became apparent that the relative acellularity of bone meant that larger quantities would actually be required in order to extract sufficient bone RNA to go on to perform analysis of expression of gene products. Using stored samples from a separate study which had involved collagen formation rates relating to hip fracture, where entire femoral neck/head was removed as part of total hip replacement surgery we established that repeated, reliable extraction of RNA was possible in samples of ≥100mg.

There are multiple potential markers for bone turnover. The process of osteoclastic bone resorption results in the liberation of bone mineral and

osteoid, which is subsequently metabolised to peptides that can be measured in serum and urine. Examples include urinary N-telopeptide (NTx) and serum C-telopeptide (CTx). Similarly, osteoid formation leads to the production of proteins such as osteocalcin and by-products of collagen synthesis that may also be measured in serum. These biomarkers have some utility in certain clinical settings such as osteoporosis but there is a paucity of data regarding use in CKD-MBD. Assay variability and poor standardisation have also limited their usefulness in the clinical setting (147). In addition, biological variability due to a large number of physiological, pathological, circadian and dietetic factors must also be considered when interpreting these results (148, 149).

The gold standard for diagnosis of bone disease remains histomorphometric assessment of bone biopsy. Despite the limitations, iPTH remains the most utilised marker for the assessment of bone status in CKD-MBD. Our limited data supports previously established limitations in its usefulness. CKD progresses to a multisystem disorder that leads to widespread organ damage. Disturbances in bone and mineral metabolism are common and biochemical and hormonal derangements apparent early. Timely, optimal management is essential to maintain the homeostatic role of the skeleton and thus minimise cardiovascular damage in our patient group. To date there is no single serological marker that adequately reflects bone activity in the uraemic setting. This is a highly interesting and complex area that requires ongoing research.

6 MYOCARDIAL CONTRACTILE FUNCTION & INTRADIALYTIC HYPOTENSION

6.1 Introduction

Despite continued advances in haemodialysis technology, intradialytic hypotension (IDH) remains highly prevalent in the chronic haemodialysis population. IDH has been reported in about 30% of sessions (75-77) and is associated with high rates of morbidity and mortality (78). The pathophysiology of IDH is multifactorial but involves inadequate compensatory mechanisms to hypovolaemia induced by ultrafiltration. These include a decrease in venous capacitance, increase in total peripheral resistance and increased cardiac output (75). Failure of any of these mechanisms predisposes patients to IDH. However, controversy exists as to which component is the most important in its development.

Additionally, there is increasing evidence implicating IDH in the pathophysiology of 'uraemic cardiomyopathy'. IDH increases the risk of myocardial hypoperfusion leading to segmental ischaemia inducing segmental left ventricular dysfunction (73), a process known as myocardial stunning (74). Myocardial stunning is cumulative and leads to irreversible left ventricular dysfunction in ischaemic heart disease (150, 151, 152).

Pharmacological stress is in routine use in the diagnosis of ischaemic heart disease, by exposing areas of ventricular dysfunction during echocardiography. Dobutamine at low dose (≤10μg/kg/min) acts predominantly as an inotrope and at higher doses exerts a more chronotropic effect. It has been shown to be safe, feasible and effective

in prognostic assessment in the general population (153). It has been shown to be safe in haemodialysis patients. Dobutamine stress echo has been reported to demonstrate differences in cardiac output, stroke index and SBP between hypotension-prone and non hypotension-prone patients (154).

As the overall age of the dialysis population increases so does the burden of cardiovascular disease. This further increases the propensity of this population to IDH. The ability to identify patients likely to be at risk of IDH would therefore allow us to target these patients with superior dialysis techniques associated with less risk of IDH, prior to delivering a period of potentially harmful IDH prone standard HD.

6.2 Aims

The aim of our study was to perform non-invasive measurement of cardiovascular parameters in a group of chronic haemodialysis patients undergoing pharmacological stress, in place of dialysis induced cardiac stress, allowing us to ascertain the dominant functional abnormalities that might predispose patients to IDH.

6.3 Methods

6.3.1 Patients

Ten non-diabetic chronic HD patients were recruited for the study. Five were classified as having been hypotension prone (HP) on dialysis and five acting as controls were known to be stable on dialysis (hypotension resistant) (HR). IDH was defined as symptoms of hypotension during HD for at least one third of dialysis sessions for greater than one year, plus either systolic blood pressure (SBP) less than 100 mmHg, or SBP reduced by at least 25%. Three patients were female and the patients in the two groups were well matched for age, dialysis vintage and baseline haemodynamics. None of the patients had a history of overt ischaemic, or significant valvular heart disease. Patients had echocardiography prior to the study. All patients were dialysing three times a week with conventional HD. Patients were excluded from the study if they were taking vasoactive medication, had a history of cardiac arrhythmias or ischaemic heart disease/acute coronary syndrome.

All patients gave written informed consent. Ethical approval for the project was granted by Derbyshire Local Research Ethics Committee.

6.3.2 Study protocol

Dobutamine-atropine stress (DAS) pulse wave analysis was performed in all patients one day following a HD session to reduce the likelihood of significant post dialysis hypovolaemia. All patients underwent clinical examination prior to commencing DAS and were subjectively deemed to be euvolaemic. Patients were studied in a fasted state and usual medications were continued. Non-invasive haemodynamic monitoring was performed throughout the study with the use of the Finometer® (Finapres Medical Systems, Arnhem, The Netherlands). Baseline haemodynamic parameters were obtained for each patient with 10 minutes of monitoring prior to the start of the Dobutamine infusion. The finger cuff was left in place for the duration of the study on the non-fistula arm. Each patient had an initial 12 lead ECG followed by continuous ECG monitoring.

Primary endpoints were change in blood pressure (BP) from baseline and percentage change in CO, Stroke Volume (SV) and Total Peripheral Resistance (TPR), in response to Dobutamine-atropine stress. Secondary endpoints were change in heart rate and BRS.

6.3.3 Dobutamine-atropine stress

DAS was performed after initial baseline haemodynamic monitoring. Dobutamine was infused intravenously via a peripheral cannula following the same protocol in each patient. Dobutamine infusion was initiated at

5μg/kg/min for 5 minutes followed by 10μg/kg/min for 5 minutes. This was defined as the "low dose" period. Following this the Dobutamine was increased by 10μg/kg/min every 3 minutes, to a maximum of 40μg/kg/min (high-dose), unless a test endpoint was reached. If a test end-point was not reached despite maximum dose Dobutamine then atropine was added intravenously to a maximum of 2mg.

Test end-points were 1) achieved target heart rate (220-age), 2) peak dose of Dobutamine/Atropine 3) chest pain or ECG evidence of cardiac ischaemia, 4) severe hyper- or hypotension (systolic blood pressure >220mmHg or <90mmHg, diastolic blood pressure >110 mmHg or <30mmHg), and 5) intolerable symptoms.

6.3.4 Finometer

The Finometer allows continuous non-invasive beat-to-beat haemodynamic monitoring. This technology uses pulse-wave analysis at the digital artery together with Modelflow™ algorithms to reconstruct a central aortic waveform (103). This provides beat-to-beat estimates of cardiac output, stroke volume and peripheral resistance as well as BP and heart rate measurements. The Finometer is useful at tracking relative change but needs calibration against another measurement for absolute values (155). In our centre we have widely utilised the Finometer to track change from baseline of haemodynamic variables in CKD patients. These have been validated against changes seen on echocardiography and have shown a strong correlation between percentage change in SV measured by the two techniques (156). As we

were interested in changes in haemodynamics, these are presented as percentage change from baseline except for BP, which is calibrated against brachial readings, where absolute values are shown (106).

BRS was calculated from the regression slope between interbeat interval and beat to beat changes in blood pressure. Multiple consecutive changes in interbeat interval in the same direction are required before a phase shift calculation (incorporated into the software) is performed. BRS measured in this way gives a compound indication of the activity of the autonomic nervous system (112, 157).

6.3.5 Statistical analysis

Results are expressed as the mean ± SE unless otherwise stated. Blood pressure and haemodynamic data with increasing Dobutamine dose were analysed with a one-way ANOVA (repeated measures) with Dunnetts test to compare results with control data before the start of Dobutamine infusion.

Unpaired t tests were used to compare averages between groups in the case of parametric data or the Mann-Whitney U test for non-parametric data. For all tests, a P value of less than 0.05 was judged to be significant (ns=not significant).

All data were analysed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA).

6.4 Results

Baseline characteristics	HR mean ± SE	HP mean ± SE	P value
Number	5	5	ns
Age	61.2± 6.0 (41-77)	65.2± 4.2 (54-75)	ns
Sex *male/female*	3/2	4/1	ns
Haemodialysis vintage- *years*	2.2±0.5 (1-3)	5.3±1.5 (1-10)	ns
Hematocrit	0.4 ± 0.01	0.4 ± 0.02	ns
LVMI ($g/m^{2.7}$)	64.3 ± 20.3	62.8 ± 15.5	ns
Hemodynamics SBP, mmHg	123.1 ± 5.8	120.8 ± 8.5	ns
MAP, mmHg	91.5 ± 5.4	88.5 ± 4.7	ns
DBP, mmHg	71.5 ± 5.5	68.2 ± 4.8	ns
HR, bpm	78.6± 3.9	85.7 ± 6.1	ns
BRS, ms/mmHg	3.6± 0.6	2.1± 0.7	ns

Table 11 Baseline clinical characteristics of hypotension resistant (HR) and hypotension prone (HP) patients. Abbreviation: LVMI, left ventricular mass index.

Baseline patient information is shown in table 11. DAS pulse-wave analysis was successfully carried out in all 10 patients. All patients required full dose Dobutamine and atropine as per the protocol to achieve target heart rate. Target heart rate was achieved in all patients without symptomatic hypotension, ischaemic ECG changes or other significant side effects.

Blood pressure response to DAS

After initiation of Dobutamine in HR patients there was no significant change in any of the measures of BP. Mean arterial pressure (MAP) increased from a baseline of 91.5 ± 5.4 mmHg to 91.8 ± 4.6 mmHg (p=ns) at peak heart rate after atropine. SBP fell from a baseline of 123.1 ± 5.8 mmHg to 121.2 ± 3.5 mmHg (p=ns) at peak heart rate and DBP increased from 71.5 ± 5.5 mmHg to 78.3 ± 4.6 mmHg (p=ns) after atropine. However, in the HP group there was a significant change in blood pressure during Dobutamine-atropine stress. SBP at baseline of 120.8 ± 8.5 mmHg fell to 95.9 ± 9.4 mmHg ($p<0.001$) at peak heart rate, MAP fell from 88.5 ± 4.7 mmHg to 74.9 ± 6.4 mmHg ($p<0.001$) and DBP from 68.2 ± 4.8 mmHg to 62.2 ± 5.7 mmHg (p=ns) at maximum heart rate.

Comparing HR to HP, the HR patients exhibited a mean SBP of 126.9 ± 2.9 mmHg, mean DBP was 75.52 ± 1.6 mmHg and mean MAP was 93.05 ± 0.7mmHg for the duration of the study after initiation of DAS. In the group defined as HP, all BP parameters were lower, mean SBP was

109.5 ± 8.1mmHg (p=ns), mean DBP was 65.26 ± 2 mmHg (p<0.05) and mean MAP was 81.27 ± 4.1 mmHg (p<0.05). BP data are summarised in figure 4.

Hemodynamic response to DAS

Heart rate increased significantly with increasing Dobutamine-atropine dose within each group. In the HR group baseline heart rate increased from 78.6 ± 3.9 beats per minute (bpm) to 147.3 ± 6.1 bpm (p < 0.001). In the HP patients baseline heart rate increased from 85.7 ± 6.1 bpm to 147 ± 3.2 bpm (p < 0.001). There were no significant differences in mean heart rates between the two groups throughout the study, HR 111.7 ± 19.1 bpm versus a mean heart rate in the HP patients of 118.5 ± 15.9 (p=ns).

SV declined in both groups throughout the study. In the HR patients SV fell by 28.2 ± 3.6% from baseline (p<0.001) and in the HP patients SV decreased by 41.2 ± 6.1% from baseline (p<0.001). In a comparison between the groups after initiation of Dobutamine in the HR patients the mean SV was 8 ± 10.3 % below baseline and in the HP the mean SV was 24.8 ± 11.3 below baseline (p=ns).

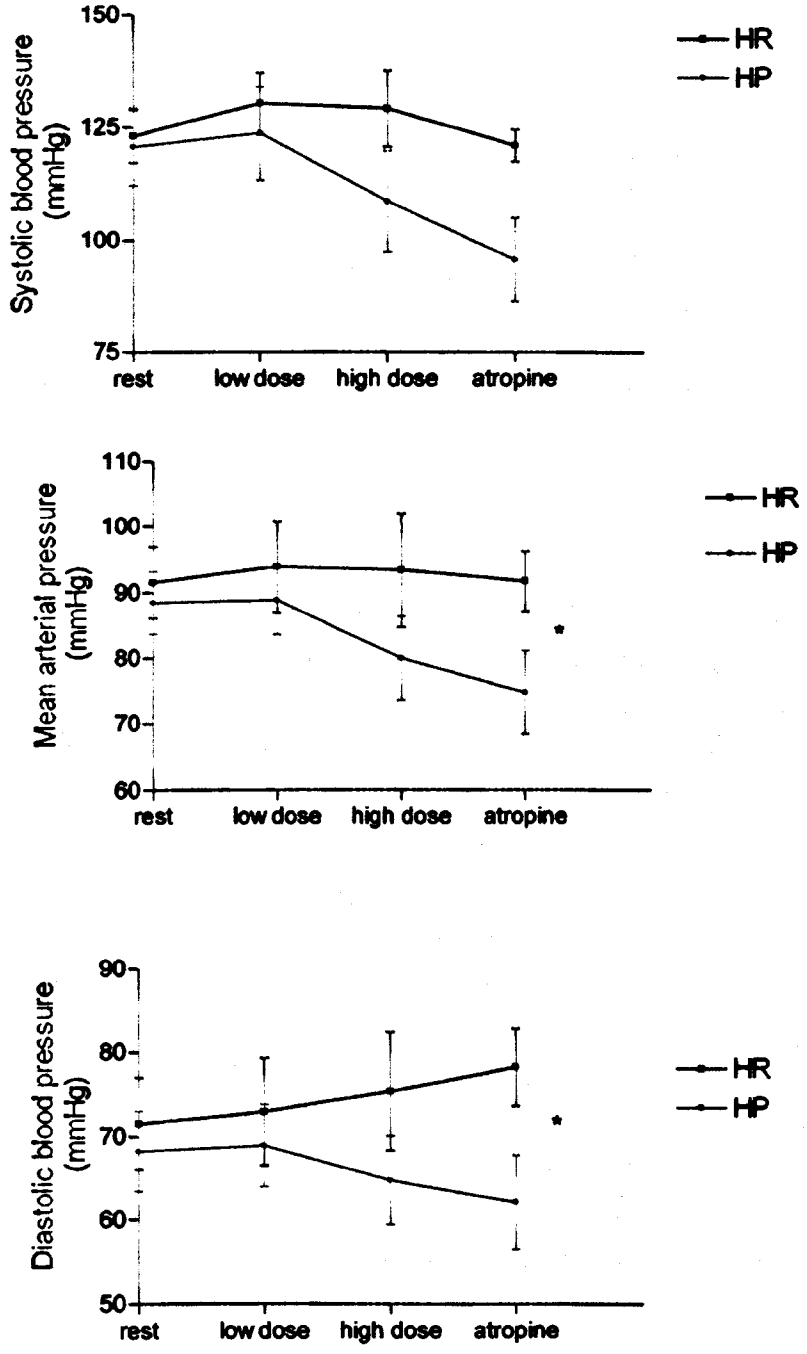


Figure 4.

Blood pressure response in HR and HP patients to Dobutamine-atropine stress expressed as absolute change. MAP and DBP was significantly higher in HR patients for duration of the study *p<0.05.

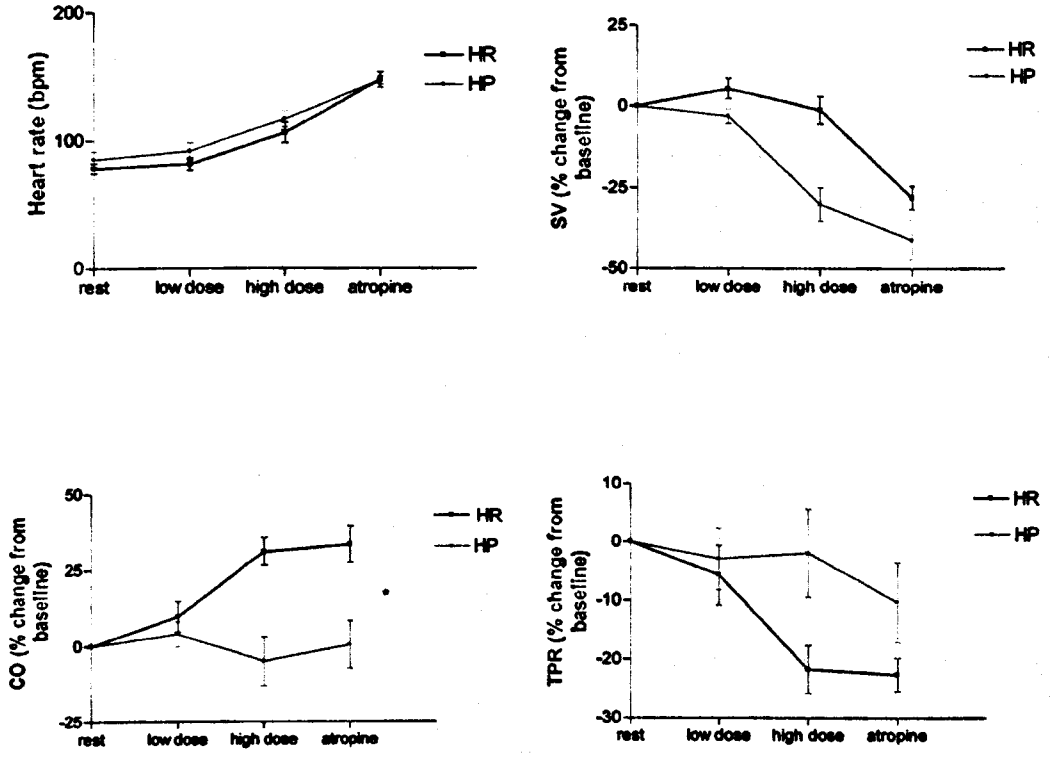


Figure 5. Systemic haemodynamics during Dobutamine-atropine stress in HR and HP patients. CO was significantly greater in HR patients *p<0.05.

	Baseline HR	Baseline HP	Low dose HR	Low dose HP	High dose HR	High dose HP	Atropine HR	Atropine HP
Heart rate *bpm*	78.6±3.9	85.7±6.1	81.9±4.8	91.9±6.7	**106.0±7.8***	**116.7±6.4***	**147.3±6.1***	**147.0±3.2***
SBP *mmHg*	123.1±5.8	120.8±8.5	130.4±6.9	123.8±10.3	129.3±8.5	108.8±11.2	121.2±3.5	**95.9±9.4***
MAP *mmHg*	91.5±5.4	88.5±4.7	93.9±6.9	88.8±5.2	93.5±8.6	80.1±6.4	91.8±4.6	**74.9±6.4***
DBP *mmHg*	71.5±5.5	68.2±4.8	72.9±6.4	68.9±4.9	75.4±7.1	64.7±5.3	78.3±4.6	62.2±5.7
SV (% change from baseline)	0	0	5.4±3.2	-3.2±2.2	-1.3±4.3	**-30.2±5.2***	**-28.2±3.6***	**-41.2±6.1***
CO (% change from baseline)	0	0	9.8±5.1	4±4.1	**31±4.6***	-5±8	**33.4±6***	0.5±7.9
TPR (% change from baseline)	0	0	-5.7±5.1	-3±5.3	**-21.7±4.2***	-1.9±7.6	**-22.7±2.9***	-10.3±6.8

Table 12. Haemodynamic response to Dobutamine-atropine stress in hypotension resistant (HR) and hypotension prone (HP) patients.

*$p<0.001$ versus baseline by ANOVA

In response to DAS in the HR group CO increased by 33.4 ± 6% above baseline ($p<0.001$) but only by 0.5 ± 7.9 % from baseline in the HP group (p=ns). During DAS in the HR patients the mean change in CO was +24.7 ± 7.5 % as compared to a mean change of - 0.2 ± 2.6 % in the HP ($p<0.05$). TPR declined in both groups during the protocol. However, TPR declined to a lesser extent in the HP group. TPR fell by 22.7 ± 2.9% from baseline ($p<0.001$) in the HR but only by 10.3 ± 6.8% in the HP group (p=ns). The mean TPR for the entire study in the HR was - 16.7 ± 5.5 % compared with a mean of - 5.1 ± 2.6 % in the HP patients (p=ns). Haemodynamic data are summarised in figure 5 and table 12.

There were no significant differences in BRS between groups at baseline. BRS increased from baseline with low dose DAS (p=ns) but then decreased with increasing DAS stress. In the HP group BRS decreased with progressively higher levels of pharmacological stress. There was a trend for mean BRS to be higher in HR patients throughout, (*$p<0.05$ with low and high dose dobutamine). BRS data are summarised in table 13.

Intervention	BRS (ms/mmHg) Hypotension Resistant	BRS (ms/mmHg) Hypotension Prone
Baseline	3.6± 0.6	2.1± 0.7
Low dose dobutamine	4.8± 0.4	1.78± 1.2*
High dose dobutamine	3.2± 0.8	1.3± 0.3*
Atropine	2.4± 0.7	1.3± 0.8

* $p<0.05$

Table 13. BRS response to Dobutamine-atropine stress in hypotension resistant (HR) and hypotension prone (HP) patients

6.5 Discussion

This is the first study in which Dobutamine-atropine stress in association with non-invasive pulse wave analysis has been used to differentiate between HR and HP patients in terms of their haemodynamic response, with a significant fall in MAP in the HP group.

Dobutamine stress is commonly used to investigate ischaemic heart disease by exposing new areas of ventricular dysfunction seen on echocardiography. Failure to increase CO was primarily due to a greater fall in SV seen in the HP patients as both groups increased their heart rate by similar amounts. This is in keeping with the findings by Poldermans et al who in their study using Dobutamine stress echocardiography were able to demonstrate that patients defined as hypotension prone were unable to increase cardiac index and that this was primarily due to a failure to increase stroke index in response to Dobutamine (154). This they defined as an impairment of myocardial contractility in the HP group. The impairment of myocardial contractile reserve as a cause of IDH is also supported by work by Nette (158). In the study by Boon et al in which they monitored haemodynamics during uncomplicated haemodialysis, a greater fall in SV was also responsible for the decline in CO seen in their patients in whom systolic blood pressure fell during haemodialysis (159). However, impairment in myocardial contractility was not thought to be relevant to the pathogenesis of IDH in the study by le EH et al (160).

Likewise, it was this inability to increase CO that was responsible for the fall in BP seen in the HP patients, as there was no significant difference in the response of TPR, which fell in both groups, but to a lesser extent in the HP group.

The difference in stroke volume response could be explained by differences in preload. However, in our study, to counter the influence volume status has on SV we studied patients on a day preceding their next dialysis session and examined them to ensure they were in a euvolaemic state. This ensured that the haemodynamic response to DAS that we observed was independent of the volume changes during ultrafiltration that have been implicated in dialysis induced hypotension.

Left ventricular hypertrophy (LVH) is a common finding amongst haemodialysis patients. It has been postulated that LVH and the subsequent diastolic dysfunction may be important in the aetiology of IDH. In our study, echocardiography pre study did not demonstrate a significant difference in left ventricular mass index. Therefore we suggest that LVH as a cause of diastolic dysfunction was not an important factor in differentiating the development of hypotension in these patients.

We excluded patients with a history of ischaemic heart disease and no patients experienced symptoms or had ECG changes that would be compatible with overt ischaemia during the study. It therefore seems unlikely that impairment of myocardial contractility could be explained by classical epicardial coronary artery disease alone in our patients. However,

just as dialysis has been suggested to induce subclinical ischaemia and myocardial stunning (150) we postulate that this process (but induced by DAS) may also be responsible for the inability to increase stroke volume, which was seen more in the HP prone patients in our study.

Repeated episodes of myocardial stunning may put patients at increased risk of dialysis induced hypotension and lead to left ventricular dysfunction associated with increased mortality. Interestingly, despite maximal doses of Dobutamine in both groups we saw falls in stroke volume, which in part was due to decreased cardiac filling time with increasing heart rate. In patients with non-ischaemic cardiomyopathy this inability to increase left ventricular ejection fraction has been shown to be a predictor of survival(161).

Autonomic dysfunction has also been considered to be an important factor in the propensity to IDH. Baroreflex sensitivity a marker of autonomic nervous system function was measured in our patients. The trend to decreasing BRS in the HP group is consistent with involvement of autonomic dysfunction in the development of IDH and is compatible with work by other groups (89,162).

Our study does have some potential weaknesses. We have only used small numbers of patients. The study should therefore be replicated with increased numbers. Furthermore, it would be interesting to monitor the same cohort of patients whilst on HD to see if the changes to haemodynamics seen during DAS are matched during HD. We do not have any direct measure of vascular function (in particular arterial stiffness),

which will also impact on response to pharmacological stress. However, this study has demonstrated that this method is not only applicable to this patient group but is also not associated with adverse effects.

In conclusion, this study demonstrates that Dobutamine-atropine stress in conjunction with non-invasive pulse wave analysis can be used to distinguish between HP and HR patients. It also provides an insight into the potential underlying pathophysiology responsible for the development of IDH. Intradialytic haemodynamic instability appears to be important in the development of recurrent cardiac injury. Worsening myocardial contractile reserve, predisposing to further instability, provides a 'vicious cycle' of escalating risk and harm within a single pathophysiological process.

7 PROSPECTIVE STUDY OF THE DETERMINANTS OF SECONDARY DEPLETION OF CARNITINE AND THE SKELETAL AND CARDIAC FUNCTIONAL EFFECTS OF L-CARNITINE SUPPLEMENTATION IN INCIDENT CHRONIC HAEMODIALYSIS PATIENTS

7.1 Introduction

Carnitine, as a substrate for carnitine palmitoyltransferase 1 (CPT1), plays an essential role in the translocation of intracellular long-chain fatty acyl groups into the mitochondrial matrix for subsequent β-oxidation (163.164). In addition, other functions of carnitine include modulating concentration of fatty acids, scavenging toxic acyl groups and facilitating their transport out of mitochondria. Impairment of fatty acid metabolism and accumulation of acyl CoA are characteristic of renal failure. Carnitine homeostasis in humans is maintained by endogenous carnitine synthesis, dietary carnitine intake, and efficient conservation of carnitine by the kidney, in order to maintain a total body content of around 120 mmol (165). Humans obtain approximately 2 to 12 $\mu mol \cdot kg^{-1}$ of carnitine per day from dietary sources such as meat and dairy products, and up to 1.2 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ by endogenous synthesis. Carnitine biosynthesis occurs predominantly in the liver and kidney. Almost all of the carnitine excreted during normal conditions occurs through the kidney (166), with more than 90% of filtered free carnitine (FC) being reabsorbed (167). In contrast, tubular absorption of acylcarnitine is limited, resulting in clearance levels between 4 and 8 times that of free carnitine, highlighting the role of carnitine in removal of potentially toxic acyl groups (168). More than 95% of the body's total carnitine (TC) store exists within skeletal muscle tissue (165) where one of its roles is to buffer excess acetyl groups from pyruvate oxidation, in a reaction catalysed by carnitine

Skeletal muscle TC content is known to be considerably reduced in ERF patients undergoing haemodialysis treatment. Haemodialysis vintage has been strongly correlated to the progression of TC depletion (169-172). However, despite 30 years of research in the area it remains unclear as to how and why this deficiency occurs. FC is freely filtered into the dialysate during a haemodialysis session and, as such, plasma TC concentration falls dramatically by up to 80% and is then replenished within a few hours of the cessation of HD (27,173,174). Thus, it has been suggested that FC is leached from tissue stores in order to maintain plasma TC concentration, resulting in the observed muscle TC deficiency (27,173). On the other hand, the calculated weekly TC loss (sum of weekly dialysate removal and urinary excretion) in HD patients (800 to 2300 μmol) has been reported to be similar, if not lower than weekly urinary TC excretion in healthy controls (1500 to 2500 μmol), leading to speculation that a reduction in dietary intake and/or endogenous synthesis of carnitine must cause muscle TC deficiency (169,173-178). Indeed, the kidney is a major site of carnitine synthesis within the body, and one might expect that ERF could well reduce endogenous carnitine synthesis. However, the above studies were conducted in patients who had been receiving HD treatment for more than 1 year, and it has subsequently been reported that a significant proportion of plasma TC depletion occurs within the first year of HD treatment, where muscle TC content is depleted by approximately 20% (172), suggesting that

intradialytic TC removal could be greater during the first year of HD treatment.

Exercise tolerance is clearly impaired in ERF patients undergoing haemodialysis treatment. Values for peak oxygen uptake (VO_2peak) in HD patients in the literature are around 20 $ml \cdot min^{-1} \cdot kg^{-1}$ and are consistently lower than age-matched controls (170, 171,179). The aetiology of this impairment in exercise capacity is likely multi-factorial, with impaired skeletal muscle blood flow (180-182), oxygen conductance (183,184), metabolism (171,185,186), and strength (179, 187) being implicated. Interestingly, the progressive decline in skeletal muscle TC content in HD patients has been correlated with exercise capacity (171), and HD patients have greatly reduced CPT1 activity compared to age-matched controls (170). Furthermore, both oral and intravenous L-carnitine supplementation have been reported to increase aerobic exercise capacity (29,188-190), muscle cross sectional area (29), muscle fibre diameter (30,191,192), and muscle strength (29).

Lack of myocardial contractile reserve is an important determinant of the haemodynamic response to haemodialysis. In a population whose cardiovascular morbidity is already significantly elevated this represents a significant problem. It is well established that inherited systemic carnitine deficiency causes cardiomyopathy (193). Patients improve dramatically with supplementation (194). Studies have also shown subnormal levels of FC in ischaemic heart disease and heart failure (195,196). Amelioration of

cardiac arrhythmias (27) and left ventricular ejection fraction (28) has been demonstrated in several studies following replacement therapy with L-carnitine in prevalent haemodialysis patients. Other risk factors associated with myocardial dysfunction found in prevalent haemodialysis patients such as lipid abnormalities, erythropoietin resistant anaemia and chronic inflammation may also be ameliorated with L- carnitine replacement (27, 197). In 2003, the National Kidney Foundation of America recommended the intravenous administration of 20mg.kg^{-1} L-carnitine three times a week for 9 to 12 months (198). This prescription was not validated and is perhaps excessive given that it is more than ten times the reported weekly TC excretion of ERF patients undergoing haemodialysis treatment.

7.2 Aims

1) To further investigate how skeletal muscle becomes TC deficient over the first year of HD.

2) Determine whether the deficiency is associated with impaired exercise tolerance.

3) Determine whether intravenous L-carnitine administration can prevent muscle TC depletion over the first year of HD treatment and any impairment in exercise capacity.

4) To observe any differential effect on cardiac function in incident haemodialysis patients who receive either 10 mg·kg^{-1} L-carnitine thrice weekly post dialysis versus placebo.

7.3 Methods

7.3.1 Subjects

Seven ERF patients participated in the study, which was approved by the Southern Derbyshire Local Research Ethics Committee in accordance with the Declaration of Helsinki. Before taking part in the study, all patients gave their written informed consent to take part in the study and were aware that they were free to withdraw at any point. Potential patients were recruited from clinics at the Renal Medicine Unit at the Royal Derby Hospital if they had been diagnosed with ERF, had a creatinine clearance of ≤10 $ml \cdot min^{-1}$, and were suitable to begin haemodialysis treatment. ERF patients on anticoagulation therapy or suffering from thrombocytopaenia were excluded due to potential bleeding risks following muscle biopsy. Subjects with exercise induced angina or a concurrent medical problem meaning that they would be unable to perform a graduated exercise test, with a history of seizures, or evidence of significant malnutrition were not included in the study. Patients were allocated to receive 10 $mg \cdot kg^{-1}$ L-carnitine (n=4; Shire Pharmaceuticals Ltd, Hampshire, UK) intravenously following each HD session (i.e. 3 times per week), or the equivalent volume of saline (n=3; control), over the first year of treatment. The demographic data of the patients and aetiology of ERF is presented in Table 14. Each subject had a baseline transthoracic echocardiogram prior to initiation of dialysis. These were carried out by cardiothoracic technicians at the Royal Derby Hospital.

7.3.2 *Experimental protocol*

Each patient reported to the laboratory no more than 1 week before, and 6 and 12 months after their first haemodialysis session, at the same time of day on each visit, and performed an incremental exercise tolerance test to volitional exhaustion. The incremental shuttle walking test is described in detail in Chapter 3. Isometric handgrip strength was measured using a dynamometer devised by Bassey *et al.* (100).

7.3.3 *Sample collection and analysis*

During each experimental visit, a muscle biopsy sample was obtained from each patients from the vastus lateralis, approximately 1 h after the exercise tests, using the percutaneous needle biopsy technique (101), and was snap frozen in liquid nitrogen less than 5 s after removal from the limb (see Chapter 3 for detailed description). These were performed by the investigator. The sample was subsequently freeze-dried at a later date and, after removal of visible blood and connective tissue, powdered and analyzed for free carnitine, acetylcarnitine, and long-chain acylcarnitine content using a modified version of the radioenzymatic method of Cederblad et al. (199). Samples were processed by Dr Francis Stephens and laboratory staff from the Centre for Integrated Systems Biology at the University of Nottingham. Values were subsequently summed in order to calculate muscle total carnitine content. In addition, plasma samples were obtained before and after the first dialysis session and before and after

haemodialysis sessions at 1, 3, 6, and 12 months. A 24-hour urine collection and a dialysate sample (from partial sampling of complete dialysate waste collection from a single 4h HD session) were also obtained at these time points. All of these samples were then stored at -80°C and analyzed at a later date for free and total carnitine concentration using the radioenzymatic assay described previously by Cederblad & Lindstedt (200). In addition, a post-HD dialysate sample was collected from 31 HD patients (age 62.1 ± 2.7 y; body mass 71.4 ± 2.5 kg) who had been on dialysis treatment for 41.4 ± 4.3 months (range 12-95 months). All analysis of these samples was carried out by staff within the Centre for Integrated Systems biology, University of Nottingham.

7.3.4 Haemodynamic Data Collection

At the initial dialysis session and at 1, 6 and 12 month dialysis sessions when samples were collected for determination of carnitine levels, systemic haemodynamic function was assessed using a Finometer (TNO Biomedical Instruments, Amsterdam, The Netherlands). The Finometer allows continuous noninvasive beat-to-beat pulse wave analysis at the digital artery using a cuff placed on the index finger providing estimates of cardiac output, stroke volume and peripheral resistance in addition to blood pressure and heart rate measurements. These parameters were recorded over an entire haemodialysis session. Detailed methods can be reviewed in Chapter 3. Results are generally reported as change from baseline except for blood pressure recordings which are recorded as absolute values.

7.3.5 Statistical analysis

Where numbers permitted, a two-way ANOVA (time and treatment effects; GraphPad Prism 4.02, GraphPad Software Inc, CA) was performed to identify differences in muscle, plasma, urine, and dialysate carnitine content, walking distance and handgrip strength and intradialytic mean arterial pressure, heart rate and percentage change in stroke volume. When a significant main effect was detected, data were further analysed with Student's *t* tests using the Bonferroni correction. Otherwise, time effects were determined using one-way ANOVA or Student's paired *t* tests.

7.4 Results

Patients

Unfortunately two of the patients in the control group dropped out of the study after 6 months, 1 of whom volunteered to return for the exercise tests. Other demographic data are shown in Table 14.

Subject	Group	Age (years)	Weight (kg)	Gender	Aetiology	Co-morbidities	Medication
CS	Control	61	66	Female	GN	IDH	Ca^{2+} blocker; ß-blocker; diuretic; aspirin; statin
SD	Control	40	90	Male	DN	Hypertension	ACEi; aspirin; statin
VM	Control	69	85	Male	GN	IDH	ACEi; Ca^{2+} blocker; diuretic; statin; phos binder
LJ	Carnitine	76	69	Male	Other	-	Ca^{2+} blocker; ß-blocker; diuretic; α-blocker; aspirin; statin; phos binder
GL	Carnitine	62	92	Male	APKD	Hypertension	ACEi; Ca^{2+} blocker; phos binder
KD	Carnitine	44	83	Male	APKD	Hypertension	ACEi; α-blocker
DP	Carnitine	79	81	Male	DN	IDH	Ca^{2+} blocker; diuretic; α-blocker; aspirin; statin; phos binder

Table 14. Subject demographic data, aetiology of ERF, co-morbidities, and medication prior to the start of the study. GN, glomerulonephritis; DN, diabetic nephropathy; APKD, adult polycystic kidney disease; IDH, intradialytic hypotension.

Plasma carnitine

Pre-HD session plasma TC concentration (Figure 6A) declined over the first 6 months of HD treatment in the 3 control patients from a mean of 65.1 ± 9.9 to 57.3 ± 8.8 $\mu mol \cdot l^{-1}$, and over 12 months in one patient (47.3 to 44.9 $\mu mol \cdot l^{-1}$). (Plasma TC normal reference range 34-78 $\mu mol \cdot l^{-1}$). In contrast, from a similar basal value of 79.1 ± 16.3 $\mu mol \cdot l^{-1}$, the i.v. administration of 10 $mg \cdot kg^{-1}$ L-carnitine to 4 patients following every HD session increased plasma TC concentration to 149.3 ± 2.4 $\mu mol \cdot l^{-1}$ after 1 month and then gradually increased to 250.2 ± 74.6 $\mu mol \cdot l^{-1}$ after 12 months, such that pre-HD plasma TC concentration was higher than the control group throughout the 12 months of HD treatment. The pre-HD plasma acyl to free carnitine ratio remained similar and constant in both control and carnitine patients after 6 (0.6 ± 0.3 and 0.3 ± 0.2 $\mu mol \cdot l^{-1}$ to 0.4 ± 0.1 and 0.4 ± 0.3 $\mu mol \cdot l^{-1}$) and 12 (0.5 (1 subject) and 0.7 ± 0.4 $\mu mol \cdot l^{-1}$, respectively) months of HD treatment. (Plasma acyl to free carnitine ratio normal reference range 0.1-0.8 $\mu mol \cdot l^{-1}$).

Plasma TC was depleted by a similar amount during the first HD session between the control and carnitine patients such that the post-HD concentration was 27.6 ± 2.8 and 35.7 ± 5.6 $\mu mol \cdot l^{-1}$, respectively (Figure 6B). However, whereas post-HD plasma TC concentration declined gradually after 6 (17.2 ± 3.7) and 12 (8.8; 1 subject) months in the control patients, it remained constantly higher in the carnitine patients (49.9 ± 8.3 and 52.0 ± 18.9 $\mu mol \cdot l^{-1}$ after 6 and 12 months, respectively).

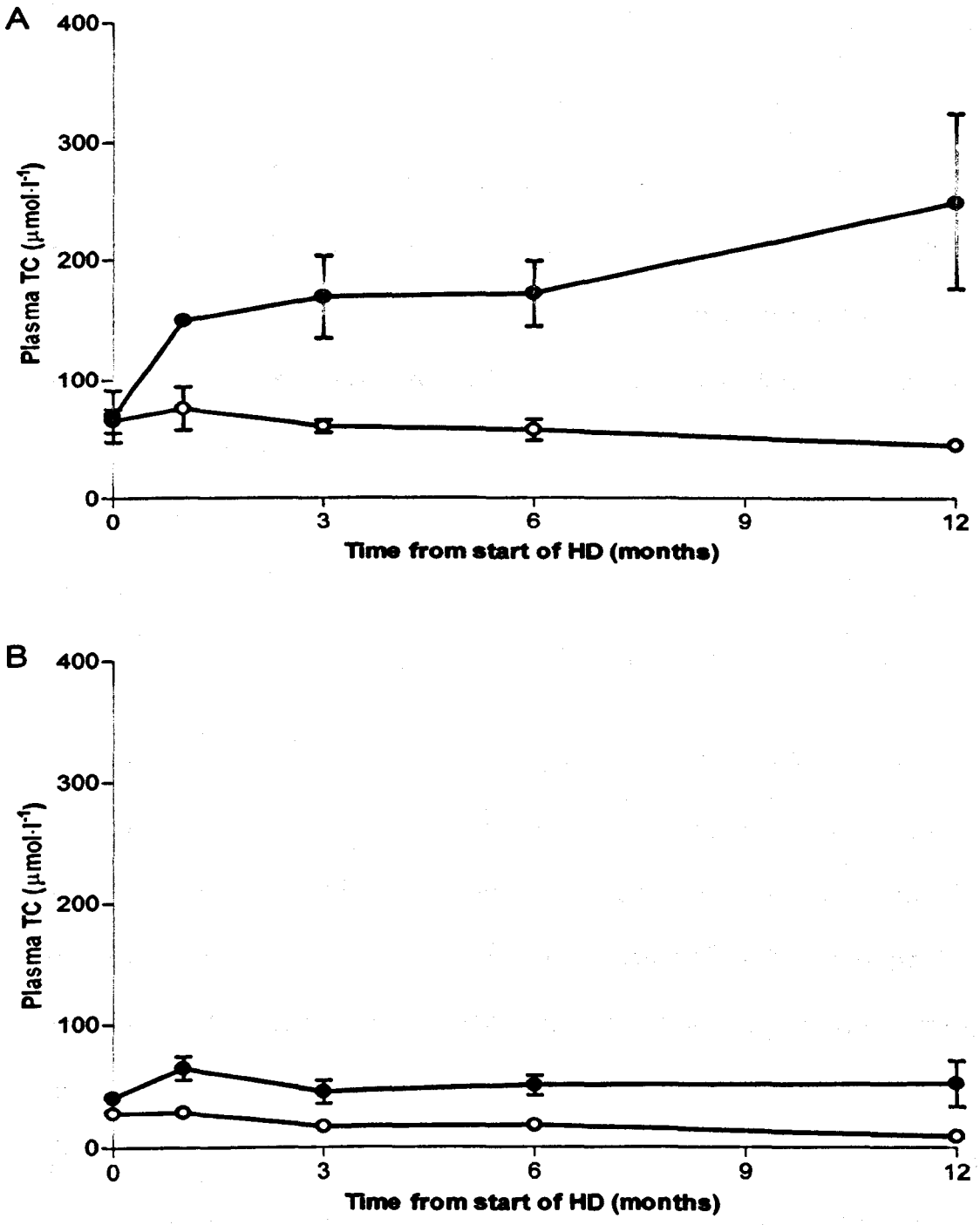


Figure 6. Plasma TC concentration before (A) and after (B) each HD session over the course of the first year of HD treatment in ERF patients receiving placebo (white circles) or 10 $mg \cdot kg^{-1}$ i.v. L-carnitine (black circles) following each HD session.

Urinary carnitine

Twenty-four hour urinary TC excretion before the first HD session was similar for the control and carnitine patients (231.3 ± 80.0 vs. 377.3 ± 36.7 µmol, respectively; Figure 7A). However, whereas urinary TC excretion declined over the first year of HD treatment in the control patients (43.7 ± 10.3 and 23.1 (1 patient) µmol for 6 and 12 months, respectively), 24 h urinary TC excretion increased to 716.4 ± 153.4 µmol after 1 month and remained constant for 12 months in the carnitine patients.

Dialysate carnitine

Dialysate TC content following the first* HD session was similar for the control and carnitine patients (481.7 ± 14.5 vs. 575.3 ± 108.4 µmol, respectively; Figure 7B). However, whereas dialytic TC removal appeared to decline over the first year of HD treatment in the control patients (1087.7 and 453.2 µmol after 6 and 12 months (1 patient), respectively), dialytic TC removal increased to 3086.4 ± 741.1 µmol after 1 month and remained constant for 12 months. In addition, the mean dialytic TC removal during a single HD session in 31 control ERF patients with a dialysis vintage of greater than 12 months was 414.5 ± 35.3 $\mu mol \cdot kg^{-1}$. There was no correlation between carnitine loss during a HD session and dialysis vintage (Figure 8).

* The duration of the first HD session was 2 h whereas all HD sessions thereafter were 4 h.

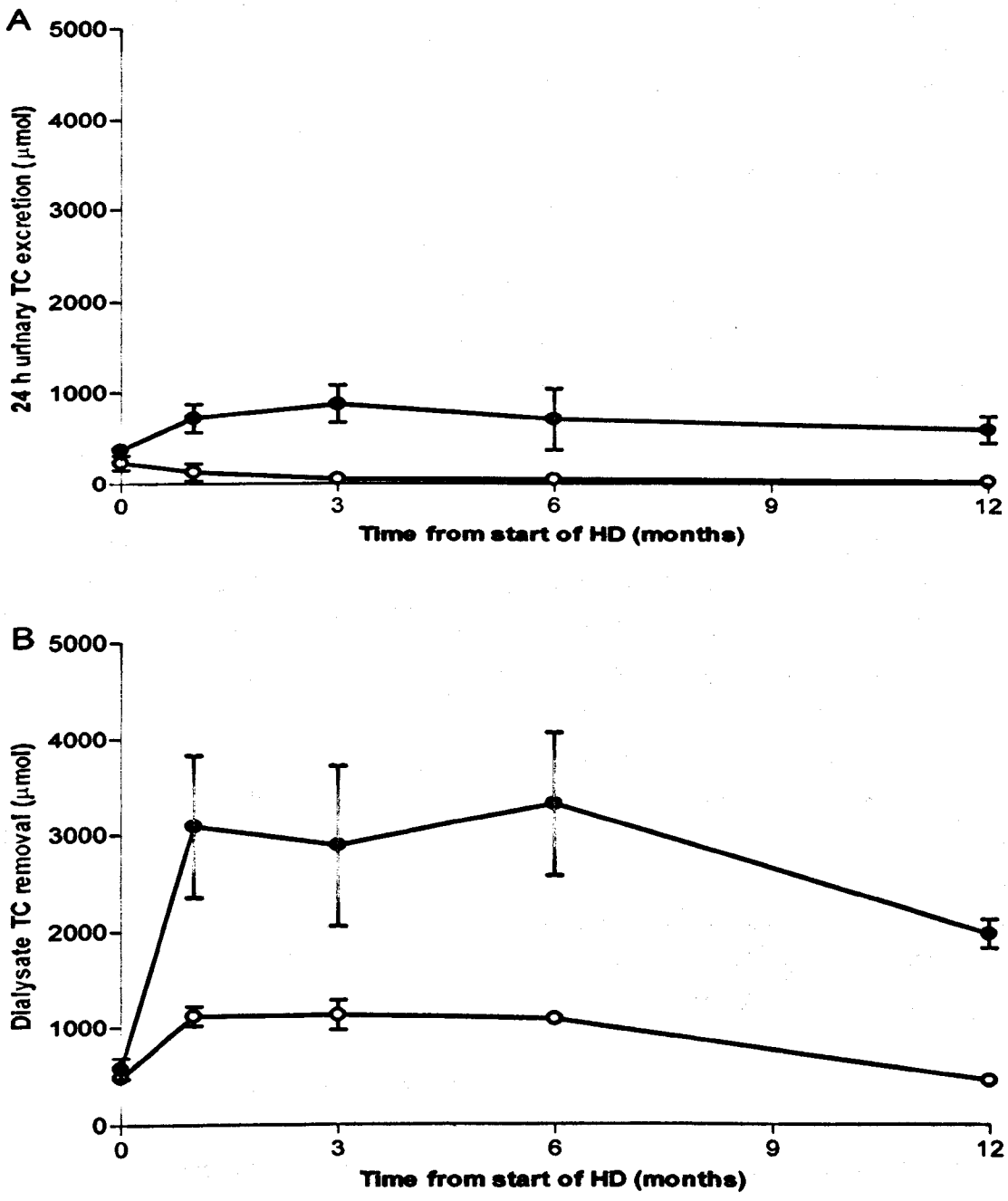


Figure 7. Twenty-four hour urinary TC excretion (A) and dialytic TC removal (B) over the course of the first year of HD treatment in ERF patients receiving placebo (white circles) or 10 $mg \cdot kg^{-1}$ i.v. L-carnitine (black circles) following each HD session.

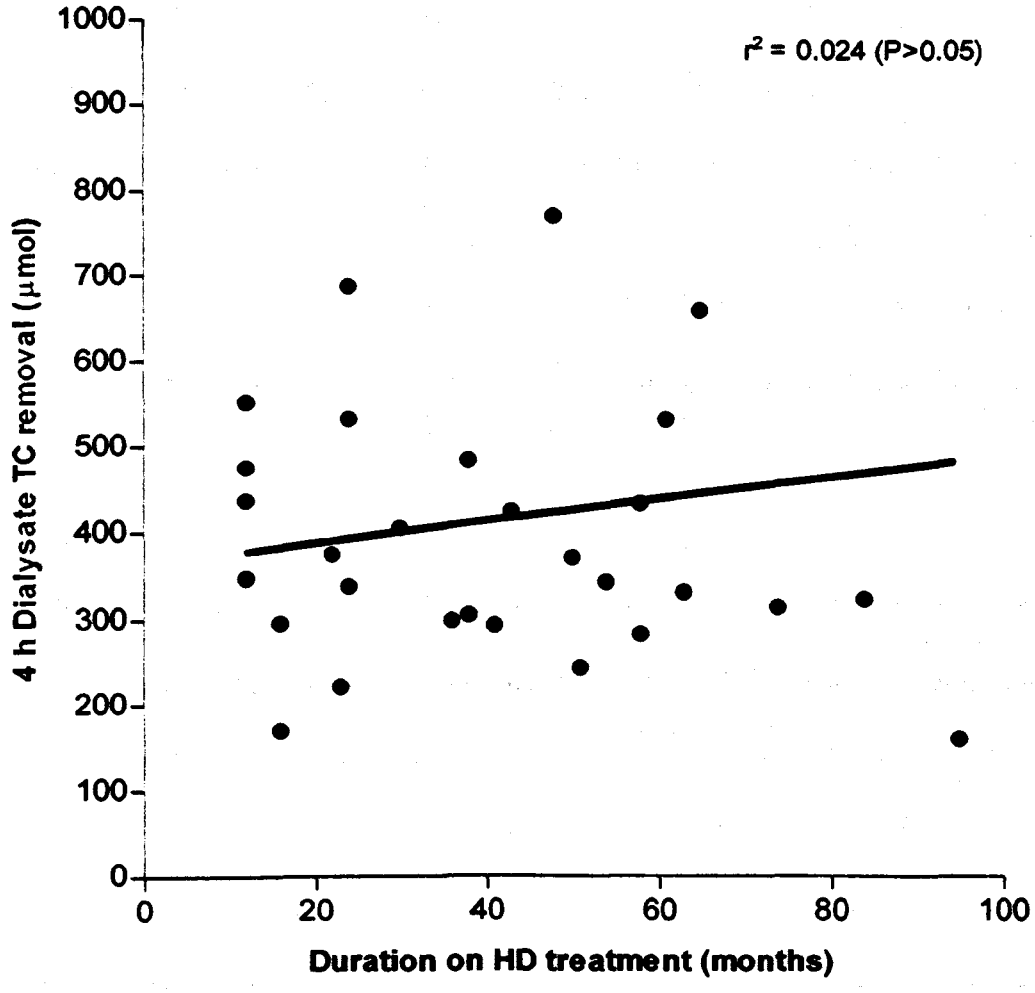


Figure 8. Relationship between dialytic TC removal during an individual 4 h HD session and number of months of receiving HD treatment in 31 ERF patients.

Muscle total carnitine

Skeletal muscle TC data are presented in Table 15 and illustrated in Figure 9. Muscle TC content decreased by 3.6 ± 0.7 mmol·(kg dm)$^{-1}$ (16%; P>0.05) after the first 6 months of HD treatment in the control group. Unfortunately, muscle biopsy samples were not obtained from two of the control patients after 12 months of HD treatment, although muscle TC appeared to decrease by a further 5% in one of the control patients. In contrast, there was no decline in muscle TC content following 6 or 12 months of HD treatment in the carnitine group, which remained at a similar value to baseline (Fig. 9).

Exercise tolerance

Total walking distance during the exercise tolerance test (Table 15) before and after 6 months of the commencement of HD treatment was similar in the control and carnitine patients. Over the first year of HD treatment total walking distance decreased in 2 of the control patients (by 30 and 60%) and 2 of the carnitine patients (by 20 and 40%), and increased in 2 of the carnitine patients (by 110 and 190%). There was a weak correlation (r^2 = 0.734; P = 0.06) between the change in muscle total carnitine content over the first 12 months of HD treatment and the change in total walking distance (Fig. 10).

Table 15. Muscle total carnitine content, total walking distance during an incremental exercise test, and handgrip strength over the course of the first year of HD treatment in 3 ERF patients receiving placebo (control) or 10 mg·kg-1 i.v. L-carnitine (carnitine) following each HD session.

		Muscle total carnitine			Walking distance			Handgrip strength		
Subject	Group	0 months	6	12	0	6	12	0	6	12
CS	Control	24.2	20.9	19.8	240	210	90	16.8	16.7	14.9
SD	Control	21.6	16.8	-	930	1020	-	59.8	58.9	-
VM	Control	22.0	19.5	-	500	360	340	39.8	32.5	30.1
Mean	*Control*	*22.6 ± 0.8*	*19.1 ± 1.2*	-	*557 ± 201*	*530 ± 249*	-	*38.8 ± 12.4*	*36.0 ± 12.3*	-
LJ	Carnitine	17.8	22.0	24.4	190	440	550	25.1	28.3	28.6
GL	Carnitine	21.1	21.8	23.5	630	560	490	40.3	41.0	43.1
KD	Carnitine	22.6	21.8	16.2	1020	920	620	60.8	66.3	68.0
DP	Carnitine	17.2	16.1	17.2	110	220	230	41.3	56.1	45.2
Mean	*Carnitine*	*19.6 ± 1.3*	*20.4 ± 1.4*	*20.3 ± 2.1*	*488 ± 211*	*535 ± 146*	*473 ± 85*	*41.9 ± 7.3*	*47.9 ± 8.4*	*46.2 ± 8.1*

Values (± *SEM*) are expressed in mmol·(kg dm)$^{-1}$, m, and kg for muscle total carnitine content, walking distance, and handgrip strength, respectively. Compared to 0 months, repeat measures at 6 or 12 months demonstrate no statistically significant change.

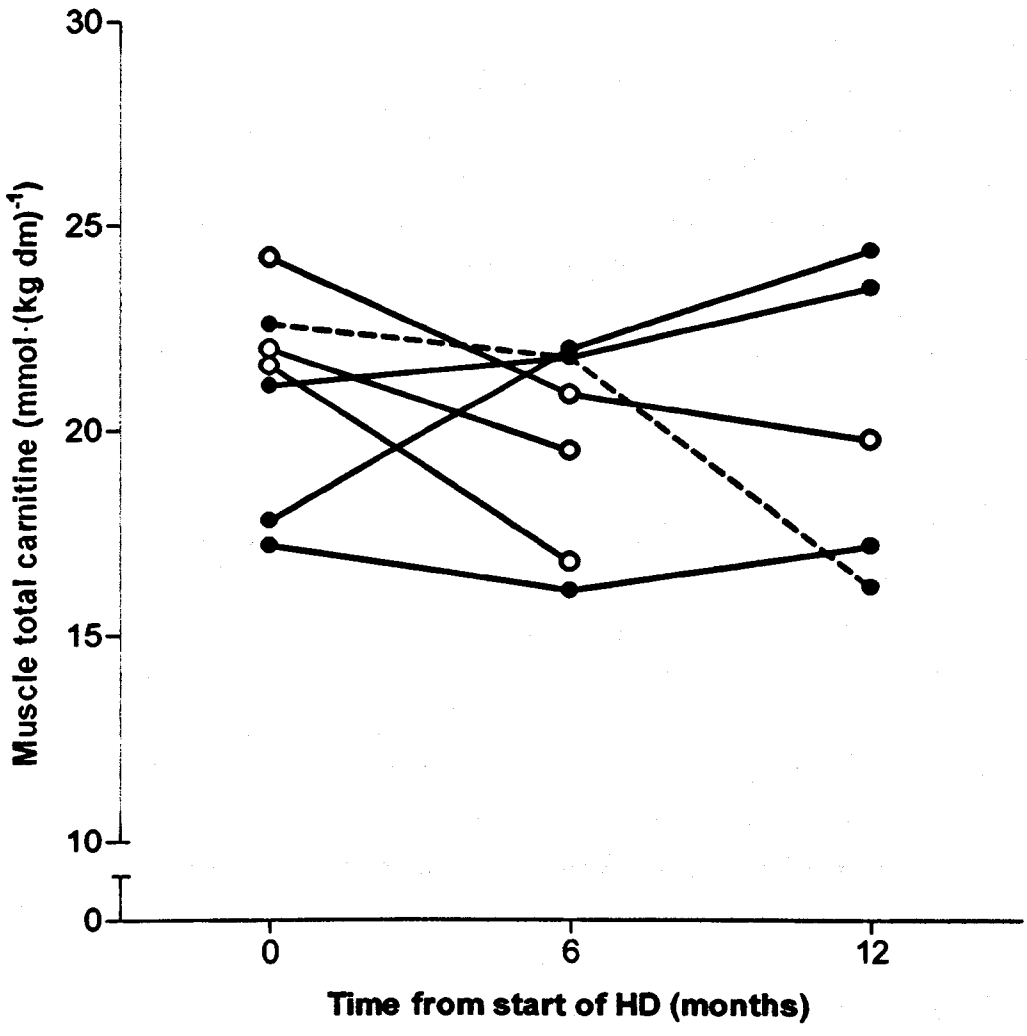


Figure 9. Muscle TC content over the course of the first year of HD treatment in ERF patients receiving placebo (white circles) or 10 mg·kg^{-1} i.v. L-carnitine (black circles) following each HD session. The dashed line represents a patient (KD) whose muscle TC store did not appear to respond to i.v. L-carnitine treatment.

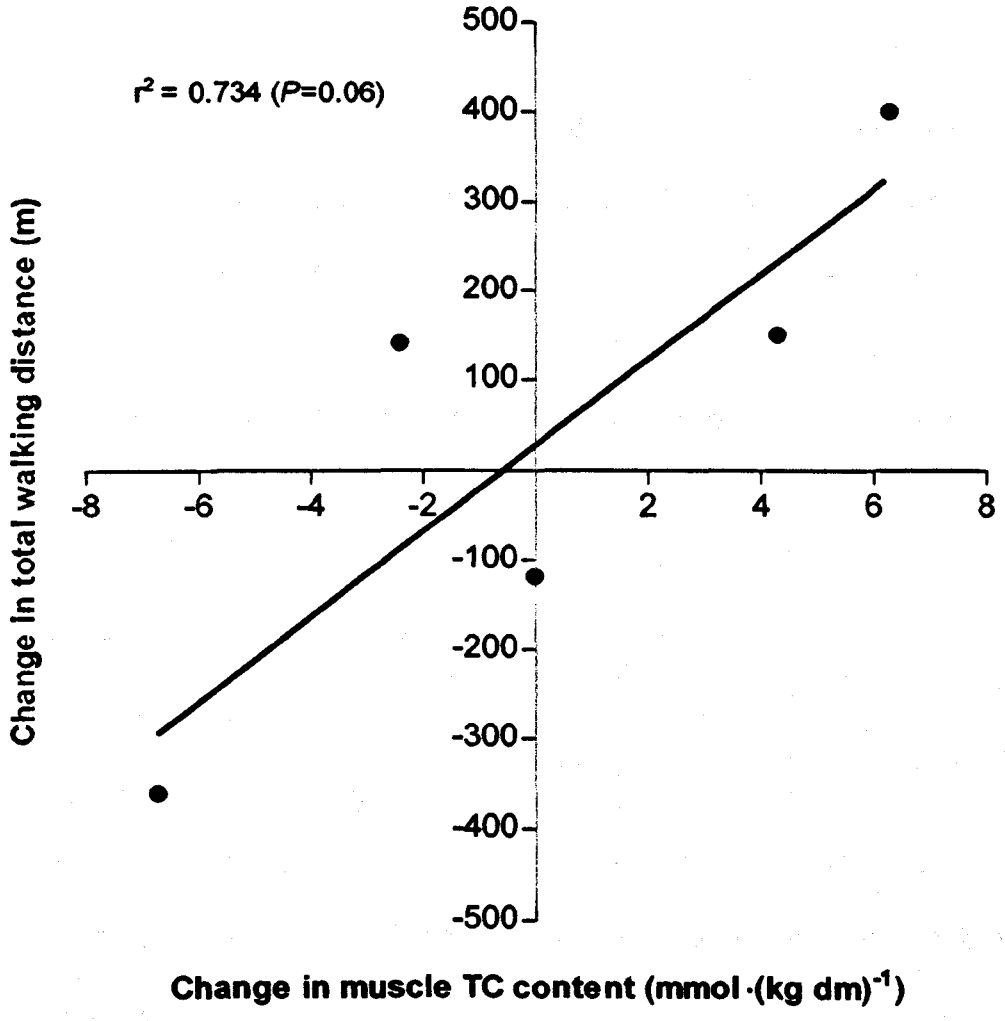


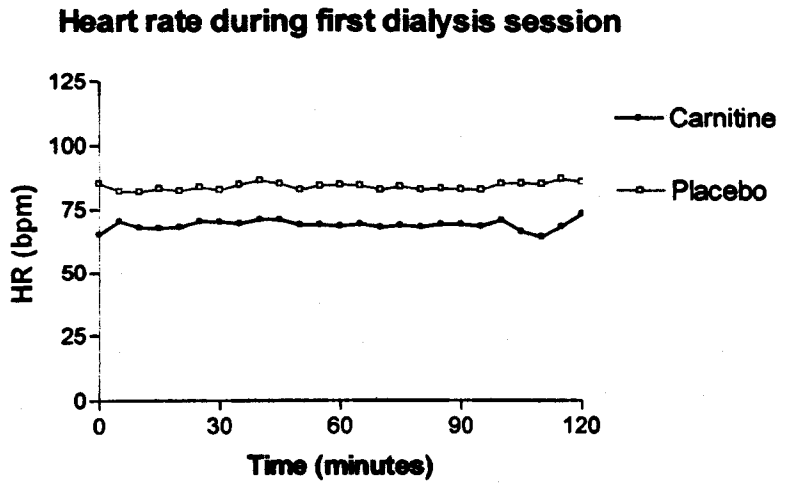
Figure 10. Relationship between the change in muscle TC content over the course of the first year of HD treatment in ERF patients receiving placebo or 10 mg·kg^{-1} i.v. L-carnitine following each HD session and the change in aerobic exercise capacity.

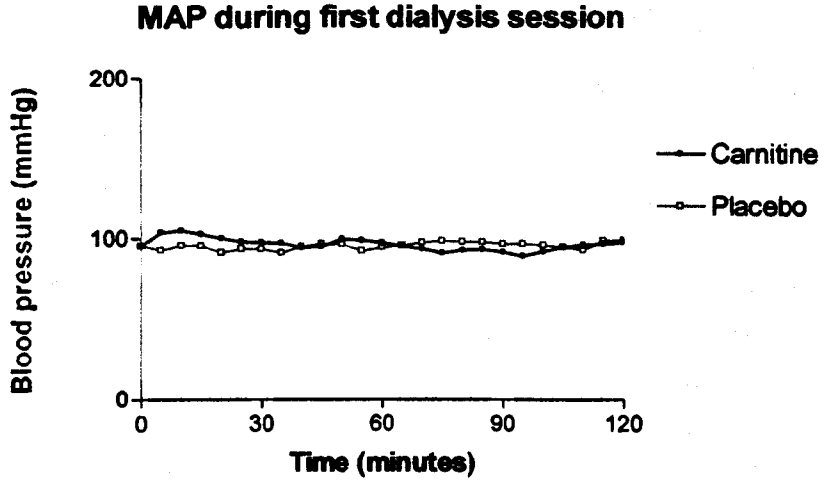
Handgrip strength

Handgrip strength data are presented in Table 15. There were no differences in handgrip strength between the control and carnitine patients before the commencement of HD treatment. Furthermore, despite handgrip strength appearing to decrease in each control patient and increase in each carnitine patient, there were no significant differences in handgrip strength over the first year of HD treatment and no correlation (r^2 = 0.001) between the change in muscle carnitine content over the first year of HD treatment and the change in handgrip strength.

Cardiovascular Data

Baseline transthoracic echocardiograpy demonstrated overall left ventricular systolic function to be within normal limits in each subject. Left ventricular ejection fraction (LVEF) was > 50%. There were no differences between heart rate (HR), mean arterial pressure (MAP) and percentage change in stroke volume (SV) from start of dialysis between the groups at the first dialysis session. Similarly no significant differences were noted between the groups for the same variables HR, MAP and SV at 6 and 12 months. (NB only one patient remained in the placebo group at 12 months). There were no significant changes noted in HR, MAP and stroke volume within the first 120 minutes of dialysis in the carnitine treated group at time 0 and 12 months. Cardiovascular data are illustrated in figures 11-13.





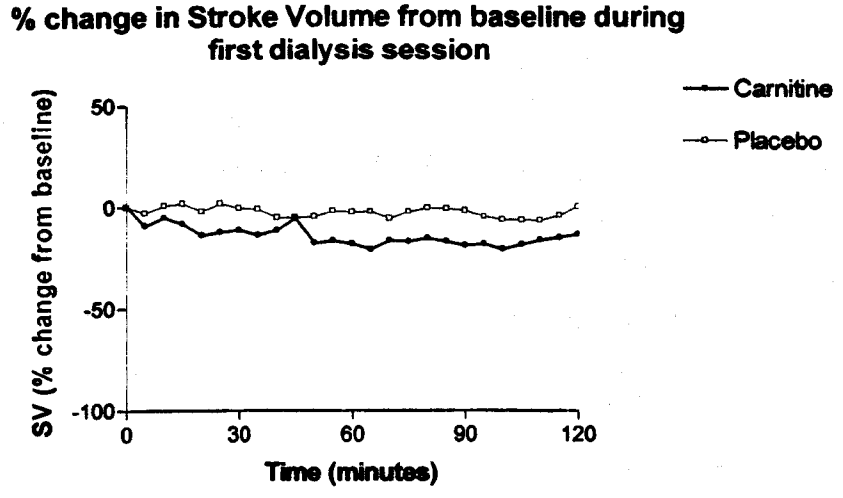


Fig.11 Heart Rate, Mean Arterial Pressure and % change in Stroke Volume from baseline between carnitine and placebo groups at initial dialysis

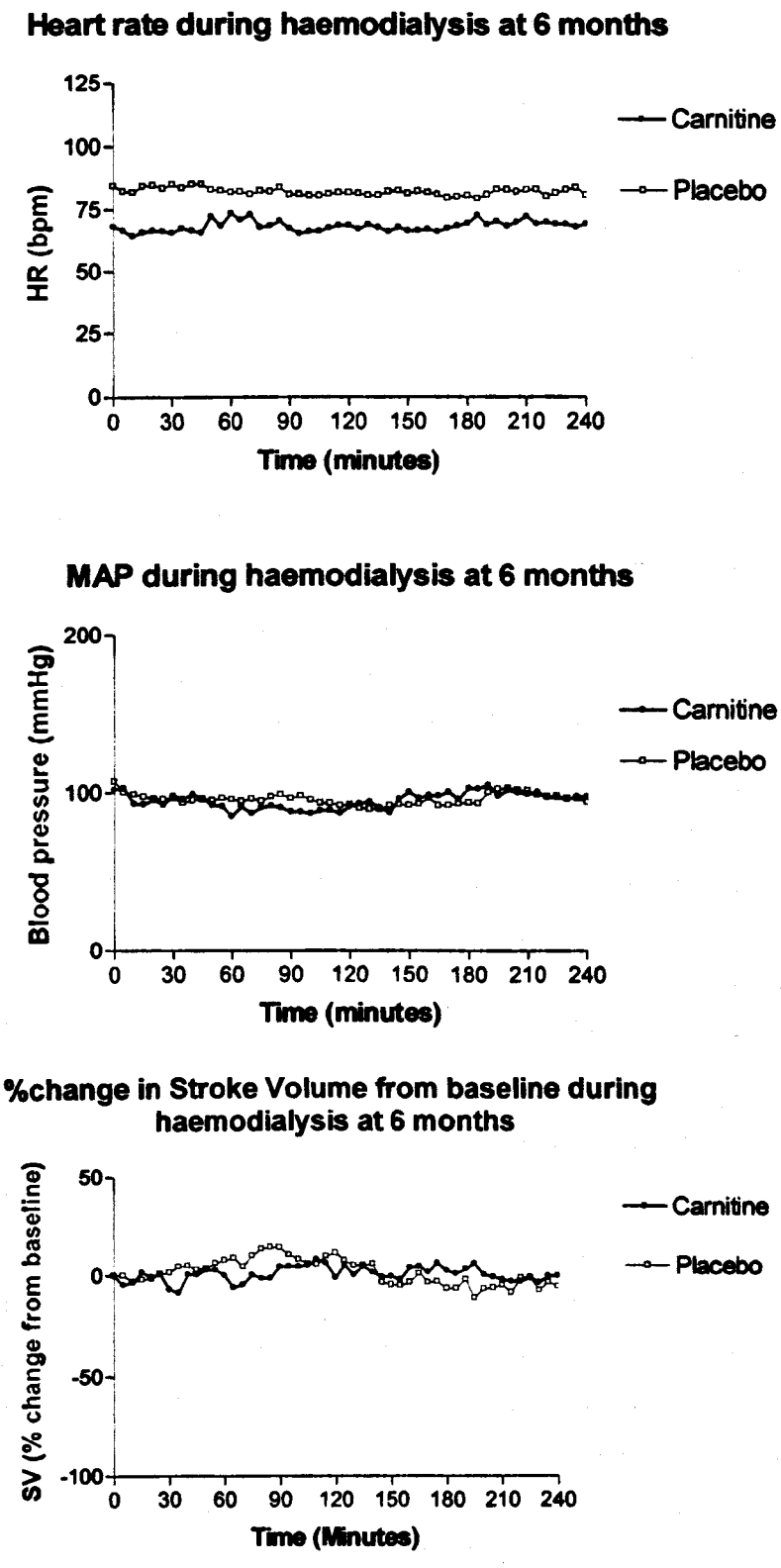
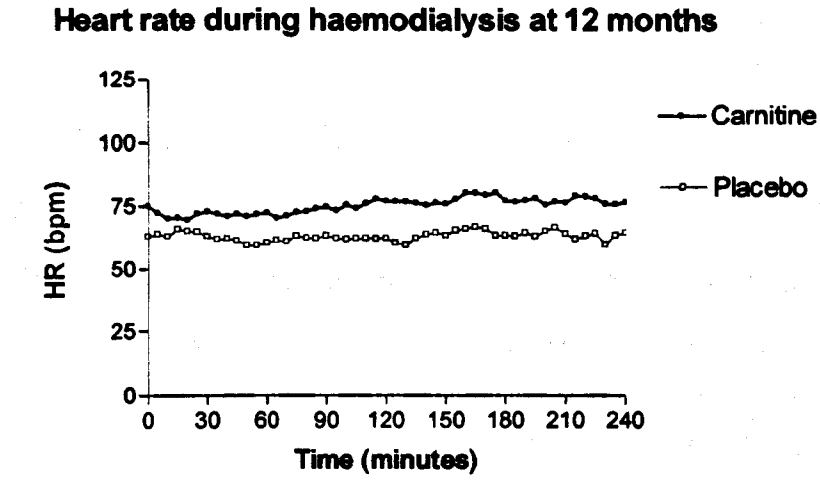
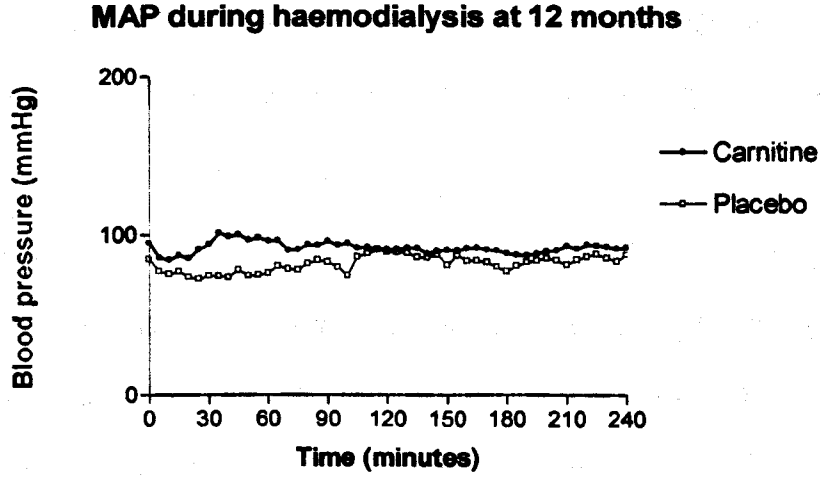


Fig.12Heart Rate, Mean Arterial Pressure and % change in Stroke Volume from baseline between carnitine and placebo groups on dialysis at 6 months





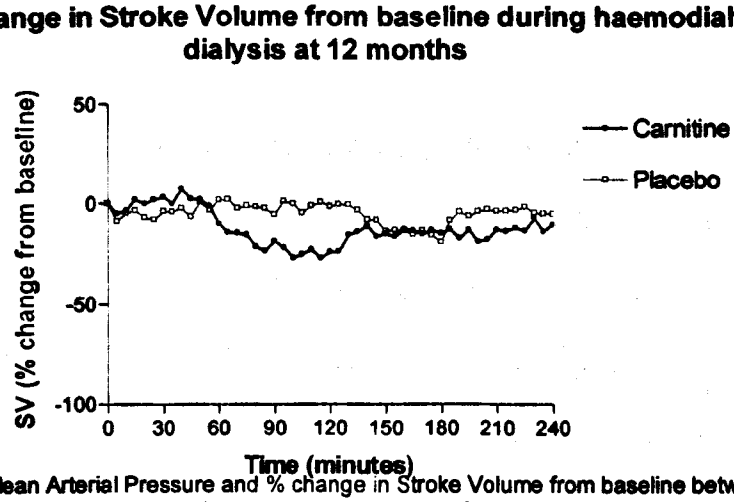


Fig.13 Heart Rate, Mean Arterial Pressure and % change in Stroke Volume from baseline between carnitine and placebo groups on dialysis at 12 months

7.5 Discussion

Skeletal muscle total carnitine content is known to be reduced in patients undergoing HD treatment, with dialysis vintage being strongly correlated to the progression of carnitine depletion (170-172). In the present study, following the first 6 months of HD treatment in 3 ERF patients, skeletal muscle TC content declined by around 15%, and by a further 5% in 1 of the patients after 12 months (Table 15, Fig. 9). This finding is in very good agreement with a study in which muscle TC content was reduced by 10 and 20% after the first 6 and 12 months, respectively, of HD treatment in 5 ERF patients (172). The principal finding of the present study was that intravenous administration of 10 $mg \cdot kg^{-1}$ L-carnitine following every HD session during the first year of HD treatment in 4 ERF patients was sufficient to prevent this observed decline in muscle TC content. A key question that has not been addressed in the literature is why and how does the muscle TC store become depleted during HD treatment?

FC is freely filtered into dialysate during HD. In the present study, over the first 6 months of HD treatment dialytic TC removal appeared to be at a steady-state value of around 1100 µmol per HD session (Fig. 7B) and daily urinary TC excretion was on average 100 µmol (Fig. 7A) in the placebo group, equating to a weekly TC loss of approximately 4000 µmol. Weekly urinary TC excretion has previously been calculated in age matched controls to be around 1700 µmol (177). Indeed, weekly urinary TC excretion before the start

of HD treatment in both groups of the present study was approximately 2000 µmol. Taken together this equates to a difference in TC excretion over 6 months of around 56 mmol between normal values and the HD patients in this study. During this period, muscle TC content was depleted by around 3 mmol·(kg wet muscle)$^{-1}$ (Table 15). This clearly suggests that the depletion of muscle TC content over the first 6 months of HD treatment is at least partly attributable to the intradialytic loss of TC during a HD session. Indeed, dialytic TC removal and muscle TC depletion within the second 6 months of the present study appeared less than in the first 6 months. After the first year of HD treatment it would appear that urinary TC excretion is negligible (Fig. 7A) (as might be expected due to decreasing urinary output) and dialytic carnitine removal is approximately 450 µmol (Fig. 7B and Fig. 8). This equates to a similar, if not lower, weekly TC loss compared to age matched controls (1350 vs. 1700 µmol). This latter observation has been reported before (169, 173, 174, 175, 178). It would also appear that after the first year of HD treatment, intradialytic TC removal remains constant over dialysis vintage (Fig. 8). Taken together these findings demonstrate, for the first time, that the rate of muscle TC depletion that occurs in ERF patients on HD treatment is greatest within the first 6-12 months of HD treatment as a result of carnitine removal during the HD procedure. Indeed, similar to the findings of Evans et al (172), muscle TC content was depleted by 20% following 1 year of HD treatment. In the patients receiving 10 mg·kg^{-1} L-carnitine (approximately 4500 µmol) following each HD

session in the present study, dialytic TC removal was at a steady state of 3000 μmol per HD session for the first 6 months of treatment (Fig. 7B), and daily urinary TC excretion averaged around 750 μmol (Fig. 7A), equating to a weekly TC loss of around 14 mmol. As weekly L-carnitine administration also equated to approximately 14 mmol for the patients in the present study, this would suggest that the loss of TC during HD was matched by the administered L-carnitine and that any excess carnitine was removed during HD. This is reflected by the finding that muscle TC content remained constant over the first year of HD treatment in the carnitine group (Table 15, Fig 9).

Plasma TC concentration before the start of HD treatment in the present study was greater than normal (mean of 73.1 ± 9.9 $\mu mol \cdot L^{-1}$ for both groups; Fig. 6A) and similar to that reported in previous studies (27,172). This is probably indicative of an inability to excrete acylcarnitine due to impaired renal function. Indeed, in agreement with other reports (27), the acyl to free carnitine ratio was greater than the normal value of 0.2 in both groups. Also in agreement with other studies (27,173,174), plasma TC concentration decreased by around 40 $\mu mol \cdot L^{-1}$ (60 to 80%) per HD session in the control patients, and by around 120 $\mu mol \cdot L^{-1}$ (55 to 75%) in the patients receiving L-carnitine (Fig. 6B). By dividing the amount of TC removed by the dialysate by the fall in plasma TC concentration, the volume of distribution for carnitine can be calculated as approximately 25L. This value is slightly higher than that reported in previous studies, which

have predicted the volume of distribution for carnitine to be equivalent to the extracellular space i.e. 20L (201), perhaps suggesting that other tissues (e.g. skeletal muscle) may release carnitine during the HD process. Indeed, it is well reported that the plasma TC compartment is replenished within a few hours following a HD session (27, 173, 174), and muscle TC content has been reported to decrease during the dialysis procedure (although the dialysate TC content could not explain the 20% decrease in muscle TC content after a single HD session;(169, 173)). Thus, it could be speculated that when the plasma TC concentration falls below a certain threshold the 100-fold TC concentration gradient between plasma and muscle cannot be maintained via the Na^+ dependent carnitine transporter (OCTN2) and TC is leached from the muscle. L-carnitine administration probably prevented any loss of TC from skeletal muscle (and possibly other tissues) in the present study as post-HD plasma TC did not fall below the normal concentration (Fig. 6B) and/or the timing of the post-HD L-carnitine injection prevented the need for the characteristic replenishment of the plasma TC pool. Another possible explanation is that the HD procedure in itself causes muscle damage and, thus, release of muscle TC into the circulation. Indeed, uremia *per se*, rather than disuse, causes atrophy and de-capillarisation as non-locomotor muscles of HD patients demonstrate these characteristics compared to matched controls (187), although the mechanism by which this occurs requires further investigation.

Exercise tolerance is clearly impaired in ERF patients undergoing HD treatment. Values for VO_2peak in HD patients in the literature are around 20 $ml \cdot min^{-1} \cdot kg^{-1}$ and are consistently lower than age-matched controls (170, 171, 179). As would be expected with such a chronic condition, the aetiology of the impairment in exercise capacity is likely multi-factorial and several causes have been suggested. For example, muscle strength is reduced in ERF patients undergoing HD. This is most probably due to the muscle atrophy that occurs in HD patients and is an accurate predictor of aerobic exercise capacity (VO_2peak, exercise duration, peak ventilation, and peak blood lactate concentration), suggesting that altered skeletal muscle function could partly explain the impaired exercise tolerance of HD patients (179). In addition, skeletal muscle blood flow is greatly reduced in response to exercise in HD patients. There are necrotic capillaries within the muscle microcirculation, and there are fewer capillaries per muscle fibre compared to age-matched sedentary controls (179-181). This would suggest that oxygen delivery to skeletal muscle during exercise limits aerobic capacity. Indeed, impaired O_2 transfer from the muscle microcirculation to mitochondria has been demonstrated during exercise in HD patients compared to age-matched sedentary controls (183,184). However, although exercise training has been reported to increase maximal workload, muscle capillarisation and muscle fibre cross-sectional area, and reduce the number of atrophic fibres (170, 187, 202), there are conflicting reports as to whether exercise training increases VO_2max (27,170,187,202,203). Furthermore, increasing

haemoglobin to a normal content in anaemic HD patients using recombinant human erythropoietin therapy appears to have little or no effect on aerobic exercise capacity (183,186,204). On the other hand, abnormal skeletal muscle energy metabolism could play a role in the impaired aerobic capacity observed in these patients. For example, at the same relative exercise intensity it has been demonstrated that HD patients have a lower intracellular pH and greater phosphocreatine (PCr) degradation (measured by magnetic resonance spectroscopy) compared to age-matched sedentary controls (185,186), and the lactate threshold is reached at a much lower workload (171). The fact that this increased anaerobic glycolysis occurs early in exercise (185) would suggest an intrinsic impairment in mitochondrial ATP production during exercise. However, it has been consistently demonstrated that mitochondrial capacity (electron transport chain activity) appears normal, if not increased, in isolated muscle mitochondria from HD patients, possibly via an adaptive mechanism (180, 187, 203, 205, 206), and muscle mitochondrial content appears normal (206). Therefore, if skeletal muscle energy metabolism were limiting to aerobic exercise capacity in HD patients, it is likely that an enzyme inhibition or substrate deficiency *per se* would be involved. In addition, the finding that some HD patients do not respond to exercise training at all, whereas renal transplant patients do (207), would suggest that the HD procedure itself impacts on the impaired exercise tolerance.

In this respect, the progressive depletion of skeletal muscle carnitine content in HD patients has been correlated with exercise capacity (171). Individuals with primary/systemic carnitine deficiency, an autosomal recessive disorder of fatty acid oxidation caused by mutations in the gene encoding OCTN2, have been reported to have a muscle TC content of less than 10% of normal, which is associated with pronounced exercise intolerance and muscle weakness (208-210). Haemodialysis patients also have greatly reduced muscle CPT1 activity compared to control (170). Furthermore, both oral and intravenous L-carnitine supplementation in ERF patients undergoing long-term HD treatment have been reported to increase aerobic exercise capacity (29,188 190, 211), muscle cross sectional area (29), muscle fibre diameter (30,191,192), and muscle strength (29). In contrast, previous studies have also failed to show any significant effect of intravenous L-carnitine administration on muscle anaerobic ATP production (measured by magnetic resonance spectroscopy) in HD patients during exercise (212). However, whether the maintenance of skeletal muscle TC content observed in the present study with L-carnitine treatment (Table 15, Fig. 8) was associated with a prevention of the decline in aerobic capacity and muscle function associated with HD treatment is unclear, mainly because we were unable to detect an effect of HD treatment on aerobic capacity in the control group (Table 15). This could be due to the small number of patients, or because muscle TC was not depleted to a content that was limiting to aerobic capacity. Nevertheless, aerobic

capacity and muscle strength appeared to be maintained in the carnitine group (Table 15) and there was a weak correlation (P=0.06; Fig. 10) between the change in muscle TC content after 12 months of HD treatment and the change in aerobic capacity (total walking distance during the incremental exercise test).

Cardiac complications are the leading cause of mortality in patients with chronic renal failure. It has been widely established that patients with ERF undergoing haemodialysis therapy exhibit low endogenous levels of L-Carnitine and elevated acylcarnitine levels. The clinical implications of this altered carnitine profile are not entirely clear. Numerous studies over the past few decades have investigated the effects of L-carnitine on cardiac dysfunction and intradialytic complications thereof, notably intradialytic hypotension. Administration of L-carnitine has been shown to be associated with a significant reduction in the number of intradialytic hypotensive events when compared with placebo (29, 30). A significant correlation between LVEF and endogenous L-carnitine levels has been reported. These patients exhibiting considerable improvement in LVEF after administration of L-carnitine post dialysis for a period of three months (213). This was particularly pronounced in patients experiencing recurrent hypotensive episodes. These symptomatic patients had a significantly lower ejection fraction than their asymptomatic counterparts. Further studies have provided similar findings (28). In the present study baseline echocardiography revealed normal left

ventricular function in all patients. Left ventricular ejection fraction was >50%. Finometer data did not demonstrate any significant differences in intradialytic MAP, HR and SV between patients treated with L-carnitine or placebo over the study period. There was also no significant difference between values observed within the carnitine group at baseline and at 12 months. Interestingly within this small study no patients demonstrated problems with episodes of significant intradialytic hypotension. This is slightly unusual and would suggest that our particular study group is not typical of the larger haemodialysis population. Fagher et al.(214), conducted a 6 week, double blind, controlled study in 28 haemodialysis patients, who received 2g IV L-carnitine after each haemodialysis session. This demonstrated no effect of L-carnitine on left ventricular function. The average value of ejection fraction was 62% at baseline however. This might imply that the greatest benefit from carnitine replacement is expected only in patients whose baseline cardiac function is significantly abnormal.

8 CONCLUSIONS, LIMITATIONS & FUTURE WORK

CONCLUSIONS, LIMITATIONS & FUTURE WORK

Chronic kidney disease is associated with profound alterations in body composition that have far reaching consequences at cellular and systemic level in addition to overall patient function. Therapeutic manoeuvres in the predialysis population to preserve eGFR may lead to an augmentation in functional anomalies. A similar paradigm exists within the dialysis population where interventions directed towards maintenance of adequate homeostasis including the dialysis process itself may actually adversely effect function at all levels.

Goal directed antihypertensive treatment in older CKD subjects

We have demonstrated that in a carefully selected group of individuals it is possible to achieve target blood pressure within older subjects with CKD and demonstrated stability in renal function at 12 months. Using the Timed get-Up and Go test as a measure of function we have demonstrated a significant decline within the CKD subjects which was not reflected in the non-CKD group. However, based on classification of the TUG testing system this decline only relates to minimal impairment. This was not associated with an increased risk of falls in this patient group. In fact the falls rate within groups (CKD, non-CKD) or taking the study group as a whole, was less than would be expected in the general population within the age range included in this study.

There were no significant differences in BIA measurements of muscle, bone or fat mass in the short-term (4 weeks) resulting from antihypertensive therapy. At twelve month follow up CKD subjects demonstrated a higher mean decline in muscle mass than non-CKD subjects although this difference did not reach statistical significance. This increased decline might be expected due to uraemic effects on muscle.

There were no significant changes in fat mass over time. BIA measured bone mass declined in both groups over the study period but this was not significantly different between groups. Based on the results from this study we therefore conclude that antihypertensive therapy does not result in significant alterations in muscle, bone or fat mass above any change that might be expected in a non-CKD population.

The major limitation with this study relates to selection bias. We have excluded subjects with background morbidities that might predispose to functional abnormalities and falls such as diabetes mellitus (eg. autonomic neuropathy, peripheral neuropathy) and those subjects already attending falls services. Indeed the greatest risk factor for falls is the presence of previous falls. However, with this particular study we have demonstrated that with careful individualised treatment goal directed antihypertensive therapy is safe and an effective intervention to preserve renal stability in subjects with CKD.

To take this work forward in the future it would be useful to involve subjects with greater degree of renal impairment. Mean eGFR in the

CKD group was only 40mls/min. Effects of features associated with uraemia such as inflammation and acidosis, which we have not really considered independently in this study are more likely to be of greater significance in subjects with higher levels of renal impairment.

Bone collagen formation rates and expression of osteotropic factors

There is an increasing evidence base demonstrating that far from being an inert structural support system the skeleton has major dynamic functions that are essential for normal homeostasis. There are clear links between skeletal dysfunction and predisposition to fracture potential, bone pain and importantly the development of vascular calcification which may also be impacted upon by our attempts to 'normalise' surrogate markers of bone turnover and provide sufficient activity to buffer the internal milieu.

Our study in male chronic haemodialysis patients is the first to compare iPTH measurement with a measure of dynamic bone turnover. In the 9 patients studied iPTH did not correlate with bone collagen FSR. Bone collagen FSR was higher than matched non-CKD controls. The significance of these findings is however severely impaired by the small numbers studied. Unfortunately due to unanticipated problems with methodology we were unable to assess the effect of serum phosphate optimisation on bone collagen FSR and any differential effect of calcium versus non-calcium containing phosphate binder medication. The reliance on a baseline skin sample

as a surrogate marker of bone turnover revealed unanticipated problems. In non-CKD subjects previous work using this specific technique had shown that collagen kinetics within skin were comparable to bone. Unfortunately we observed that levels of ^{15}N within skin at baseline were far in excess of those post ^{15}N labelled proline found in bone and therefore it was not possible to estimate uptake. The reasons for this are unclear. Further studies using alternate markers or investigation of uraemic effects on skin collagen are required before this technique can be applied in this setting again but the concept of measuring dynamic bone function over a period of hours involving single bone biopsy would be very appealing to improve in our understanding of the effects of uraemia and its consequent treatment on overall bone function. The relative acellular nature of bone, small samples obtained in this study and instability of RNA made observation of expression of gene products of bone turnover difficult. Larger samples would help with this problem but increase the potential risks of bone biopsy. Recent development of newer preservatives to prevent the denaturing of RNA might prevent this problem.

The interplay of effects of uraemia on the skeleton is complex. Given the invasive nature of bone biopsy the search for adequate serological and urinary markers to demonstrate skeletal activity is extremely important. To date assessment of bone health remains reliant on multiple factors.

Intradialystic hypotension remains highly prevalent in the chronic haemodialysis population, the resulting myocardial hypoperfusion, segmental ischaemia and subsequent myocardial dysfunction (myocardial stunning) lead to irreversible left ventricular dysfunction. We have demonstrated that in a group of chronic haemodialysis patients Dobutamine atropine stress with simultaneous non-invasive measurement of cardiac parameters can be performed without untoward side effects and used to differentiate correctly between those known to be hypotension prone and those who are not. Failure to increase cardiac output was primarily due to a greater fall in stroke volume in the hypotension prone group as both groups were able to increase their heart rate by similar amounts.

This study is again limited by a small number of subjects involved and should be repeated with greater numbers. In the future this technique could be applied to a clinical setting in order to identify patients with low myocardial functional reserve, without exposing them to recurrent episodes of dialysis induced hypotension and resultant permanent myocardial injury, with earlier utilisation of less haemodynamically stressful dialysis treatments.

Carnitine depletion and skeletal and myocardial functional effects of carnitine replacement in incident chronic haemodialysis patients

The characteristics of cardiovascular disease and progressive impairment of exercise capacity have been linked to reductions in serum L-carnitine levels and skeletal muscle carnitine. Major carnitine stores are within skeletal muscle and cardiac muscle where its main role is in the translocation of fatty acids into mitochondria for β-oxidation and eventually ATP production. During dialysis carnitine is readily filtered and not replaced. Plasma carnitine levels are subsequently replenished by skeletal and possibly myocardial stores. We have demonstrated that the characteristic decline of muscle TC content observed in ERF patients is greatest within the first 6-12 months of HD treatment and is associated with a substantial intradialytic removal of TC. The reason for the greater loss of TC from the muscle store into the dialysate during the first year of HD is not known and warrants further investigation. It is unclear from the present study whether it results in impaired aerobic capacity on muscle function. After the first year of HD treatment, factors other than dialytic TC loss, such as a decrease in dietary carnitine intake and/or decrease in endogenous synthesis (particularly in the failing kidney) most likely contribute to the progressive depletion of the muscle TC store. This also requires further investigation. Another important finding from the present study was that intravenous

administration of 10 $mg \cdot kg^{-1}$ L-carnitine following every HD session (i.e. 3 times per week) is more than sufficient to prevent the decline in muscle carnitine content over the first year of HD treatment, and also appears to maintain exercise tolerance. In the context of muscle TC content, the guideline from the National Kidney Foundation of America recommending the intravenous administration of 20 $mg \cdot kg^{-1}$ L-carnitine three times a week for 9 to 12 months (198) was excessively high.

Regarding cardiovascular function, the present study does not demonstrate any measurable benefit. The study is severely impaired by a very small number of participants. This is similar to many other previous studies. Further larger scale investigation, with more standardised dose of carnitine and assessment methods of cardiovascular function are required.

Summary statement

The effects of uraemia on body composition and function are innumerable. Although commonly utilised therapeutic interventions within the CKD population aim to ameliorate some of the abnormalities found our increasing knowledge base serves to illustrate deleterious effects of these treatments and how much more we have to learn. This thesis merely scratches the surface of the problem and much more work is required to begin to comprehend the consequences of the failing kidney and the modifications we can make to produce the best outcome for our patients.

9 ABBREVIATIONS

ACEi	Angiotensin converting enzyme inhibitors
acylCoA	Acyl Coenzyme A
AHT	Antihypertensive therapy
APKD	Adult Polycystic kidney disease
ARB	Angiotension II receptor antagonists
BIA	Bioelectrical impedance analysis
BMI	Body Mass Index
BP	Blood pressure
BRS	Baroreflex sensitivity
BSU	Bone Structural Units
CAPD	Continuous Ambulatory Peritoneal Dialysis
cCalcium	corrected calcium
CFR	Collagen formation rate
CKD-MBD	Chronic Kidney Disease-Mineral Bone Disorder
Cm	Centimetre
CO	Cardiac Output
CPT1	Carnitine palmitoyltransferase 1
CrCl	Creatinine clearance
CRP	C-reactive protein
CSA	Cross sectional area
CSR	Collagen Synthesis Rate
Ct	Cycle threshold
CT	Computed tomography
CTA	Clinical Trial Approval
CTx	C-telopeptide
CVD	Cardiovascular Disease
DAS	Dobutamine-atropine stress

DBP	Diastolic Blood Pressure
DEXA	Dual Energy Xray Absorptiometry
DN	Diabetic nephropathy
DNA	Dioxyribonucleic acid
ECF	Extra cellular fluid
ECW	Extra cellular water
EDTA	Ethylenediaminetetraacetic acid
eGFR	estimated Glomerular Filtration Rate
ERF	Established Renal Failure
FBC	Full Blood count
FC	Free Carnitine
FGF23	Fibroblast growth factor 23
FSR	Fractional Synthetic Rate
g	grams
GC-C-IRMS	Gas chromatography-combustion-isotope ratio mass spectrometry
GC-MS	Gas Chromatographic-Mass Spectrometric (analysis)
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
GN	Glomerulonephritits
GP	General Practitioner
HD	Haemodialysis
HP	Hypotension Prone
HR	Hypotension Resistant
IDH	Intradialytic Hypotension
IGFI	Insulin like growth factor I
IHD	Ischemic Heart Disease
IL-6	Interleukin-6
i PTH	intact PTH
Kg	Kilogram

K/DOQI	Kidney Disease Outcomes Quality Initaitive
L	Litres
LVEF	Left Ventricular Ejection Fraction
LVH	Left Ventricular Hypertrophy
LVMI	Left Ventricular Mass Index
M	Molar
m^2	metres squared
MAP	Mean Arterial Pressure
MDRD	Modification of diet in renal disease
MHRA	Medicines and Healthcare products Regulatory Agency
MIA	Malnutrition-Inflammation-Atherosclerosis complex
min	minute
ml	millilitres
mmHg	millimetres of mercury
mRNA	Messenger RNA
NaCl	Sodium Chloride
NAP	N-acetyl-n-propyl esters
NHS	National Health Service
ns	Not significant
NTx	N-telopeptide
NYHA	New York Heart Association
OCTN2	Na dependent carnitine transporter
OFC	Osteitis Fibrosa Cystica
OPG	Osteoprotegerin
PCR	Polymerase Chain Reaction
PTH	Parathyroid Hormone
RANKL	Receptor activator NFkB ligand
RNA	Ribonucleic Acid
RT-PCR	Reverse transcriptase polymerase chain reaction
s	Seconds

SBP	Systolic Blood Pressure
SV	Stroke Volume
TBW	Total Body Water
TC	Total Carnitine
TNF-α	Tumour necrosis factor- α
TPR	Total peripheral resistance
TUG	Timed get-Up and Go
U&E	Urea & Electrolytes
u PCR	urine Protein:Creatinie ratio
VO2 Peak	Values for peak oxygen uptake

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