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THE HAEMODYNAMIC AND CARDIOVASCULAR EFFECTS OF DIALYSIS

by

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Thesis submitted to the School of Medical and Surgical Sciences

University of Nottingham for the degree of

DOCTOR OF MEDICINE

09 August 2007

for

Sara, Evie and William

Table of Contents

Abstract

Patients on dialysis are subject to hugely elevated rates of cardiovascular mortality. This thesis describes research work focusing on the large scale haemodynamic changes that occur during dialysis and how they may negatively impact on the cardiovascular system. Our results show that the haemodynamic disturbances that occur during haemodialysis are of sufficient magnitude to cause left ventricular (LV) regional wall motion abnormalities, reflecting subclinical myocardial ischaemia (myocardial stunning). This is pertinent as in non-dialysis patients repeated episodes of myocardial stunning lead to chronic heart failure, and in dialysis patients the presence of LV dysfunction dramatically increases the risk of death. We also explore how the haemodynamic effects of dialysis and the genesis of LV regional wall motion abnormalities can be ameliorated by using various interventions comprising of biofeedback dialysis (Hemocontrol® and Diacontrol®), cooling the dialysate and acetate free paired haemodiafiltration (PHF). We also examine the haemodynamic and metabolic effects of peritoneal dialysis (both continuous ambulatory and automated peritoneal dialysis) and show that these are much greater than previously thought. We also investigate possible mechanisms underlying these changes, namely alterations in cardiac filling and systemic glucose absorption leading to hyperinsulinaemia, and go on to examine the differential effects of the commercially available peritoneal dialysis solutions. Finally, we examine whether regional LV function is affected by the haemodynamic changes of CAPD.

Declaration

Except where acknowledged, I declare that this thesis is entirely my own work and is based upon research carried out in the Department of Vascular Medicine, University of Nottingham and Department of Renal Medicine, Derby Hospitals NHS Foundation Trust between June 2004 and August 2006.

Nicholas M Selby.

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Publications and abstracts arising from this thesis

Peer reviewed publications

- NM Selby, JO Burton, LJ Chesterton, CW McIntyre. Dialysis induced regional left ventricular dysfunction is ameliorated by cooling the dialysate. Clin J Am Soc Nephrol 2006; 1: 1216-1225
- NM Selby, SH Lambie, PG Camici, CS Baker, CW McIntyre. Occurrence of regional left ventricular dysfunction in patients undergoing standard and biofeedback dialysis. Am J Kid Dis 2006; 47: 830-841
- NM Selby, J Fialova, JO Burton, CW McIntyre. The haemodynamic and metabolic effects of hypertonic-glucose and amino-acid based peritoneal dialysis fluids. Nephrol Dial Transplant 2007; 20: 870-879
- NM Selby, CW McIntyre. A systematic review of the clinical effects of reducing dialysate temperature. Nephrol Dial Transplant 2006; 21: 1883-1898
- NM Selby, S Fonseca, L Hulme, RJ Fluck, MW Taal, CW McIntyre. Hypertonic glucose based peritoneal dialysate is associated with higher blood pressure and adverse haemodynamics as compared with Icodextrin. Nephrol Dial Transplant 2005; 20(9): 1848-53
- NM Selby, CW McIntyre. The acute cardiac effects of dialysis. Semin Dial 2007; 20(3): 220-228
- NM Selby, S Fonseca, L Hulme, RJ Fluck, MW Taal, CW McIntyre. Automated peritoneal dialysis is associated with significant systemic haemodynamic effects. Perit Dial Int. 2006; 26: 328:335
- NM Selby, MW Taal, RJ Fluck, CW McIntyre. Effects of Acetate-Free Double-Chamber Hemodiafiltration and Standard Dialysis on Systemic Hemodynamics and Troponin T Levels. ASAIO J 2006; 52: 62-69
- NM Selby, MW Taal, CW McIntyre. A comparison of progressive conductivity reduction with Diacontrol and standard dialysis. ASAIO J 2007; 53(2): 194-200

Oral presentations

- NM Selby, J Fialova, JO Burton, CW McIntyre. CAPD with hypertonic glucose solutions induces hyperglycaemia and is associated with an adverse haemodynamic response in non-diabetic PD patients. Renal Association 4th May 2006 (abstract ref: RA6040)

- NM Selby, MW Taal, CW McIntyre. Reduction of dialysate sodium concentration using conventional HD or prescribed end dialysis plasma conductivity (Diacontrol). Renal Association 4th May 2006 (abstract ref: RA6041)
- NM Selby. Haemodynamic and metabolic effects of peritoneal dialysis. PEAK conference, Vienna 31st March 2006
- NM Selby, RJ Fluck, MW Taal, CW McIntyre. Acetate-free paired haemodiafiltration is associated with improved systemic haemodynamics and lower troponin-T levels as compared with standard dialysis. Renal Association 7th April 2005 (abstract ref: RA5097)

Poster presentations

- NM Selby, RJ Fluck, MW Taal, CW McIntyre. Automated peritoneal dialysis is associated with significant systemic haemodynamic effects. Renal Association 5th April 2005 (abstract ref: RA5096), ERA-EDTA 6th June 2005 (abstract ref: MP419)
- NM Selby, RJ Fluck, MW Taal, CW McIntyre. Hypertonic glucose-based peritoneal dialysate is associated with higher blood pressure and adverse haemodynamics as compared with icodextrin. Renal Association 7th April 2005 (abstract ref: RA5093), ERA-EDTA 6th June 2005 (abstract ref: MP420)
- LJ Chesterton, NM Selby, T Bennett, CW McIntyre. Baroreflex sensitivity is important in blood pressure control during haemodialysis and is enhanced by dual chamber paired haemodiafiltration. Renal Association 7th April 2005 (abstract ref: RA5325), ERA-EDTA 6th June 2005 (abstract ref: MP394)
- NM Selby, RJ Fluck, MW Taal, CW McIntyre. Acetate-free paired haemodiafiltration is associated with improved systemic haemodynamics and lower troponin-T levels as compared with standard dialysis. ERA-EDTA 5th June 2005 (abstract ref: SP298)
- NM Selby, SH Lambie, PG Camici, CS Baker and CW McIntyre. Dialysis-Induced Left Ventricular Dysfunction: A Potential Mechanism for Heart Failure? American Society of Nephrology Congress 10th Nov 2005 (abstract ref: TH-PO728)
- NM Selby, RJ Fluck, MW Taal, CW McIntyre. Automated Peritoneal Dialysis Is Associated with Significant Systemic Haemodynamic Effects. American Society of Nephrology Congress 11th Nov 2005 (abstract ref: F-PO557)
- NM Selby, MW Taal, CW McIntyre. No difference in the systemic inflammatory response seen with acetate free dialysate as compared to standard bicarbonate dialysis. Renal Association 5th May 2006 (abstract ref: RA6043)
- NM Selby, J Fialova, JO Burton, CW McIntyre. The haemodynamic effects observed during PD fluid drainage and instillation do not adversely affect regional LV function or troponin T levels. Renal Association 5th May 2006 (abstract ref: RA6039), ERA-EDTA conference July 16th 2006 (abstract ref: SP715)

- NM Selby, J Fialova, JO Burton, CW McIntyre. CAPD with hypertonic glucose solutions induces hyperglycaemia and is associated with an adverse haemodynamic response in non-diabetic PD patients. ERA-EDTA conference July 16th 2006 (abstract ref: SP716)
- NM Selby, MW Taal, CW McIntyre. Reduction of dialysate sodium concentration using conventional HD or prescribed end dialysis plasma conductivity (Diacontrol). ERA-EDTA conference July 16th 2006 (abstract ref: SP678)
- NM Selby, CW McIntyre. A systematic review of the clinical effects of reducing dialysate fluid temperature. ERA-EDTA conference July 16th 2006 (abstract ref: SP478)
- JO Burton, NM Selby, LJ Chesterton, Christopher W McIntyre. Haemodialysis induced myocardial stunning is reduced by the use of cooled dialysate. American Society of Nephrology Congress, 18th Nov 2006 (abstract ref: TH-PO297)
- NM Selby, J Fialova, JO Burton, CW McIntyre. CAPD with hypertonic glucose solutions induces hyperglycaemia and is associated with an adverse haemodynamic response in non-diabetic PD patients. American Society of Nephrology Congress, 20th Nov 2006 (abstract ref: TH-PO776).

1. Introduction

1.1 Prevalence of renal disease

Established renal failure (ERF) is defined in the current UK guidelines for the management of chronic kidney disease as an estimated glomerular filtration rate of <15ml/min/1.73m^2 [1]. ERF or chronic kidney disease stage 5 (CKD 5) is the complete or near complete failure of the kidneys to perform their normal functions. These functions comprise of excretion of waste products, regulation of fluid and electrolyte balance, acid-base homeostasis and secretion or metabolism of hormones (in particular erythropoietin, renin and cholecalciferol). CKD 5 implies that kidney function is impaired to a degree that is unlikely to be able to sustain life in the longer term and so renal replacement therapies are often instituted.

ERF presents an increasingly large health and economic burden to the NHS. Currently, 626 patients per million population (37,050 in total) with ERF are treated in the UK at a cost of approximately £22,000 per patient per year [2, 3]. Furthermore, the ERF population is consistently increasing, with predicted growth rates between 4-8% per year for at least the next 15 years [2, 4]. In particular, the rate of increase of ERF is most rapid in the elderly. Transplantation remains the gold standard for treating ERF, but with a shortage of donor kidneys and an increasingly older ERF population many of whom are unfit for transplantation, dialysis remains the treatment for the majority. 39% of ERF patients in the UK are treated with haemodialysis, and 15% with peritoneal dialysis [2].

1.2 Dialysis modalities

Dialysis works by the principle of diffusion, with solutes removed from the blood down a concentration gradient across a semi-permeable membrane (which prevents loss of larger proteins and blood cells).

1.2.1 Haemodialysis

Haemodialysis involves moving patients' blood through an extracorporeal circuit, with the blood flowing along one side of a semi-permeable membrane and dialysis fluid (dialysate) flowing along the other side. A typical dialysis machine is shown in figure 1-a. Patients receive haemodialysis intermittently, typically three times a week for 3-4 hours at a time.

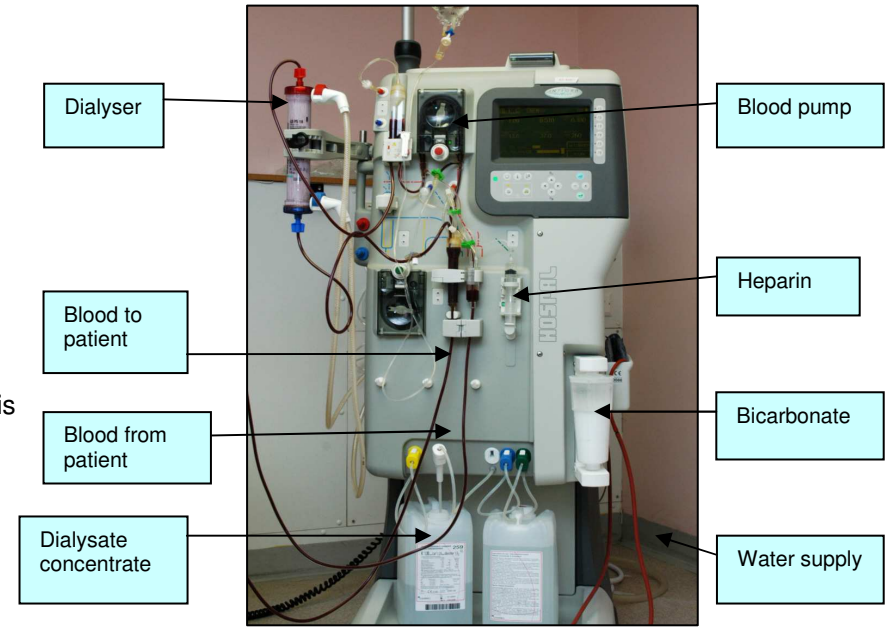


Figure 1-a.
Standard haemodialysis monitor

1.2.2 Peritoneal dialysis

Peritoneal dialysis involves the instillation of dialysate into the abdomen to allow effective dialysis to occur across the membrane lining the abdominal cavity. Peritoneal dialysis is continuous and fluid exchanges can either be performed manually, usually four times a day (continuous ambulatory peritoneal dialysis, CAPD) or can be done automatically overnight (automated peritoneal dialysis, APD) which allows a greater number of fluid exchanges to be performed whilst the patient sleeps.

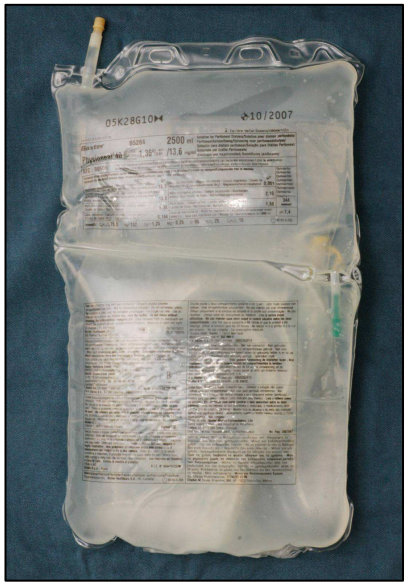


Figure 1-b.
Peritoneal dialysate.

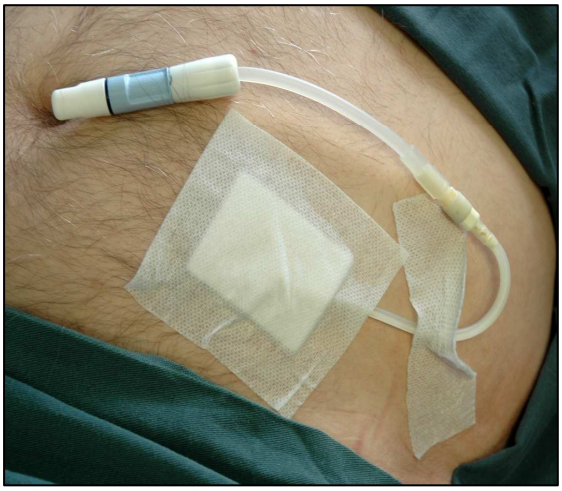


Figure 1-c.
Tenckhoff catheter.

1.3 The history of dialysis

1.3.1 Haemodialysis

Haemodialysis (HD) was first described in the early 1900s. However, it was not until the 1960s that HD became a viable technique for the long-term treatment of patients with ERF. This was enabled by the development of a reliable and safe Teflon arteriovenous shunt that allowed adequate rates of blood flow, and also by the availability of heparin to prevent clotting in the extracorporeal circuit (as opposed to the unpredictable natural hirudins). Although viable, early HD was beset with problems. Low efficiency of the dialysers in terms of solute removal led to long treatment times. Ultrafiltration (UF) was controlled manually by adjustment of the blood and dialysate pumps to create a pressure gradient that led to unpredictable UF rates. Blood leaks in the dialyser and lines were common and there were concerns about bacterial contamination of the dialysate fluid. Also, it was not possible to produce a commercially viable dialysate solution that contained bicarbonate as a buffer because bicarbonate and calcium precipitate at a pH greater than 7.4. Therefore, non-physiological acetate was employed as a buffer that diffused into the patient and was subsequently converted into bicarbonate by the liver. However, acetate transfer into the patient strongly predisposes to intradialytic symptoms, in particular hypotension (IDH), headaches and hypoxia [5].

Significant advances have been made over the subsequent years. The advent of hollow fibre dialysers industrialised the process of dialysis and greatly increased the ease of setting up a dialysis circuit. As hollow fibre dialysers became more efficient treatment times have become shorter, although shorter dialysis sessions with greater UF rates may themselves contribute to IDH. Dialysis membranes are now synthetic, which improves their biocompatibility. Bicarbonate-based dialysis became possible, initially using concentrated bicarbonate solutions although these solutions were prone to bacterial contamination. Subsequently dry bicarbonate concentrates were developed that overcame the problems of precipitation, microbial growth and the storage of large amounts of bicarbonate solution. In the 1980s the concept of Kt/V, a measure of the amount of urea cleared by dialysis in relation to body size, became widely accepted. Importantly, it was shown that too little dialysis (as measured by a low Kt/V) was associated with increased mortality [6, 7]. Finally, various modifications of the dialysis technique have become common practice. The most widespread of these is haemodiafiltration (HDF), a technique based on additional convective clearance due to high UF volumes and subsequent fluid reinfusion. HDF may confer benefit in terms of stability on dialysis and greater clearance of some uraemic middle molecules.

However, despite these technological advances HD remains an imperfect treatment. Dialysis only partially replicates the removal of fluid and uraemic toxins by the kidney, and does not replace any of its hormonal functions. The intermittent nature of dialysis dictates repeated, rapid shifts

between fluid overload and euvolaemia, and IDH remains a complication of up to a third of treatments. In addition, the cardiovascular death rate of haemodialysis patients is extraordinarily high [8].

1.3.2 Peritoneal dialysis

The earliest report of instillation of fluid into the peritoneal cavity for therapeutic purposes was in 1743, when Christopher Warrick instilled Bristol water and claret wine to treat ascites. The first description of successful dialysis using the peritoneum as a semi-permeable membrane was in 1923. The dialysate consisted of an electrolyte solution designed to mimic normal plasma, tempered by a series of compromises to allow production and stability under storage conditions of the fluids. These meant that glucose was used as the main osmotic agent, buffered with lactate alone (that produced a low pH). This allowed sterilisation of the fluids by heat with avoidance of caramalisation of the glucose. Early peritoneal dialysis (PD) techniques were hampered by high rates of infection and the requirement for repeated placement of catheters. In addition, early PD was an intermittent hospital-based technique, with some patients needing up to 60 hours per week of in-patient dialysis. PD became a viable technique to treat large numbers of patients with ERF in the late 1970s following the development of the Tenckhoff catheter (polyethylene catheter with Dacron cuffs designed to be tunnelled subcutaneously) and a simple, commercially available closed system for drainage and instillation of the fluid which minimised the chances of bacterial contamination (see figures 1-b and 1-c). These advances

allowed PD to be performed on an out-patient basis. Currently, it is still convention to use glucose as the osmotic agent, although different glucose concentrations have become available to allow tailoring of UF volumes. These conventional fluids employ lactate as the buffer, and this renders the fluids acidic. Again bicarbonate has traditionally been avoided due to problems with precipitation. Low pH results in increased infusion pain [9] and directly effects neoangiogenesis and mesothelial cell damage [10]. Furthermore, due to the acidic nature of the fluids and the heat sterilisation process, such fluids also contain glucose degradation products (GDPs) that also have been shown to have undesirable effects both on the peritoneum and systemically after absorption [11].

Continuous ambulatory peritoneal dialysis (CAPD) and automated peritoneal dialysis (APD) are now well established techniques in the treatment of ERF. Recent improvements to the technique have also been made, largely based around the development of more biocompatible dialysate fluids. There are those that do not contain glucose and therefore have very few or no GDPs; these comprise of Icodextrin (Extraneal®, a large molecular weight glucose polymer that is not systemically absorbed) and 1.1% amino acid solution (Nutrineal®, which may have beneficial effects on protein malnutrition). However, both of these solutions use a lactate buffer. Newer glucose-based fluids have also been developed with bicarbonate as a buffer, present in a separate chamber and mixed immediately prior to instillation. These fluids have a neutral pH and therefore also have much lower levels of GDPs as compared to

conventional solutions. Current commercially available peritoneal dialysis fluids are summarised in table 1-a.

However, peritoneal dialysis remains imperfect. Peritonitis remains a real problem, and over time the peritoneal membrane undergoes diabetiform change. Eventually, the peritoneum fails as an adequate selectively permeable membrane [12]. This is in part due to the bioincompatible nature of conventional fluids with an acidic pH and the presence of GDPs. However, the presence of glucose is also strongly implicated and supraphysiological glucose concentrations still occur with the newer dialysate solutions (Icodextrin and Nutrineal are only licensed for once daily administration). In addition, glucose and possibly GDPs are systemically absorbed, which in the case of glucose leads to weight gain and hyperinsulinaemia [13]. Registry data have suggested that conventional solutions may even have a negative impact on patient survival, although this must be interpreted within the limitations of a retrospective observation study [14]. As with HD, PD patients are also exposed to greatly increased rates of cardiovascular death. This is present at similar level to HD despite the differences between the dialysis techniques [15].

	Potential drawbacks	Potential Benefits
Single bag solutions		
Glucose containing, lactate buffer • Dianeal® (Baxter)	• Low pH • High GDP content • Poor peritoneal membrane biocompatibility • Infusion pain • Local and systemic glucose exposure	• Ease of manufacture • Low cost
Icodextrin containing, lactate buffer • Extraneal® (Baxter)	• Hypersensitivity • Low pH • Licensed for single daily use only • Lactate containing	• Sustained ultrafiltration • Preservation of residual renal function • Replacement for hypertonic glucose solutions • Reduced hyperglycaemia • Improved short term systemic haemodynamic profile • Desirable effects on metabolic profile and body composition
Amino acid containing, lactate buffer • Nutrineal® (Baxter)	• Low pH • Licensed for single daily use only (to avoid exacerbation of uraemic symptoms and acidosis)	• No GDPs • Avoid systemic and peritoneal glucose exposure • Peritoneal membrane protection • Enhance nutrition
Multibag solutions		
Lactate buffered • Balance® (Fresenius) • Gambrosol Trio® (Gambro)	• More physiological pH, but not neutral • Local and systemic glucose exposure	• Lower GDP levels • More physiological pH • Improved peritoneal membrane biocompatibility • Preserved membrane defence
Lactate/ bicarbonate buffered • Physioneal® (Baxter)	• Local and systemic glucose exposure • Does not eliminate peritoneal lactate exposure	• Lower GDP levels • More physiological pH • Improved peritoneal membrane biocompatibility • Preserved membrane defence • Reduced infusion pain
Bicarbonate buffered • bicaVera® (Fresenius)	• Local and systemic glucose exposure	• Lower GDP levels • More physiological pH • Improved peritoneal membrane biocompatibility • Preserved membrane defence • Improved correction of acidosis

Table 1-a. Summary of the characteristics of currently available PD solutions.

1.4 Cardiovascular disease in haemodialysis patients

It is well recognised that dialysis patients display hugely elevated rates of cardiac mortality, at least thirty-times greater than age-matched controls (figure 1-d, [8]). Although there are undoubtedly several mechanisms that contribute to this, the development of cardiac failure (which occurs in as many as 25-50% of haemodialysis patients) confers a particularly bleak prognosis [16]. Such high levels of cardiovascular disease are in part due to the high prevalence of 'traditional' risk factors (diabetes, hypertension, hyperlipidaemia, smoking, and physical inactivity). However, treating these traditional risk factors in dialysis patients has been disappointing; for example no benefit was observed with cholesterol lowering in diabetic haemodialysis patients [17]. The management of hypertension is particularly contentious, with no clear evidence that aggressive blood pressure lowering leads to a reduced risk of mortality [18, 19].

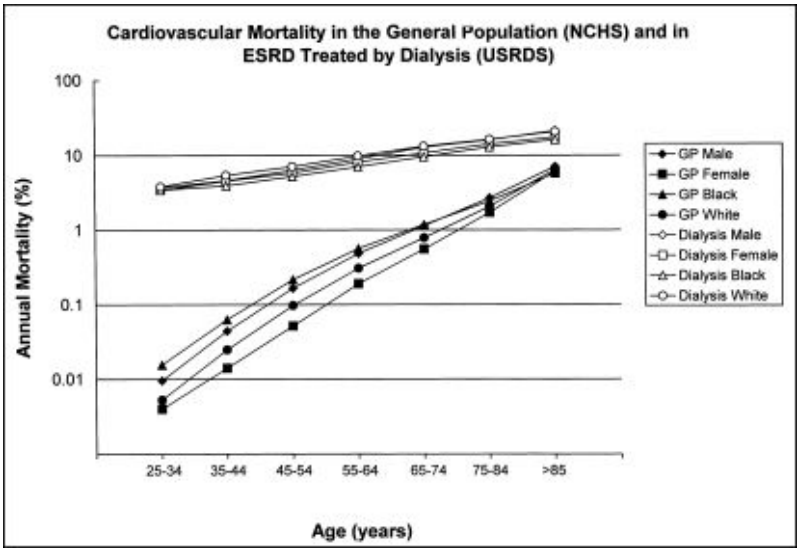


Figure 1-d.
Graph showing the cardiovascular mortality rates by age and ethnicity for dialysis patients and healthy controls [8]. The y-axis is a logarithmic scale, emphasising the huge excess of cardiovascular death seen with dialysis patients.

Therefore this excess of cardiovascular disease in dialysis patients must be explained by the presence of unique metabolic and haemodynamic derangements, the so-called 'uraemic' risk factors. These uraemic risk factors are less well defined but are multiple. Vascular calcification with increased vessel stiffness is common, is associated with the development of left ventricular hypertrophy (LVH) and also independently predicts mortality [20]. The presence of LVH itself predicts a worse long term outcome, and is associated with an increased propensity to cardiac arrhythmias [21]. In addition to vascular calcification, hypertension also leads to LVH [22], as does recurrent volume overload with rapid fluid shifts (that occur on dialysis). Hyperparathyroidism and anaemia have also been implicated in the genesis of LVH (although the issue of anaemia remains controversial as interventional studies have not always demonstrated that an improvement in haemoglobin concentration reduces LVH [23, 24]). Cardiac mortality in dialysis patients has also been linked to chronic inflammation, often manifest as hypoalbuminaemia and elevated C-reactive protein (CRP) levels. Inflammation is associated with accelerated atherosclerosis, vascular calcification and increased muscle catabolism [25]. Certainly, the elevation of several cytokines (in particular CRP and interleukin-6) have also been shown to be associated with an increase in mortality [26, 27]. In addition, it is possible that risk factors exist that are directly related to the dialysis procedure.

It has long been suspected that myocardial ischaemia may be precipitated by haemodialysis, with the first report of silent ST segment

depression during dialysis dating back to 1989 [28]. However, this concept of dialysis induced subclinical ischaemia (occurring without acute atherosclerotic plaque rupture) has received remarkably little attention, despite its theoretical plausibility. Short intermittent haemodialysis treatments by necessity lead to high UF rates, and as such exert significant haemodynamic effects; 20-30% of dialysis treatments are complicated by IDH [29-31]. In conjunction with this, haemodialysis patients are particularly susceptible to myocardial ischaemia. In addition to the high prevalence of coronary artery atheroma [32, 33], diabetic dialysis patients have been shown to have a reduced coronary flow reserve (CFR) even in the absence of coronary vessel stenoses [34]. There is preliminary evidence that the same phenomenon is also seen in non-diabetic dialysis patients [35]. In part this may be due to LVH, present in 74% of patients on dialysis [36]. LVH leads to both structural and functional reductions in the myocardial microcirculation; a capillary-myocyte mismatch has been described, and the presence of LVH per se reduces CFR even in the absence of large vessel disease [37]. In addition, the presence of concentric LVH renders the ventricle more sensitive to acute changes in filling pressure, exactly as occurs during haemodialysis [38]. Increased peripheral artery stiffness is also recognised to have an adverse effect on myocardial perfusion and reduces the ischaemic threshold [39]; therefore, LVH in tandem with increased vascular stiffness leads to a propensity to reduced subendocardial blood flow [40]. In addition, dysregulation of blood pressure control due to abnormal baroreflex sensitivity [41] and

vasoregulatory failure leading to the increased reliance of blood pressure on cardiac output [42] also increase the risk of myocardial hypoperfusion.

Since the initial report by Zuber et al [28], there have been a further nine studies that have demonstrated silent ST segment depression occurring during dialysis [43-51]. These studies report the occurrence of dialysis induced ST depression at rates that vary between 15 and 40%. However, there has been ongoing debate as to whether these electrocardiographic abnormalities reflect silent ischaemia or changes in electrolyte concentrations. Other than this, there has been only one subsequent study that has demonstrated ischaemia using an alternate technique. Singh et al assessed dialysis induced ischaemia using sestamibi single photon emission computed tomography (SPECT) [52]. In an unselected group of ten dialysis patients who were not known to have coronary artery disease, seven developed perfusion defects during dialysis. Importantly, concurrent ST depression occurred with the perfusion defects in only three patients, suggesting that electrocardiographic assessment alone may underestimate the incidence of dialysis induced ischaemia. However, none of these studies included an intervention to attempt to reduce the frequency of dialysis induced ischaemia, nor did any search for potential long term sequelae on LV function.

In non-renal patients, transient myocardial ischaemia may lead to LV dysfunction that can persist after the return of normal perfusion. This

prolonged dysfunction is known as myocardial stunning [53]. Myocardial stunning has been demonstrated in patients with coronary artery disease after exercise and dobutamine stress, and recent studies have provided evidence that repetitive episodes of ischaemia can be cumulative and lead to prolonged left ventricular dysfunction (myocardial hibernation) [53-59]. Myocardial stunning is therefore increasingly thought to be a causative mechanism for heart failure, with stunning and hibernation existing as part of a single spectrum (figure 1-e). Therefore, if myocardial ischaemia is induced by haemodialysis then the process of haemodialysis itself, repeated thrice weekly, may potentially contribute to chronic cardiac damage in this patient group. However, all of the available human data concerning myocardial stunning has been derived from patients with large vessel coronary artery disease and normal renal function.

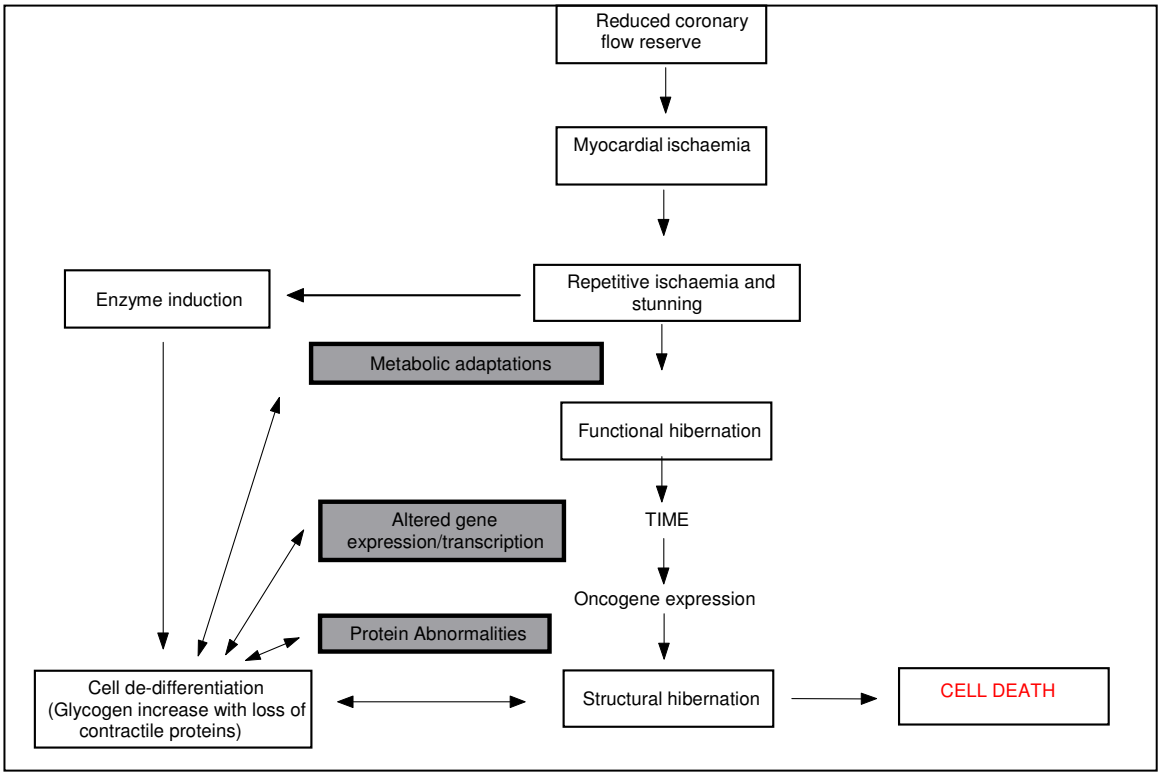


Figure 1-e.
Repeated episodes of transient myocardial ischaemia lead to maladaptive intracellular changes resulting in reduced myocardial function, hibernation and eventually cell death [60].

1.5 Dialysis induced changes in biomarkers of cardiac damage

It is well recognised that cardiac troponins are often elevated in dialysis patients, and that elevated levels independently predict mortality [61, 62]. Although this trend is seen for both troponin T (cTnT) and troponin I (cTnI), the data are more compelling for cTnT due to the larger number of studies, the uniformity of assays and greater homogeneity of cut off points employed. In addition, cTnT is elevated more frequently than cTnI, suggesting this molecule may be a more sensitive marker [61, 63]. These findings have previously been summarised in an excellent meta-analysis [62]. Although there was initial debate about the origin of elevated troponins in renal disease, it is now clear that the troponins are cardiac in origin, and it is the intact molecule (as opposed to smaller fragments) that are detected by the current assays [64].

However, there is continuing debate as to whether troponin rises acutely following dialysis. Several authors have reported significant rises in cTnT post dialysis consistent with acute myocardial cell injury [63, 65, 66]. Others have found no difference in pre and post dialysis cTnT or cTnI levels, or found that any difference disappears after correction for haemoconcentration [67-69]. Although it is convenient to collect the post dialysis blood sample at the end of the dialysis session, plasma troponin levels may only become elevated after 6 to 12 hours following an episode of ischaemia. Therefore, these discrepant results may arise from the

timing of the second blood sample, which in all of these studies was immediately post dialysis.

There is a much smaller literature concerning acute effects of dialysis on creatinine kinase (CK) and creatinine kinase MB (CKMB). CK and CKMB are chronically elevated less often in dialysis patients as compared to troponins [70-75]. Elevations in CK have not been shown to have long-term prognostic implications [76, 77], although there have been some data to show that chronically elevated CKMB levels do predict cardiac events and mortality, albeit to a lesser extent than troponins [70]. CK does not appear to rise with dialysis and although one author has reported a fall in CK post dialysis, this is not consistent with its large molecular weight [68]. There have been suggestions that CKMB may rise acutely following dialysis [66, 78], although this has not been a universal finding [71]. It has been previously demonstrated that CKMB rises acutely following dialysis complicated by IDH, but remains unchanged when patients are stable [78]. Although this is also consistent with subclinical myocardial injury, the consistency of this finding (with blood sampling immediately post dialysis) requires confirmation by other studies as CKMB may also take up to six hours to rise following an ischaemic insult.

1.6 Intradialytic hypotension

IDH remains a significant cause of morbidity in the haemodialysis population, occurring in 20-30% of treatments [31]. In addition, a fall in blood pressure during dialysis predicts mortality [79]. Furthermore, IDH could potentially contribute to myocardial hypoperfusion during dialysis.

The initiating factor in the aetiology of IDH is the rate of UF exceeding the plasma refill rate, leading to a reduction in circulating volume. Hypotension occurs when this reduction in blood volume surpasses the compensatory mechanisms of the cardiovascular system. As such, there are several factors of importance that can influence the genesis of IDH (summarised in figure 1-f).

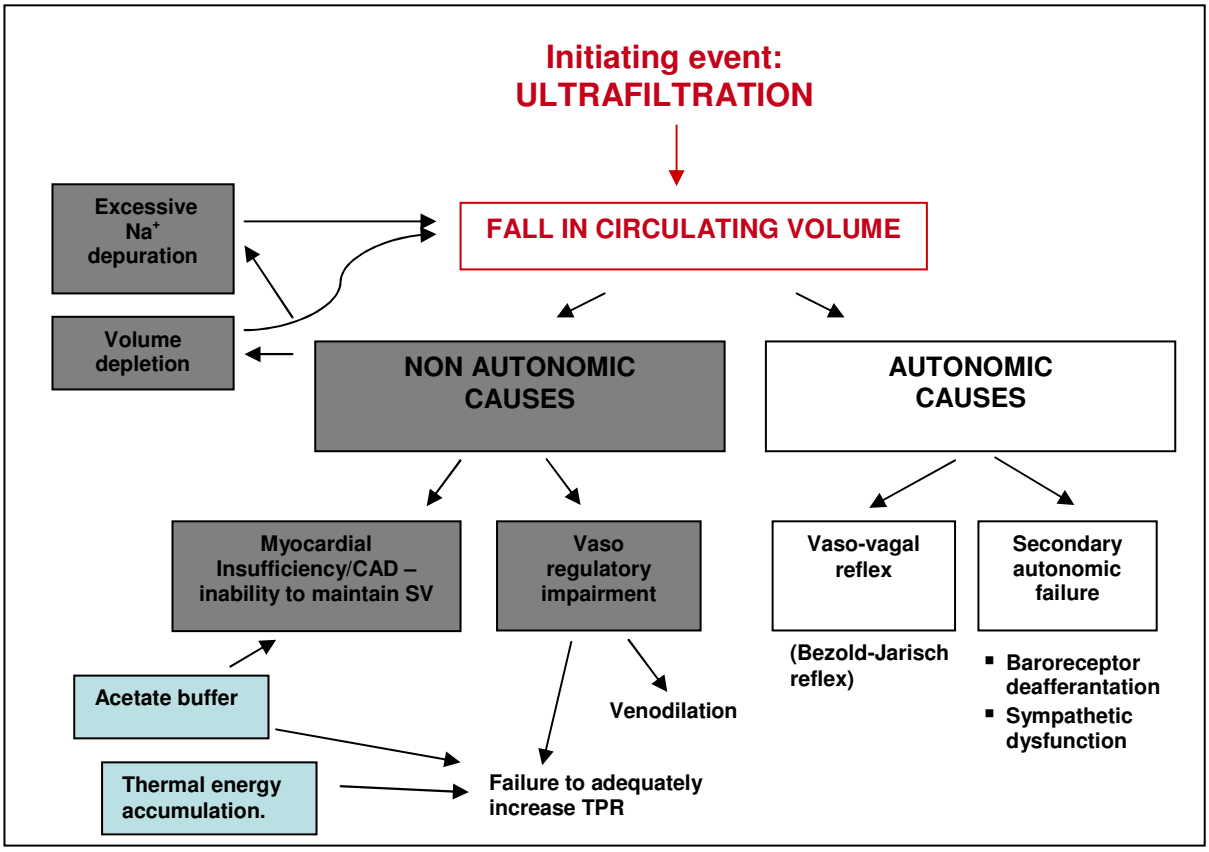


Figure 1-f.
Summary of the many inter-related factors that lead to intradialytic hypotension.
TPR = total peripheral resistance, SV = stroke volume, CAD = coronary artery disease.

1.6.1 Haemodynamic response to dialysis

Dialysis with ultrafiltration provokes several cardiovascular responses [30]. Stroke volume (SV) and cardiac output (CO) fall, traditionally thought to be in response to reduced ventricular refill rates that reflect falling blood volume. In response to this, total peripheral resistance (TPR) rises in an attempt to maintain blood pressure (BP). This rise in TPR appears to be mediated by changes in small artery compliance [80], which are in turn due to activation of the sympathetic nervous system [81]. Often there is little change in heart rate (HR). At the end of dialysis BP is largely dependent on CO, suggesting that the adaptive vasoconstriction response is often maximal [42]. The rising TPR (which leads to a fall in pressure in the venous capacitance vessels, thereby increasing venous recoil) and the rise in sympathetic outflow combine to cause venoconstriction with centralisation of blood volume. However, in many dialysis patients these compensatory responses are impaired. Many patients have cardiac failure and LVH, both of which influence SV, CO and therefore BP; a stiff, hypertrophied ventricle is much more sensitive to acute changes in preload (as occurs during dialysis) as compared to those without LVH. This is manifest in some patients that are prone to IDH, in whom SV appears to decline to a greater degree as compared to stable patients [29]. Autonomic neuropathy is associated with an increased propensity to IDH and is present in many patients on dialysis [82, 83]. In addition, considerable variability in venous compliance exists. Therefore, as long as haemodialysis is a short intermittent treatment there will be a requisite for high UF rates and IDH will remain a prevalent

complication. As such, there have been many different attempts to try and modify the dialysis technique to improve cardiovascular response and reduce IDH.

1.6.2 Relative blood volume and ultrafiltration rate

UF rate is important in determining blood volume [84]. Studies have shown that IDH occurs once the reduction in blood volume exceeds a critical threshold that is specific for each patient, although there remains a small but significant proportion of patients for whom this relationship between blood volume and IDH does not exist [85]. It is possible to reduce the rate of UF by extending the length of dialysis sessions and although this approach may be efficacious, it is often unacceptable to patients. Other than reducing the interdialytic weight gain, which to a large extent is patient-dependent, a form of biofeedback dialysis has been designed to preserve blood volume to an extent that avoids hypotension (Hemocontrol®, Gambro-Hospal, Mirandola, Italy) [86]. Relative blood volume (RBV) is monitored by an optical absorption biosensor, and significant falls (defined on an individual basis) are countered by temporarily reducing the UF rate and increasing the dialysate sodium conductivity. This is done within defined limits to ensure that total ultrafiltration and sodium depuration are unaffected. Several studies have shown biofeedback dialysis to be effective in reducing IDH frequency in both IDH prone and resistant patients [87-89].

1.6.3 Dialysate temperature

Standard dialysis (with a dialysate temperature of 37^0C) leads to an increase in patient body temperature [90]. The reasons for this are not entirely clear, but may include heat transfer to the patient from warm dialysate (especially as many dialysis patients have low baseline core temperatures), reduced heat loss from the skin due to vasoconstriction or possibly increased thermogenesis from an inflammatory response to a blood-membrane reaction. Cooling the temperature of the dialysate has been shown to reduce the incidence of IDH [90]. However, cooling the dialysate remains a relatively under utilised technique. In part this may be due to fears of causing unacceptable symptoms of cold and shivering, as empirical reduction of dialysate temperature may in some patients lead to excessive cooling. In addition, there has also been concern that cooling the dialysate will lead to a reduction in dialysis adequacy due to peripheral solute sequestration as a result of greater vasoconstriction. More recently, a biofeedback device has been developed (BTM®, Fresenius, Bad Homburg, Germany). This device measures the temperature of blood in the arterial and venous bloodlines leaving the fistula and makes a correction for recirculation. It is able to calculate energy transfer and can make constant adjustments to the dialysate temperature during dialysis in response to the calculated body temperature. BTM can therefore be programmed in a variety of ways; it can deliver a predetermined energy balance (either negative balance or thermoneutral dialysis where energy balance is neutral), or it can deliver a prescribed change in body temperature (programmed cooling or

isothermic dialysis where patient's body temperature is maintained). Isothermic dialysis requires a negative energy balance to maintain body temperature. By reacting to an individual patient's core temperature, BTM and in particular isothermic dialysis should theoretically avoid severe thermal symptoms.

Dialysis techniques that use proportionally greater degrees of convection to remove uraemic toxins (haemofiltration, HF or haemodiafiltration, HDF) have also been linked to improvements in stability during dialysis (in addition to their other potential advantages on solute clearance) [91]. However, there is accumulating evidence that most, if not all of this benefit on stability is due to thermal effects. When dialysis and HDF are matched for thermal energy transfer, low flux dialysis is equivalent in terms of IDH frequency and changes in systemic haemodynamics to both pre and post dilution HDF [92-94].

1.6.4 Dialysate sodium concentration

Dialysate sodium levels also influence IDH. Complete removal of the inter-dialytic accumulation of sodium is a key requirement of haemodialysis. Failure to do so leads to greater thirst, increased interdialytic weight gains (IDWG) and hypertension, which in turn impact on cardiovascular outcomes [95]. Furthermore, large IDWG also predispose to IDH. Current practice of a fixed dialysate sodium for all patients may not only result in repeated failure to adequately remove the interdialytic sodium load, but could lead to intradialytic diffusion of sodium into those patients with lower pre-dialysis plasma sodium levels [96].

Empirical reduction of dialysate sodium to below plasma water sodium enhances sodium removal by diffusion, and may lead to a reduction in total body sodium. However, in the early period of dialysis this may result in an excessive reduction in plasma tonicity with resultant symptoms and hypotension. Although most studies show that this approach results in improvements in blood pressure (BP), reductions in IDWG and fewer symptoms of thirst, others do not report such differences or find the reverse [97-103]. Furthermore, the greatest benefits seen at the lowest dialysate sodium conductivities (13.0mS/cm) were only achievable in a limited number of patients due to intradialytic symptoms [102].

A fixed dialysate sodium does not always lead to the same amount of sodium depuration as pre dialysis plasma sodium levels vary both between and within individuals [104]. Individualising dialysate sodium concentration based on pre dialysis sodium levels does avoid sodium loading during dialysis [96, 97]. However, this method is time consuming and requires ongoing monitoring of pre-dialysis sodium levels. A more sophisticated approach is a biofeedback system (Diacontrol®, Gambro-Hospal, Mirandola, Italy) that returns plasma conductivity (as a surrogate marker of plasma sodium concentration) to a prescribed level. Diacontrol® (DC) aims to achieve total removal of the interdialytic sodium load by returning the patient to a predefined post-dialysis sodium level, and therefore a fixed total body sodium by the end of each dialysis (assuming dry weight is also attained). This is achieved by online monitoring of plasma conductivity, which reflects plasma sodium [105]. Automatic adjustment of the dialysate conductivity is then performed via a negative

feedback loop to deliver the prescribed end-dialysis plasma conductivity. Therefore, the interdialytic sodium is completely removed even when pre dialysis sodium levels vary. Diacontrol® (DC) has been shown to repeatedly achieve prescribed end dialysis plasma conductivities within very tight limits at settings above 14.0mS/cm [105]. However, although early studies suggested improved haemodynamic stability with this technique [106-108], clinical evaluation of DC remains relatively limited. The improved stability was thought to be due to the biofeedback system maintaining dialysate sodium levels at a higher level early in the dialysis session, thereby avoiding rapid reductions in plasma tonicity. This is shown in figure 1-g.

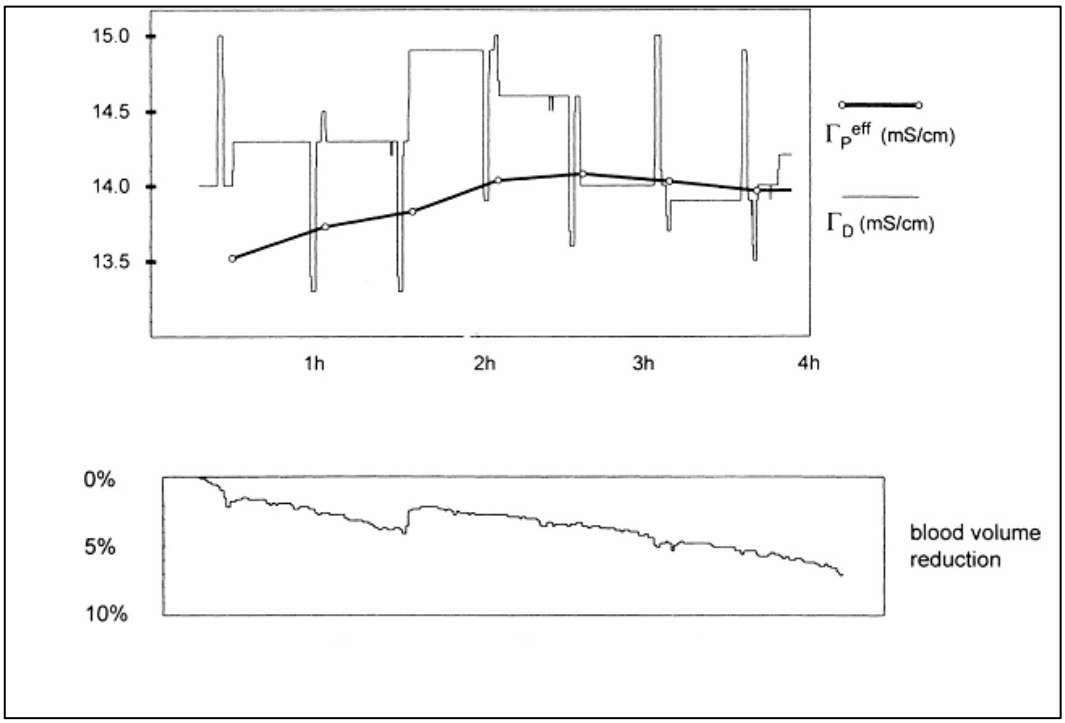


Figure 1-g.
Diacontrol. The upper figure shows plasma (Γ_p^{eff}) and dialysate (Γ_D) conductivity. Note the higher time averaged dialysate conductivity in the early part of the dialysis session, and that blood volume rises at 90min corresponding to the increase in dialysate conductivity.

1.6.5 Buffer composition

Historically, acetate was used as the sole buffer in haemodialysis. However, it was recognised that acetate was an aetiological factor in IDH, and switching from acetate to bicarbonate as the principle buffer improved cardiovascular stability and reduced intradialytic symptoms [5]. However, the aetiology of acetate-induced IDH remains contentious. Acetate has been shown to cause vasodilatation that is mediated by nitric oxide release, but has also been shown to reduce myocardial contractility [109-112]. Many of these studies were carried out in animal models, and debate remains about validity of the results from some of the studies in humans. Despite this, standard bicarbonate based dialysis is not acetate free. Small amounts of acetate (3-4mmol/l) are required to prevent precipitation of calcium carbonate, and although the concentration of bicarbonate in dialysate is relatively large compared to that of acetate, the difference in dialysate/plasma concentration gradients is much less because plasma acetate levels are usually very low (<100μmol/l) [113]. Therefore, standard bicarbonate based dialysis can still result in significant transfer of acetate to the patient, making up as much as 25-49% of the buffer load [113, 114]. The original solution to this problem was the development of acetate-free biofiltration (AFB). AFB is a haemodiafiltration technique in which a post-dilution bicarbonate infusion is used in combination with base-free dialysate. This technique is significantly more complex than standard dialysis, and also incurs problems due to storage and handling of the bicarbonate re-infusion fluid. Nonetheless, AFB has been shown to be associated with less IDH and an

improvement in pre-dialysis BP [115, 116]. However, an alternate acetate free dialysis method has now been developed. Paired haemodiafiltration (PHF, Bellco, Mirandola, Italy) is a novel online technique that employs a double chamber dialyser (consisting of a high flux dialyser and ultrafilter, see figure 1-h) that allows re-infusion to take place inside the dialyser. PHF is designed to be used with dialysate that is completely acetate free. In place of acetate, the dialysate concentrate contains hydrochloric acid to prevent calcium precipitation (this is converted to water and sodium chloride during online dialysate preparation) and there is no requirement for a sterile bicarbonate infusion. PHF is therefore much easier to perform as compared to AFB. One previous study has shown that PHF is a feasible technique [117], but there are no studies examining the clinical and haemodynamic response to acetate-free PHF.

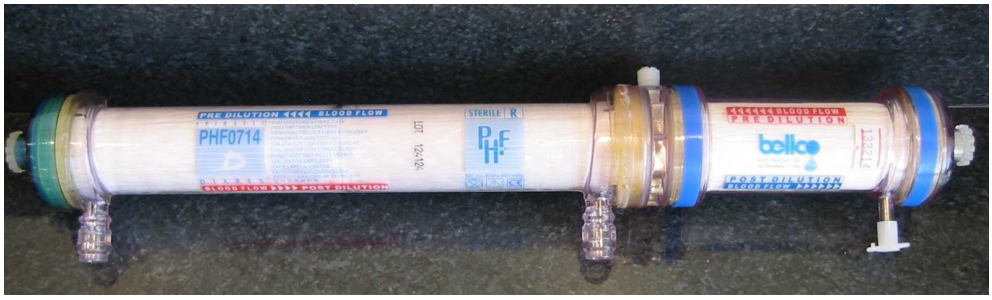


Figure 1-h.
Double chamber dialyser used for PHF. The smaller chamber is the ultrafilter for the reinfusate, the larger chamber is the high flux haemodiafilter.

1.7 Cardiovascular effects of peritoneal dialysis

CAPD and APD are widely used treatments for ERF. Overall and cardiovascular death rates are similar in haemodialysis and peritoneal dialysis populations [15]. Some uraemic risk factors, such as LVH and vascular calcification exist in both HD and PD patients; however, there are many differences between haemo- and peritoneal dialysis. In contrast to haemodialysis, UF and solute removal take place continuously and at a much slower rate. CAPD is generally regarded as being better tolerated from a cardiovascular point of view, but as compared to haemodialysis there is a relative of paucity of research in this area. One recent study suggested that haemodynamic changes in response to CAPD are greater than previously thought when examined during a 30min dwell of a standard peritoneal permeability analysis [118]. Other work on this subject dates back more than 20 years, used intermittent monitoring techniques and produced conflicting data [119-121]. It is also conceivable that there may be variation in the haemodynamic response to the wide selection of commercially available peritoneal dialysis solutions. These fluids differ in the strength and type of osmotic agent (glucose, Icodextrin, amino acids), their buffer type and biocompatibility. The different tonicities of the glucose based solutions produce different amounts and rates of ultrafiltration. However, there are no data available on the haemodynamic effects directly attributable to glucose-containing or alternative dialysate fluids, or whether buffer type and biocompatibility of the fluid exert an effect.

In contrast to haemodialysis, peritoneal dialysis may be associated with greater metabolic perturbation. Glucose is systemically absorbed leading to impaired insulin sensitivity and weight gain [13]. In non-diabetic cohorts, insulin resistance and excursions of plasma glucose to outside of the normal range in response to an oral glucose load are recognised as predicting cardiovascular death [122, 123]. Furthermore, there is also some evidence that GDPs disappear from the peritoneal cavity and are systemically absorbed [124, 125]. This is of importance as some GDPs have been shown to directly cytotoxic and to increase the production of advanced glycation end-products, another factor implicated in atherogenesis [11, 126-128].

In addition, there is a theoretical link between haemodynamic and metabolic changes induced by peritoneal dialysis using glucose containing dialysate. BP has been shown to rise in response to hyperglycaemia in several studies in both non diabetic and diabetic patients, while glucose and insulin exert independent effects on systemic haemodynamics [129, 130]. Marfella et al demonstrated a rise in BP during hyperglycaemia, during both normal and elevated insulin levels [130]. BP was returned to baseline with a glutathione infusion, suggesting the direct effect of hyperglycaemia on haemodynamics may be mediated by free radical production leading to reductions in nitric oxide. Equally, hyperinsulinaemia during euglycaemic clamp experiments has been shown to cause elevations in HR, SV and CO whilst also causing a fall in TPR [131]. It is not currently known whether GDPs are active in this way.

As seen in haemodialysis patients, elevated cTnT levels in CAPD patients independently predict mortality [132, 133]. Elevated cTnT levels also correlate with increasing LV mass index (LVMI). However, the predictive power of an elevated cTnT on mortality is independent of its association with increased LVMI or elevated CRP. The reasons behind elevated cTnT levels in peritoneal dialysis patients have not been elucidated. Some authors postulate that they may reflect subclinical myocardial damage due to undetected coronary artery disease, a combination of LVH and microvascular coronary disease or direct injury to myocardial cells (uraemic toxins, stretching due to fluid overload, or hypoxia) [132]. However, if the process of peritoneal dialysis is associated with significant haemodynamic disturbance then this may also impact on cTnT release.

APD is an increasingly utilised modality in the treatment of ERF, used in preference to continuous ambulatory peritoneal dialysis to improve dialysis adequacy or for lifestyle reasons. Residual renal function (RRF) is extremely important in maintaining adequacy in peritoneal dialysis patients, and also in helping maintain fluid and electrolyte balance. In addition, maintenance of RRF has been shown to affect survival [134, 135]. However, several studies have implicated APD in increasing the rate of decline of RRF as compared to CAPD, although the reasons for this remain unexplained [136-138]. Some authors have postulated that APD causes greater shifts in osmotic load and exerts more intensive ultrafiltration as compared to CAPD, and this in turn may potentially

cause adverse haemodynamic effects [136, 139]. However, there are no published data reporting the haemodynamic response to APD, despite the recent work showing CAPD to be associated with significant haemodynamic disturbance [118].

To summarise, relatively little is known about the effects of peritoneal dialysis on short term haemodynamic and myocardial function, although recent data suggest that such effects may have previously been underestimated. In the face of an excess of cardiovascular death it is important to search for novel cardiac risk factors, including those potentially related to the dialysis procedure itself. This is increasingly important as there are now several different opportunities to modify the technique of PD, in particular by altering the glucose content and biocompatibility of dialysate fluids.

2. Aims of thesis

This thesis has been planned to test the following hypothesis:

The haemodynamic stress of dialysis therapies leads to subclinical myocardial ischaemia and stunning, which may be novel risk factors for the development of uraemic cardiomyopathy.

To do this, the following interrelated research questions will be addressed:

- Is it possible to confirm that dialysis induced myocardial ischaemia and stunning occurs?
- If so, can we intervene to reduce dialysis induced myocardial stunning by improving haemodynamic stability?
- Which novel haemodialysis technologies are effective in improving haemodynamic stability and reducing IDH?
- How extensive are the haemodynamic effects of peritoneal dialysis, and are these affected by the type of dialysate fluid used?
- Does myocardial stunning occur in response to the haemodynamic changes of CAPD?

3. Generic Methods

The following techniques are used in more than one chapter, and are therefore described fully only once.

3.1 Non-invasive continuous haemodynamic monitoring

The Finometer (Finapres Medical Systems, Arnhem, The Netherlands) is a tool for blood pressure and haemodynamic monitoring (figure 3-a). It 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 [140] 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 [141]. 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 a 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 normals, unstable intensive care patients and in cardiac surgery patients, a proportion of whom had vascular calcification [142-144]. 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 [145]. Validation of the Finometer in dialysis patients is presented in chapter 4.

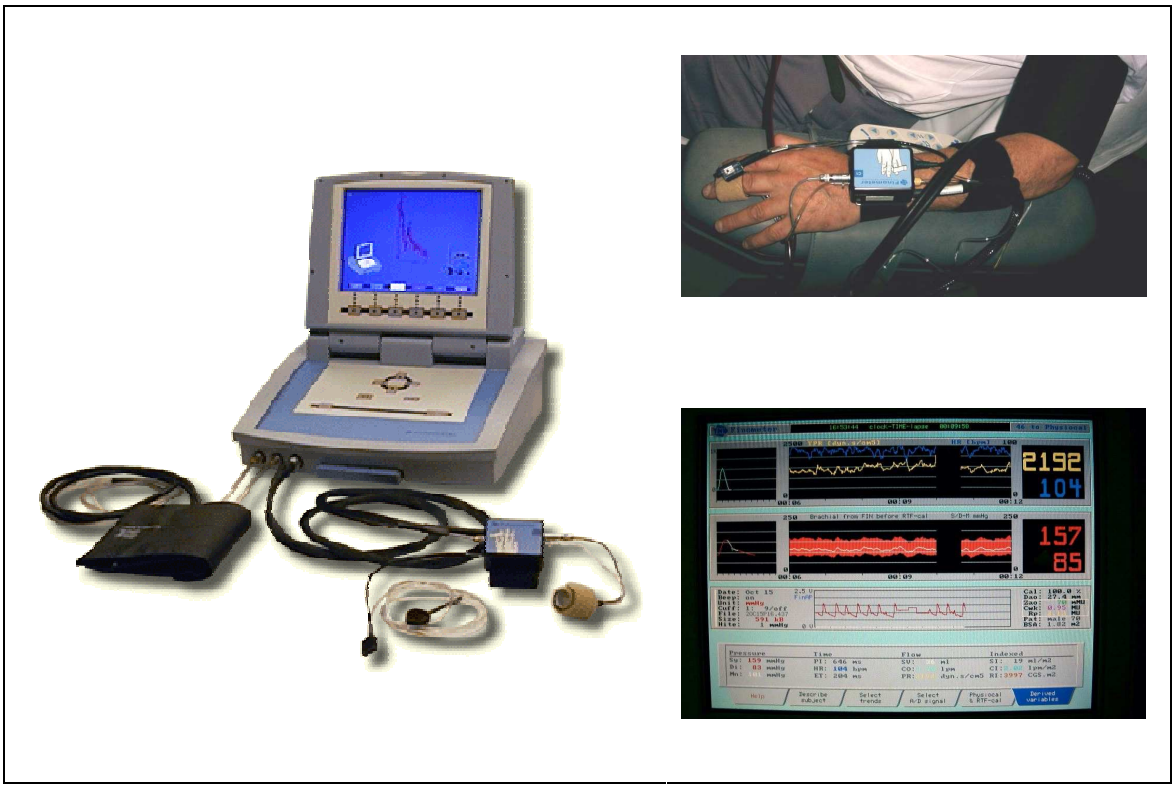


Figure 3-a.
Non-invasive continuous haemodynamic monitoring with the Finometer.

3.2 Echocardiography

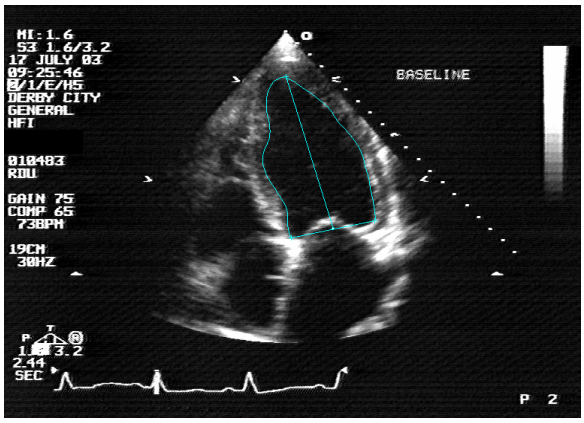
We used two-dimensional echocardiography to assess regional LV function as a marker of ischaemia. The development of new LV regional wall motion abnormalities (RWMAs) during physiological or pharmacological stress occurs in response to ischaemia and its onset precedes that of symptoms and electrocardiographic changes. This principle underlies dobutamine stress echocardiography [146].

LV regional wall motion was assessed by recording standard apical views before, during and after dialysis procedures and then performing specialised semi-automated analysis on the images. Images were acquired using commercially available equipment (1.5-3.6 MHz 3S probe, Vivid 3®, GE medical systems, Sonigen, Germany). A single experienced technician (who was blinded to dialysis modality) carried out all examinations with the patients in the left lateral position. Timing of the echocardiography varied between studies but is stated in each relevant chapter. Standard apical 2-chamber and 4-chamber views (to visualise the LV endocardial border in two planes at 90^0 to each other) were recorded onto super-VHS videotape for off-line analysis.

Videotaped images were subsequently analysed using a personal computer based digitising programme (Echo-CMS, MEDIS, Leiden, The Netherlands) as previously described [147]. Three consecutive heartbeats were analysed for each time point (extrasystolic beats were excluded). Endocardial borders (excluding papillary muscles) were traced semi-automatically for each videoframe of the 3-beat sequence, and any

anomalies corrected manually (see figure 3-b). Maximal displacement of the endocardial border from a centrepoint was then measured over each of 100 chords around the LV wall, corrected for end-diastolic LV circumference and expressed as percentage shortening fraction (SF), as shown in figure 3-c. Each apical view was divided into five segments and SF for the chords in each segment was averaged so ten regions of the left ventricle were assessed at each time point. New RWMAs were defined as those segments that demonstrated a decline in SF of >20% from baseline. This technique uses endocardial borders as the sole marker of abnormal contraction, and therefore could be criticised as it does not take account of wall thickening or transmyocardial heterogeneity. However, this method does have the advantage that it is repeatable and quantitative.

We also used echocardiography to measure cardiac dimensions and global LV function. Ejection fraction (EF) was calculated using LV volumes at end systole and end diastole, measured by the biplane disk method. Left atrial volume, which has been used as a marker of diastolic function in haemodialysis patients [148], was calculated using single-plane Simpson's method from the apical 4-chamber view and indexed for body surface area. M-mode echocardiography was used to measure LV dimensions and LVMI was calculated from each patient's original baseline images using the Devereux formula corrected for height$^{2.7}$.



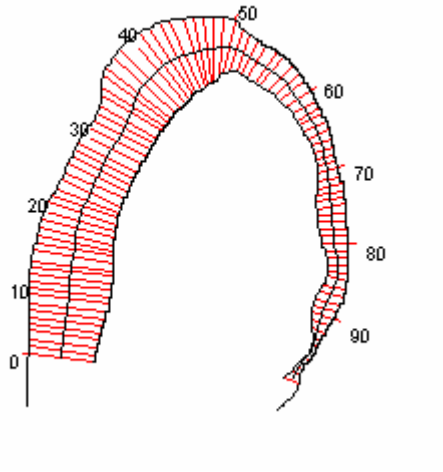


Figure 3-b. Figure 3-c.
Echo CMS. Figure 3-b shows semi-automatic tracing of the endocardial border. The movement of the endocardial border is then measured over 100 chords around the left ventricle – this is shown in figure 3-c with each red line representing a measurement.

3.3 Dialysis methodology

3.3.1 Blood volume measurement

Blood volume measurement was performed by dialysis monitors in several of our experiments. Measurement of the change in the concentration of blood components (blood cells, proteins, haemoglobin) during dialysis reflects the balance between fluid removal from the circulation (ultrafiltration) and the plasma refill rate. There are two commonly available methods to measure blood volume - optical and ultrasonic. The optical method measures either haemoglobin concentration or the haematocrit by measuring the light absorbance of blood at two different frequencies. Measurement at two frequencies

allows correction for the degree of oxygenation, which can alter the light absorbance characteristics of blood.

The ultrasonic method calculates the total protein concentration in blood (plasma proteins and haemoglobin) by measuring the velocity of sound in blood (which is determined by protein concentration) and compares this to the velocity of sound in isotonic saline. For this method, the temperature of the blood and saline must be accurately fixed, and the haematocrit is deduced from the total protein concentration using simple formulae.

In our experiments, we used the optical method that has been commercially developed and incorporated into dialysis monitors [149].

3.3.2 Conductivity measurement and ionic dialysance

The conductivity of a substance is defined as the ability to carry electrical current. In dialysate fluid, the conductivity is determined by the concentration of ions capable of carrying electrical current, providing the temperature remains constant. This is almost entirely dependent on sodium concentration, although the other ions (such as potassium and calcium) that are present in much smaller quantities do exert a small effect. Therefore, by measuring conductivity of the dialysate it is possible to derive a value for dialysate sodium concentration [150]. This technique has a variety of applications. A conductivity monitor can be placed at the dialysate inlet and outlet ports of the dialyser and by changing the inlet conductivity by a set amount and measuring the resultant change in

dialysate outlet conductivity, it is possible to calculate ionic dialysance (value representing the amount of electrolytes that have passed from plasma to dialysate). From the degree of ionic dialysance that occurs at a set dialysate conductivity, it is possible to calculate plasma conductivity and this can be used a surrogate for plasma sodium concentration. As the transfer characteristics of sodium and urea are similar, the ionic dialysance also reflects the clearance of urea. Therefore, using this technology it is possible to estimate from each dialysis session the ionic mass balance (amount of sodium removed), the dialysis adequacy for small solute removal (Kt/V, providing the patient's volume of distribution is known) and the plasma conductivity at the end of the treatment [150].

3.3.3 Dialysis technique

All dialysis described in this thesis was performed using Hospal Integra® monitors (Gambro-Hospal, Mirandola, Italy) except for chapter 6, in which Bellco Formula 2000® monitors were used. In all cases, we used low-flux polysulphone dialysers either 1.8m^2 or 2.0m^2 as per individual patients' usual prescription (LOPS® 18/20, Braun Medical Ltd, Sheffield, UK) unless otherwise stated. Dialysate contained sodium 138mmol/l, potassium 1mmol/l, calcium 1.5mmol/l, magnesium 0.5mmol/l, bicarbonate 32mmol/l, glucose 1g/l and acetate 3mmol/l. All treatments were of 4 hours duration and anti-coagulation was achieved with unfractionated heparin. Dialysate flow was 500ml/min, dialysate temperature was set at 37^0C and blood flow was 250-460ml/min. For

paired sessions, care was taken to ensure blood flows were similar for each patient. Net fluid removal was set on an individual basis according to ideal dry weight. No patients underwent sodium or ultrafiltration profiling.

3.4 ELISAs

Enzyme linked immunosorbent assays (ELISAs) were used to quantitatively detect cytokines, proteins and hormones. Briefly, a monoclonal antibody against the molecule to be detected is adsorbed onto the surface of microwells. Samples or standards are then added to the wells and the molecule binds to these solid phase antibodies. A labelled antibody against the molecule is then also added and this also binds to the molecule to form a sandwich (solid phase antibody - test molecule - labelled antibody). After incubation, the wells are washed to remove unbound labelled antibody and a chromogenic reagent is added that reacts with the labelled antibody to produce a colour change. A stop solution is added to prevent excess colour development and the absorbance of each well is measured spectophotometrically. The absorbance is proportional to the concentration of test molecule present. A standard curve is drawn and values for the concentration of samples in each well can then be calculated. We used standard ELISA kits that provided precoated 96 well plates for detection of the following molecules.

3.4.1 Insulin (Biosource, Nivelles, Belgium)

Blood was collected from patients in EDTA tubes and immediately centrifuged at 3500rpm for 10min. The plasma was then removed and frozen at -80^{0}C until time of assay. Samples were centrifuged again upon thawing at 17,000rpm for 3min to remove particulates. The INS-EASIA kit uses two monoclonal antibodies directed against distinct epitopes of insulin. 50µl of each calibrator solution, standards and samples were pipetted in duplicate into the precoated wells. 50µl of anti-insulin antibody conjugated with horseradish peroxidase (HRP) was then added to each well. The plate was then incubated at room temperature for 30min on a horizontal plate shaker (set at 700rpm). Each well was then aspirated and washed three times using 400µl of wash solution. 200µl of chromagen tetramethylbenzydine (TMB) solution was then added to each well and the plate incubated for 15min at room temperature on a horizontal shaker. 50µl of stop solution (H_2SO_4) was then added to each well and absorbances read at 450nm (reference filter 650nm).

3.4.2 C-reactive protein high sensitivity (DRG diagnostics, Marburg, Germany)

Serum was isolated from blood collected in plain tubes and prepared as for the insulin ELISA. Before assaying, the serum was diluted 100-fold. The C-reactive protein (CRP) high sensitivity ELISA uses monoclonal mouse antibody for the solid phase and a goat anti-CRP antibody labelled with HRP. The methods were similar to the insulin ELISA but with the

following differences: 10µl of calibrators and samples and 100µl of HRP-antibody were used, samples and antibody were incubated for 45min, wells were washed five times with distilled water, 100µl of TMB and stop solution were used.

3.4.3 Interleukin-6 (Diaclone, Besancon, France)

Interleukin-6 (IL6) was measured in plasma that was isolated as above. 100µl of samples and calibrators were pipetted in duplicate into each well. 50µl of anti-IL6 antibody conjugated to biotin was then added to each well, and the plate incubated at room temperature for one hour. The wells were then washed three times using 300µl of wash solution. 100µl of streptavidin-HRP was then added to each well and the plate incubated for 30min. The wells were then washed in the same way and 100µl of TMB substrate added. The plate was then incubated for a further 15min. 100µl of stop solution was added and the plates read at 450nm (reference 650nm).

3.4.4 Tumour necrosis factor alpha (Bender MedSystems, Vienna, Austria)

Tumour necrosis factor alpha (TNFα) was measured on plasma isolated as for insulin ELISA. The TNFα kit employed a monoclonal solid phase antibody and a biotin conjugated polyclonal antibody, and also required a tyramide signal amplification step. 50µl of diluent, 50µl of samples or

calibtrators and 50µl of biotin-conjugate were added to each well. The plate was then incubated at room temperature for two hours on a horizontal shaker at 100rpm. Wells were then aspirated and washed six times with wash solution. 100µl of streptavidin-HRP was then added to each well and the plate incubated at room temperature for one hour on the horizontal shaker. Wells were aspirated and washed as previously. 100µl of the first amplification solution was added to each well and the plate incubated for 15min at room temperature. Wells were again washed and 100µl of the second amplification solution added. The plate was incubated for 30min before a further washing step and addition of 100µl of TMB solution to each well. The plate was incubated at room temperature for 10min before 100µl of stop solution was added to each well and absorbance measured as previously.

The amplification step consisted of the addition of biotinyl-tyramide. This was converted by HRP to free radicals that bind any protein in the well, with the amount of bound biotinyl-tyramide proportional to the amount of HRP initially present. The second amplification solution contains further streptavidin-HRP that binds biotin sites created during the biotinyl-tyramide reaction, thus multiplying the total number of available HRP molecules for the subsequent reaction with TMB.

4. Results: occurrence of regional left ventricular dysfunction in patients undergoing standard and biofeedback dialysis

4.1 Introduction

This chapter describes a study to determine whether standard haemodialysis with ultrafiltration is capable of inducing new LV regional wall motion abnormalities (RWMAs), reflecting subclinical myocardial ischaemia. To test the hypothesis that maintaining intradialytic blood pressure and improving the haemodynamic tolerability of dialysis reduces the development of LV RWMAs we compared standard dialysis (HD) to a form of biofeedback dialysis (BFD, Hemocontrol®). Hemocontrol® (Gambro-Hospal, Mirandola, Italy) is a system that has been designed to preserve blood volume to an extent that avoids hypotension. Significant falls in relative blood volume (defined on an individual basis) are countered by temporarily reducing the UF rate and increasing the dialysate sodium conductivity. This is done within defined limits to ensure that total ultrafiltration and sodium depuration are unaffected. Several studies have shown BFD to be effective in reducing IDH frequency in both IDH prone and resistant patients [87-89].

4.2 Methods

4.2.1 Patients

Eight chronic haemodialysis patients prone to IDH were recruited. All were male and had been on dialysis for more than 12 months. All had LV hypertrophy (defined as LV mass index of >51g/$m^{2.7}$) on analysis of baseline echocardiograms, and there was a high prevalence of atherosclerotic vascular disease. Six patients were treated with aspirin, one with clopidogrel and five were taking statins. Individual patient characteristics are shown in table 4-a.

4.2.2 Study protocol

Upon entry to the study, patients had their dry weight confirmed with reference to clinical examination. Following this, dry weight and anti-hypertensive medications remained unchanged for the duration of the study. Patients were then randomised to group A or B. Group A patients were commenced on standard thrice weekly HD, whereas group B patients started thrice weekly BFD treatment. Both groups underwent one week of the dialysis therapy before undergoing a monitored session during one of the midweek dialysis sessions of the second week, consisting of serial echocardiography and non-invasive haemodynamic monitoring (using the Finometer). At the end of the second week, patients then crossed over to the other dialysis modality thereby acting as their

own controls. After a further week on the alternate modality, patients underwent a second monitored session.

All patients gave informed consent prior to commencement, and ethical approval for the project was granted by Derbyshire Research Ethics Committee.

Patient	Age	Months on dialysis	Cause of ESRF	Vascular disease	IHD	LVMI ($g/m^{2.7}$)	Angiogram	Anti-BP drugs
1	68	45	APKD	Y	Y (angina)	63.8	N†	Diltiazem 180mg od
2	58	40	Obstructive uropathy	N	N	94.2	N	
3	53	15	Crescentic GN	Y	Y (angina)	86.9	N	Nifedipine 60mg od
4	72	47	Unknown	Y	Y (MI)	79.7	Y*	ISMN 30mg od
5	69	59	Diabetes	Y	Y (MI x 3)	86.5	N	Lisinopril 40mg od
6	73	36	ARVD	Y	N	94.6	N	Atenolol 50mg od Lisinopril 20mg od
7	71	13	ARVD	Y	N	96.4	N	
8	80	22	Diabetes	Y	Y (angina)	80.8	N	
Mean ± SD, or total number (%)	8 ± 8.6	34 ± 16		7 (88%)	5 (63%)	85.4 ± 10.7	1 (13%)	

Table 4-a.
Patient demographics. APKD= adult polycystic kidney disease, GN= glomerulonephritis, ARVD= atherosclerotic renovascular disease. IHD = ischemic heart disease, LVMI = LV mass index.
* Angiogram result for patient 4: Circumflex occluded with some retrograde filling, right coronary artery occluded with good retrograde filling, left anterior descending artery some atheroma but no occlusive disease.
† Patient 1 had recently had a dipyridamole stress test, showing a fixed apical perfusion defect with reversible inferior wall hypoperfusion.

4.2.3 Haemodialysis details

Dialysis was performed as described in chapter 3. For standard HD, dialysate sodium conductivity was set at 13.6mS/cm. For BFD, conductivity limits were set at 13.0mS/cm and 14.0mS/cm. Automatic adjustment of dialysate conductivity by the dialysis monitor during Hemocontrol® has been shown to achieve equivalent overall dialysate conductivity and therefore equal change in plasma water sodium concentration [86]. Limits for relative blood volume were set on an individual basis depending on measurements taken during the week prior to echocardiographic assessment.

4.2.4 Echocardiography

Echocardiography and subsequent analysis were performed as described in chapter 3. Images were recorded prior to commencing dialysis (baseline), at 120min and 240min during dialysis and 30min after dialysis was finished (recovery). 10 regions of the left ventricle were assessed for the development of new RWMAs at each time point. We calculated mean SF for all ten segments ($SF_{(mean)}$) and for those segments that developed new RWMAs ($SF_{(WMA)}$). Peak stress was defined for each patient as the point during the first monitored dialysis session when most RWMAs were present (either 120min or 240min). When comparing dialysis modalities, the same time point was used in the second dialysis session.

4.2.5 Finometer

The Finometer was used as described in chapter 3. In addition, baroreflex sensitivity was also calculated from the regression slope between continuous interbeat interval and beat-to-beat BP changes. Three consecutive changes in the R–R interval in the same direction were required before a phase shift calculation (incorporated into Finometer software) was performed. Baroreflex sensitivity measured in this way is a composite marker of the overall activity of the autonomic nervous system [151].

Although the Finometer is becoming increasingly used to assess chronic dialysis patients [29, 30, 118] it has not been validated in patients with ERF. This may be important as dialysis patients are subject to a variety of unique pathophysiological processes affecting the vascular tree that may impact upon the reliability of results from the Finometer. We therefore carried out work to verify the accuracy of the Finometer in dialysis patients.

4.3 Validation of the Finometer in dialysis patients

We performed simultaneous Finometer and echocardiographic measurements of stroke volume (SV) in chronic dialysis patients. We selected echocardiography as a standard as it is a widely accepted technique and is non-invasive. Invasive haemodynamic measurement techniques carry a small but significant risk of serious complications and in view of the available evidence supporting the accuracy of the Finometer it was considered unethical to use these as a comparator.

We studied 13 unselected dialysis patients, four on haemodialysis and nine on CAPD, all of whom had been on dialysis for more than four months. The HD patients were examined before and at the end of a standard four-hour dialysis session (with ultrafiltration). The CAPD patients were examined before and after an exchange, where waste dialysate fluid was drained out of and 2.5L of fresh fluid drained into the abdominal cavity. 2-dimensional echocardiography was performed as described in chapter 3. A single experienced technician carried out all measurements with the patients in the left lateral position. Images were subsequently analysed and SV derived using the biplane disk method. Finometer values are a time average mean for a 5min period during which echocardiograms were acquired. As the Finometer is accurate in tracking change, data are expressed as percentage change from baseline (baseline data derived a 10min period before dialysis was commenced).

4.4 Statistical analysis

Results are expressed as mean ± SD unless otherwise stated. Echocardiographic, BP and haemodynamic data were analysed using one-way analysis of variance (ANOVA) with a design for repeated measures and Bonferroni's test to correct for multiple comparisons. The frequencies of IDH and of new RWMAs occurring during each dialysis modality were compared using Poisson regression. For other data, the paired *t*-test was used after significant deviations from a normal distribution were excluded with the Kolmogorov-Smirnov test. An alpha error at $P<0.05$ was judged to be significant.

4.5 Results

4.5.1 Validation of the Finometer in dialysis patients

We found a good correlation between percentage change in SV as derived by the Finometer and by echocardiography ($r^2 = 0.64$, $p<0.0001$). Bland-Altman analysis demonstrated a mean difference of 1.7% change from baseline between SV measured by the two techniques, with limits of agreement of ± 9.3%. These data are shown in figure 4-a.

This is the first validation of the Finometer in dialysis patients. These results support the premise that the Finometer is a useful and accurate monitoring tool in this patient group.

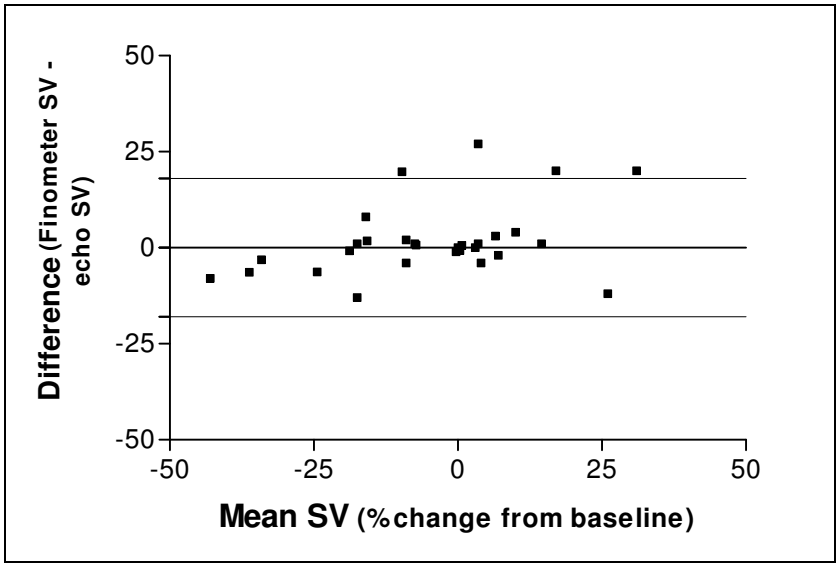


Figure 4-a.
Bland-Altman plot of stroke volume (SV) measured as percentage change from baseline by the Finometer and by echocardiography in chronic dialysis patients.

4.5.2 Blood pressure data

During standard HD, systolic BP (SBP) was 135 ± 30.8 mmHg, diastolic BP (DBP) was 73.2 ± 13.9 mmHg and mean arterial pressure (MAP) was 93.9 ± 19.8 mmHg. During BFD all three BP parameters were higher - mean SBP was 143.1 ± 21.1 mmHg ($p<0.001$), mean DBP was 76.4 ± 12.3 mmHg (p=ns) and mean MAP was 100.1 ± 3.3 mmHg ($p<0.001$). BP gradually declined during the second half of HD treatments whereas BP was maintained during the second half of BFD sessions, accounting for the difference in mean BP values. BP data are summarised in figure 4-b.

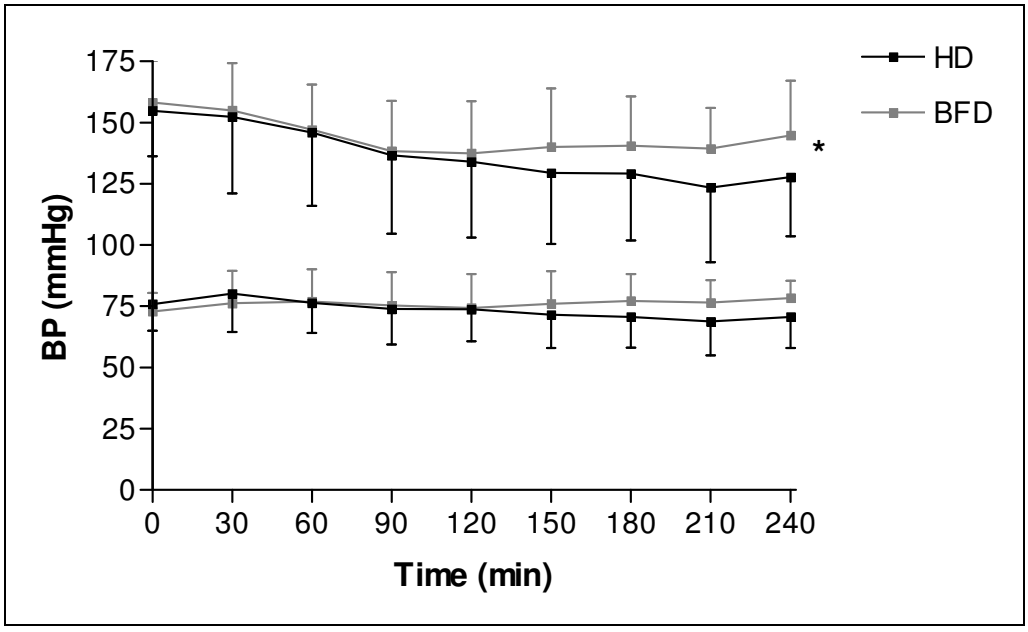


Figure 4-b.
Population blood pressure (systolic and diastolic) data during standard (HD) and biofeedback (BFD) dialysis. For clarity, mean arterial pressure is omitted, but this was also significantly higher during BFD.
* $p<0.001$ by ANOVA.

IDH was defined as any SBP of less than 100mmHg, or a fall in SBP of >10% from baseline in association with the classical symptoms of hypotension (dizziness, cramps, flushing). There were no episodes of symptomatic hypotension during the 16 monitored sessions, but we observed 24 asymptomatic episodes of IDH with HD as compared with 12 during BFD (OR 2.0, 95% CI 1.01 to 4.4). These findings were in context of a slightly greater volume of ultrafiltration (UF) during BFD (1.91 ± 1.38 litres) as compared with HD (1.54 ± 1.19 litres), although this difference was not statistically significant. Individual UF volumes and body mass index are shown in table 4-b.

Patient	**BMI (kg/m^2)**	**UF vol HD (l)**	**Indexed UF vol HD**	**UF vol BFD (l)**	**Indexed UF vol BFD**
1	22	0.4	1.8	0.5	2.3
2	24	1.3	5.4	2	8.2
3	29	2.2	7.6	3.2	11.0
4	21	1.2	5.7	1.8	8.5
5	23	0.5	2.2	0.4	1.8
6	24	2.7	11.1	3.3	14.1
7	27	0.4	1.5	0.4	1.5
8	29	3.6	12.4	3.7	12.6
Mean ± SD	24.9 ± 3	1.54 ± 1.19	6.0 ± 4.2	1.91 ± 1.38	7.5 ± 5.1

Table 4-b.
Ultrafiltration (UF) volumes, body mass index (BMI) and UF volumes indexed to BMI for individual patients during both types of dialysis.

4.5.3 Echocardiographic data

Throughout the study, all patients were in sinus rhythm and none had significant valvular disease or pulmonary hypertension. SF at baseline in all regions was compared on an individual basis for each type of dialysis. There were no significant differences in baseline SF in any of the patients. This was done to ensure repeatability of images and measurement technique, and also to ensure the RWMAs that persisted at 30min post dialysis were not permanent.

All eight patients developed RWMAs at peak stress during HD, as compared to seven patients during BFD. More RWMAs developed during HD as compared to BFD, with a total of 42 RWMAs occurring during HD as compared to 23 RWMAs during BFD (OR 1.8, 95% CI 1.1 to 3.0). There was also a difference when comparing the rate of unaffected regions between dialysis modalities (OR 0.60, 95% CI, 0.39 to 0.91). By 30min post HD, 32 (76%) of the affected segments demonstrated a complete or partial resolution while after BFD 15 (65%) of the RWMAs improved. However, at 30min post HD 24 (30%) of affected regions still had SF >20% less than baseline, and following BFD this figure was similar at 23 (29%, OR 1.0, 95% CI 0.59 to 1.83). These data are summarised in figure 4-d and one representative patient's regional wall motion is shown in figure 4-c.

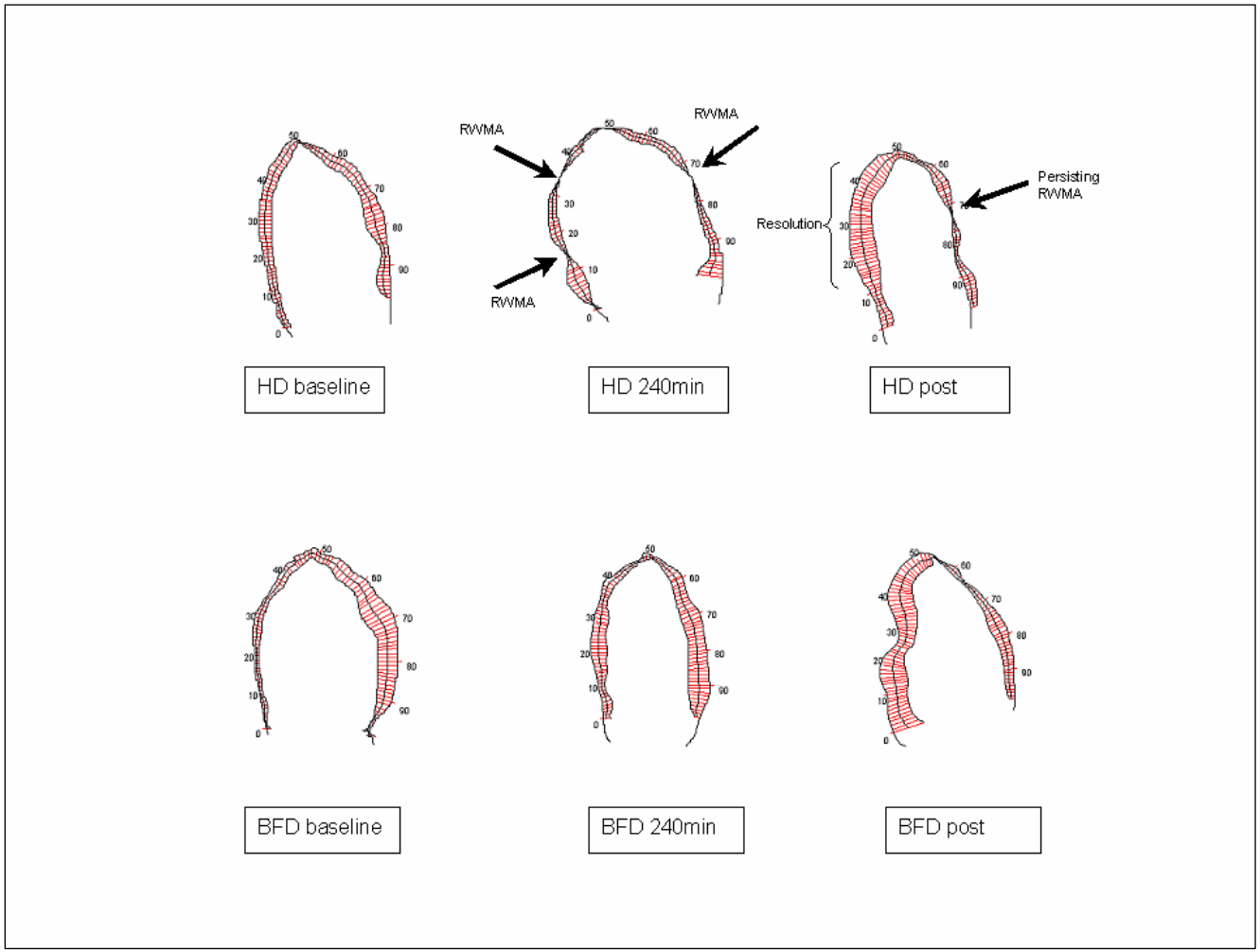


Figure 4-c.
Analysis of left ventricular wall motion (2 chamber view) of one representative patient (patient number 3) during standard (HD) and biofeedback controlled (BFD) dialysis. Wall motion is measured over each of 100 chords (red lines) around the ventricular wall. Baseline traces are similar. By 240min, 3 new regional wall motion abnormalities (RWMA, arrows) have developed during HD, but the same regions are unaffected during BFD. 30min post HD, 2 of the RWMAs have resolved and 1 persists (arrow).

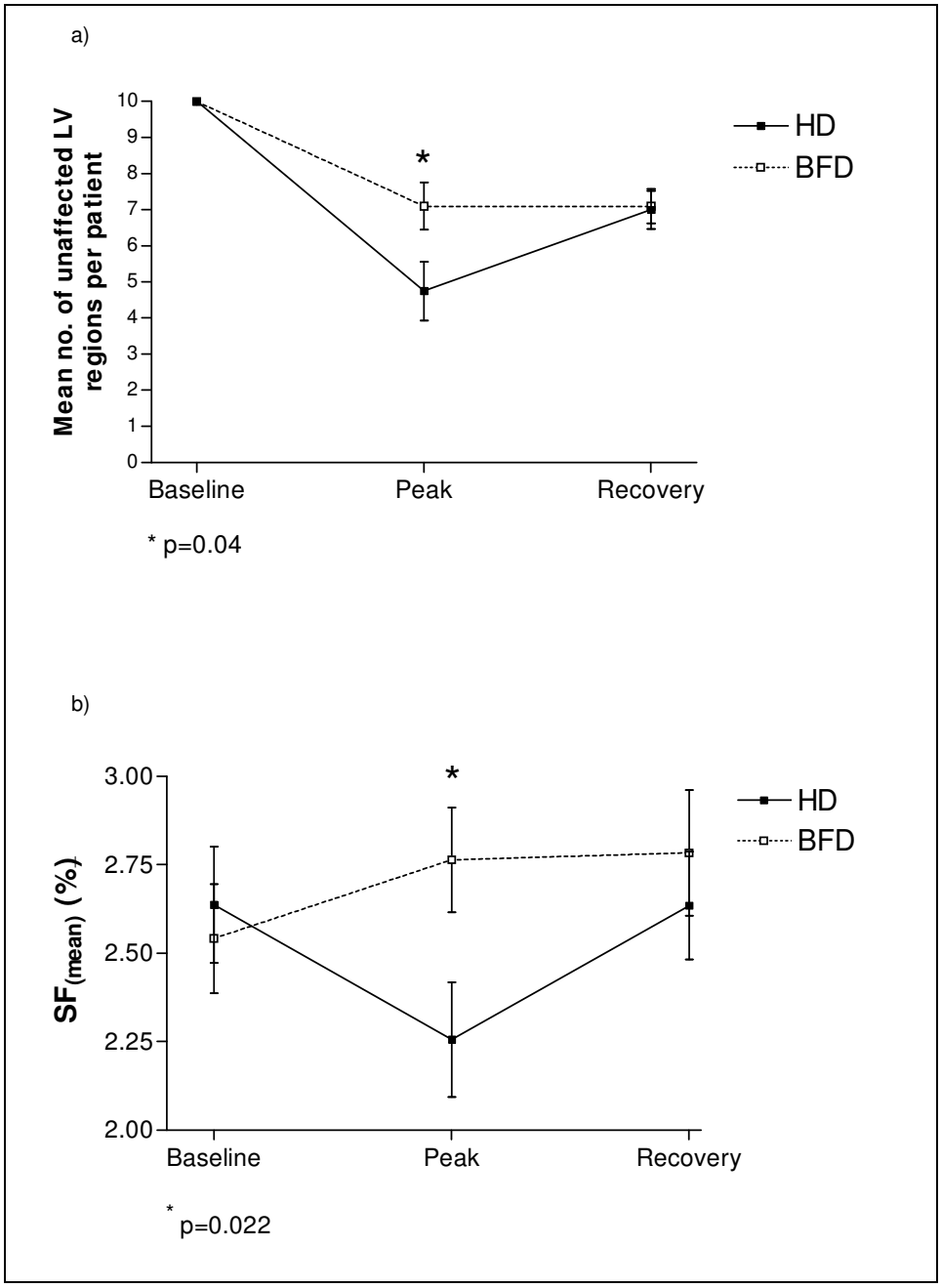


Figure 4-d.
a) Mean number of *un*affected LV regions during standard (HD) and biofeedback dialysis (BFD). Only new RWMAs were counted and therefore all regions are scored as 'unaffected' at baseline. Baseline is before start of dialysis, peak stress is the point at which most RWMAs were present during dialysis, and recovery is 30min post dialysis. Data are expressed as mean ± SE.

b) Overall mean regional LV function (SF) during standard (HD) and biofeedback dialysis (BFD). Data are expressed as mean ± SE.

Table 4-c plus figures 4-d, 4-e and 4-f summarise the data for $SF_{(mean)}$, $SF_{(WMA)}$ and EF. $SF_{(WMA)}$ declined at peak stress during both types of dialysis and then improved in recovery. $SF_{(WMA)}$ did decrease by a larger percentage from baseline during HD (-43 ± 15.1%) as compared to BFD (-37.8 ± 16.0%), but this difference did not reach statistical significance (p=0.19). In view of this trend towards a more severe reduction in $SF_{(WMA)}$ and also the greater number of RWMAs, $SF_{(mean)}$ declined to a significantly greater degree at peak stress during HD (-10.3 ± 48.4% from baseline) as compared to BFD (+13.5 ± 48.4%, p=0.022). At baseline and recovery there were no statistically significant differences in either $SF_{(mean)}$ or $SF_{(WMA)}$ between the two types of dialysis.

	EF (%)	$SF_{(mean)}$ (%)	$SF_{(WMA)}$ (%)
HD			
Baseline	50.1 ± 10.7	2.64 ± 1.5	2.98 ± 1.7
Peak	48.7 ± 12.3	**2.26 ± 1.4**†	**1.69 ± 1.0***
Recovery	53.4 ± 13.3	2.64 ± 1.3	**2.38 ± 1.3***‡
BFD			
Baseline	46.1 ± 12.3	2.54 ± 1.4	3.12 ± 1.6
Peak	53.1 ± 12.1	2.76 ± 1.3	**1.90 ± 1.0***
Recovery	54.4 ± 15.4	2.78 ± 1.6	**2.67 ± 1.7***‡

† p<0.05, * p<0.001 versus baseline by ANOVA ‡ p<0.001 versus peak by ANOVA

Table 4-c.
Global (EF) and regional (SF) LV function during standard (HD) and biofeedback dialysis (BFD). Baseline is before start of dialysis, peak stress is the point at which most RWMAs were present during dialysis, and recovery is 30min post dialysis.

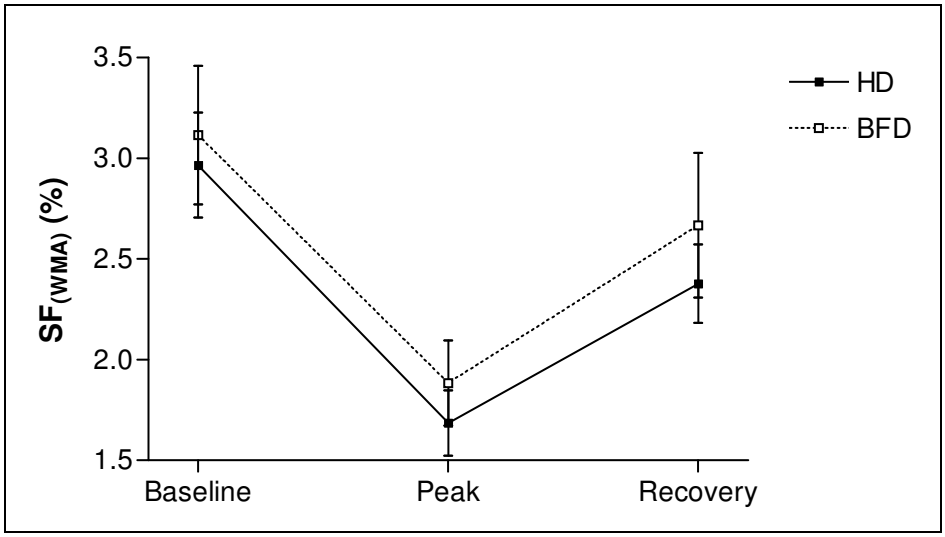


Figure 4-e.
Mean regional LV function (SF) in those regions that developed new RWMAs during standard (HD) and biofeedback dialysis (BFD). Data are expressed as mean ± SE.

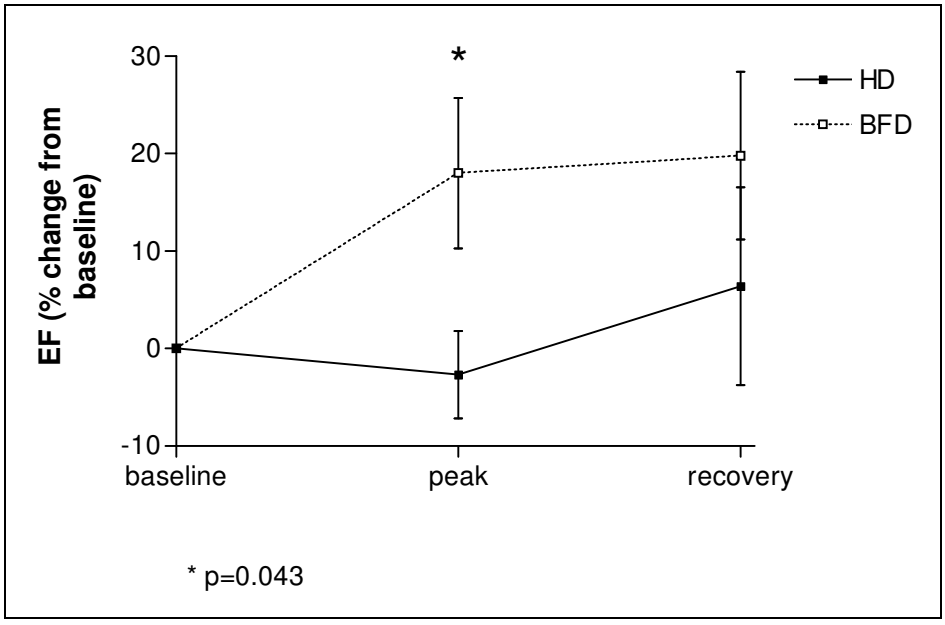


Figure 4-f.
Global LV function (EF) during standard (HD) and biofeedback dialysis (BFD). Data are expressed as mean ± SE.

At peak stress, EF was significantly lower during HD as compared to BFD (p=0.043), while no difference was found during recovery. Table 4-d shows the remaining LV and LA dimensions before and after HD and BFD. There were no differences in any of the measurements when comparing the dialysis modalities.

	Pre dialysis		**End dialysis**		***p*-value**
	HD	**BFD**	**HD**	**BFD**	
LV major axis diastole (cm)	8.4 ± 1.1	8.2 ± 1.0	8.1 ± 0.8	8.0 ± 0.6	0.79
LV major axis systole (cm)	7.1 ± 1.3	7.1 ± 1.0	7.0 ± 1.0	6.9 ± 0.6	0.97
LV minor axis diastole (cm)	5.2 ± 1.0	5.2 ± 0.8	5.1 ± 1.1	5.4 ± 0.8	0.92
LV minor axis systole (cm)	4.6 ± 1.0	4.3 ± 1.3	4.5 ± 0.7	4.3 ± 0.9	0.9
LA diameter (cm)	4.5 ± 0.7	4.5 ± 0.6	4.4 ± 0.6	4.4 ± 0.7	0.99
LA indexed volume (ml/m^2)	33.0 ± 12	33.1 ± 11	30.5 ± 12	30.8 ± 11	0.95

Table 4-d.
Echocardiographic measurements of cardiac dimensions at the start and end of dialysis. LV = left ventricle, LA = left atrium. LA volume was calculated by Simpson's rule from single plane (apical 4 chamber) and indexed for body surface area.

4.5.4 Haemodynamic data

Baseline haemodynamic data were compared between the two dialysis modalities to ensure there were no systematic errors; no differences in any of the variables were observed.

Pulse rate increased throughout the entire study period by a mean of +5.0 ± 2.5% above baseline during BFD, whereas pulse rate changed very little during HD (mean +0.4 ± 2.4%, $p<0.01$). Stroke volume declined during both treatments, but to a significantly lesser extent during BFD. Mean stroke volume for the entire HD session was -26.2 ± 7.2% from baseline, as compared to a mean of -20.2 ± 7.3% ($p<0.001$) during BFD. Cardiac output showed a similar pattern, declining during both treatments but less so during BFD. Mean cardiac output was -26.4 ± 7.2% during HD as compared to a mean of -18.2 ± 7.8 during BFD ($p<0.001$). Mean peripheral resistance during HD was +33.4 ± 11.2% above baseline, which was higher than the mean of +28.6 ± 12.2% during BFD ($p<0.05$). Mean baroreflex sensitivity was higher during HD at 7.3 ± 5.6ms/mmHg, as compared to a mean of 5.6 ± 3.4ms/mmHg during BFD ($p<0.001$). Baroreflex sensitivity also displayed more variability during HD (coefficient of variability 76.4%) as compared to BFD (coefficient of variability 60.7%) signifying increased autonomic activation during the former modality. Haemodynamic data are summarised in figure 4-g.

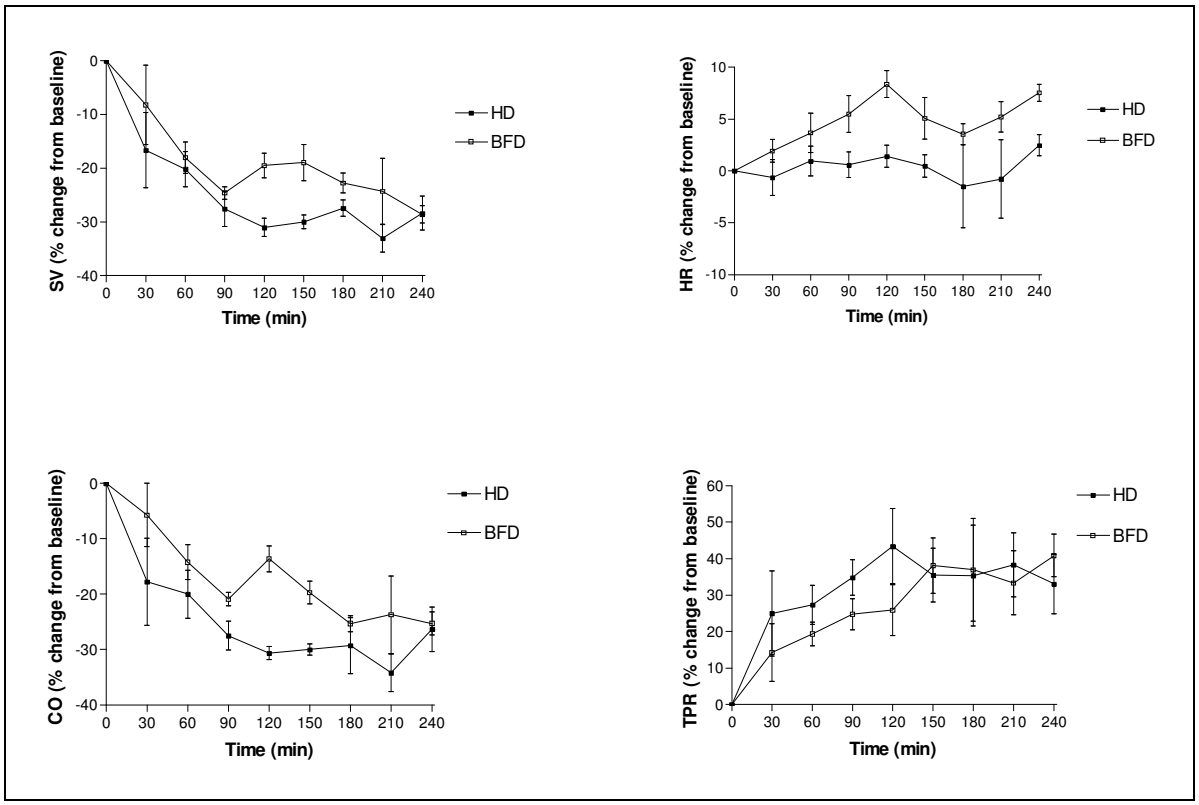


Figure 4-g.
Systemic haemodynamics during standard (HD) and biofeedback (BFD) dialysis. During BFD, there was a greater increment in pulse ($p<0.001$) and a smaller decline in stroke volume and cardiac output ($p<0.001$ for each). As a result, peripheral resistance increased to a slightly lesser extent during BFD ($p<0.05$).

4.6 Discussion

This study demonstrates that reversible reductions in LV regional wall motion occur during standard haemodialysis and to a significantly lesser extent during biofeedback dialysis. Although we did not measure blood flow before and after haemodialysis, we believe that regional LV dysfunction that develops during the procedure is most likely due to myocardial ischaemia. Previous studies have also suggested that dialysis can induce subclinical myocardial ischaemia [47, 52], but this is the first to suggest that this phenomenon can be ameliorated.

The development of new LV RWMAs during physiological or pharmacological stress occurs in response to ischaemia and its onset precedes that of symptoms and electrocardiographic changes. This principle underlies dobutamine stress echocardiography [146]. Subclinical ischaemia is therefore the likely cause of the LV RWMAs that we have demonstrated in response to the stress of dialysis. The majority of affected regions demonstrated some degree of improvement by 30min after dialysis, and SF at baseline was similar in each individual when comparing the two dialysis sessions, which indicates that these regions do not sustain irreversible damage over a short timescale. However, around a third of regions had a persistent reduction in SF at 30min post dialysis. Transient myocardial ischaemia may lead to LV dysfunction that can persist despite the return of normal perfusion. This is known as myocardial stunning [53]. Stunned myocardium can take up to 24-48

hours to recover function following an ischemic insult (which matches the inter-dialytic interval) [57]. Therefore our results would be consistent with the hypothesis that stunning occurred in our patients, with RWMAs persisting despite conditions where any perfusion abnormalities would be expected to have resolved. However, for conclusive evidence of myocardial stunning, myocardial blood flow and LV function need to be measured simultaneously.

The smaller number of RWMAs that occurred during BFD as compared with standard HD suggests less segmental myocardial ischaemia. BFD works on the principle of a negative feedback loop, designed to preserve blood volume to an extent that avoids hypotension [86]. Changes in ultrafiltration rate and dialysate conductivity are made when relative blood volume falls below a set limit, but in theory before blood pressure drops. Several studies have shown that BFD reduces IDH frequency in patients who are both prone and resistant to IDH, and this was also confirmed by our results [87, 88]. In our study, BP was significantly higher during BFD despite a trend towards higher ultrafiltration volume. This higher BP appeared to be due to a smaller decline in stroke volume and cardiac output and a higher pulse during BFD. One possible explanation for the higher stroke volume and cardiac output during BFD is a better-preserved blood volume, leading to improved cardiac filling. Baroreflex sensitivity was also lower and displayed less variability during BFD indicating less autonomic activity. This implies less haemodynamic stress during BFD. The higher BP, fewer IDH episodes or improved systemic

haemodynamics all have the capacity to lessen episodes of myocardial hypoperfusion as compared with standard HD.

The large number of new RWMAs seen in our patients may reflect their demographics. All were prone to IDH, all had LVH and seven of the eight had documented atherosclerosis, although these are not uncommon findings in chronic dialysis patients. One weakness of our study is that the patients did not undergo coronary angiography, which would have provided information about the degree and extent of large vessel coronary disease to correlate with the echocardiographic data. However, there are plausible mechanisms other than large vessel obstructive coronary disease that may predispose to myocardial hypoperfusion. Coronary flow reserve is dependent on not only large vessel patency but on microvascular disease as well, which also reduces the ability to increase blood flow to myocardium during increased demand. Specific microvascular disease has been described in dialysis patients, likely due to the high prevalence of diabetes, hypertension, LVH and vascular calcification [152]. In addition, acute severe stress can induce stunning despite normal coronary anatomy [153] but although haemodialysis is associated with sympathetic activation and a hyperadrenergic state [81, 154], none of our patients had a phenotype that resembled acute severe stress. Finally, it is possible that the autonomic nervous system may affect ventricular function during dialysis. Altered autonomic function, which is a common finding in dialysis patients, affects both IDH frequency and ventricular contractility [155, 156]. We observed differences in

baroreflex sensitivity between dialysis modalities, but this study did not assess the direct effect of the autonomic nervous system on LV function.

There was a significant difference in percentage change from baseline in EF between the dialysis modalities, which was due to the trend for EF to fall at peak stress with HD and rise with BFD. The trend for EF to rise with BFD appeared to be due to the fact that SF in some unaffected LV regions increased during peak stress. This phenomenon was also seen during HD, but to a lesser extent. This corresponds to the better preservation of stroke volume and cardiac output during BFD. Again the reasons behind these changes are not explained, but may suggest that those regions without ischaemia are better able to increment function in the short term in response to the haemodynamic stress of dialysis.

Our study does have some potential weaknesses. Patient numbers are small, so our results need to be replicated in a larger number of patients. In addition, ours was a short-term study and therefore any effect of dialysis-induced RWMAs on long-term cardiac dysfunction is at present purely speculative.

In conclusion, this study demonstrates that reversible segmental myocardial dysfunction occurs during dialysis. We also show that by improving intradialytic BP with biofeedback dialysis this occurs less, thereby suggesting that this phenomenon may be a target for intervention.

5. Results: the effects of cooling the dialysate on regional left ventricular function and systemic haemodynamics

5.1 Introduction

In chapter 4, we demonstrated subclinical ischaemia occurring in response to the stress of haemodialysis. We also showed that it was possible to reduce the frequency and severity of this phenomenon by using biofeedback dialysis (Hemocontrol®) to improve the haemodynamic tolerability of dialysis. However, because of the relatively small study size we wanted to confirm the findings of dialysis induced LV RWMAs in an additional group of patients.

We also wished to re-examine the hypothesis that maintaining intradialytic blood pressure and improving the haemodynamic tolerability of dialysis reduces the development of LV RWMAs. Hemocontrol® requires a relatively complicated prescription, is not widely available and works by reacting to changes in relative blood volume (RBV) that may not predict intradialytic hypotension (IDH) in all patients [85]. Although there have been many different strategies employed in an attempt to reduce IDH, reducing the temperature of the dialysate is one of the most simple [90]. Cooling the dialysate has been shown to be effective and is universally available at no additional cost.

Therefore, again using the development of reversible abnormalities in regional LV function as a marker of subclinical myocardial ischaemia, we performed a study to examine whether the improved haemodynamic tolerability of cool temperature dialysis leads to a reduction in the frequency of dialysis induced ischaemia, as compared to standard dialysis.

5.2 Methods

5.2.1 Patients

10 patients on chronic haemodialysis who were prone to IDH were recruited for a randomised cross-over study. Four patients were male and all had been on dialysis for longer than six months. All patients dialysed via native arteriovenous fistulae and all were anuric. Remaining characteristics are shown in table 5-a.

Patients were defined as IDH prone if they had episodes of IDH in more than 30% of dialysis sessions in the month before recruitment to the study. IDH was defined as systolic blood pressure (SBP) $\leq$ 100mmHg even in the absence of symptoms, or a fall in SBP > 10% of the pre dialysis reading in association with any of the classical symptoms of hypotension (headaches, cramps, light-headedness).

Patients were excluded if they had significant symptomatic cardiac failure (NYHA ≥ 3), had previously received a cardiac transplant or if it was not possible to obtain echocardiographic images of sufficient quality to allow meaningful analysis.

Patient	Age	Months on dialysis	Cause of ESRF	Vascular disease	IHD	LVMI ($g/m^{2.7}$)	Angiogram	BP lowering drugs
1	83	11	Myeloma	Y	N	85.0	N	
2	71	60	Unknown	Y	Y (angina)	52.1	N	Felodipine 10mg od, Atenolol 50mg od
3	72	33	Diabetes	N	N	55.3	N	Valsartan 160mg od
4	71	19	Diabetes	Y	Y (MI)	96.7	Y*	Diltiazem 90mg od
5	42	44	Sarcoid	N	N	25.7	N	
6	65	52	Diabetes	N	N	52.1	N†	
7	60	6	APKD	N	N	51.3	N	
8	66	6	Diabetes	Y	N	61.9	N	Diltiazem 300mg, Doxazosin 4mg od, Irbesartan 150mg od
9	55	17	Unknown	N	N	55.8	N	Nifedipine 60mg od, Ramipril 10mg od
10	68	40	Anti GBM	N	N	46.3	N	
Mean ± SD, or total number	65 ± 11	29 ± 19		4 (40%)	2 (20%)	58.2 ± 20	1 (10%)	

Table 5-a.
Patient demographics. APKD= adult polycystic kidney disease. IHD = ischemic heart disease, LVMI = LV mass index.
* Angiogram result for patient 4: Diffuse three vessel disease, not suitable for intervention.

† Patient 6 had had a dipyridamole stress test 12 months prior to entering the study which was negative.

5.2.2 Study protocol

Upon entry to the study, patients had their dry weight confirmed with reference to clinical examination. Following this, dry weight and anti-hypertensive medications remained unchanged for the duration of the study. Patients were then randomised to two groups. Group A patients were commenced on standard thrice weekly bicarbonate-based dialysis with a dialysate temperature of 37^0C (HD_{37}), whereas group B patients started thrice weekly dialysis with a dialysate temperature of 35^0C (HD_{35}). Patients, but not dialysis unit staff, were blinded to the intervention. Both groups underwent one week of the dialysis therapy before undergoing a monitored session during one of the dialysis sessions during the second week. At the end of the second week, patients then crossed over to the other dialysis modality thereby acting as their own controls. After a further week on the alternate modality, patients underwent a second monitored session on the same day of the week as the first study session.

For each monitored dialysis treatment, serial echocardiography was performed and non-invasive haemodynamic monitoring was undertaken using a Finometer (as described in chapter 3). The finger cuff was left in place for the entire session, and the non-fistula arm was used. To obtain baseline values, monitoring was commenced 30min prior to commencement of dialysis. Also before dialysis, patients had segmental multi-frequency bioimpedance performed (InBody® BS20, Seoul, Korea) to assess intracellular (ICW), extracellular (ECW) and total body water (TBW). Body temperature was recorded before and after each session

using a digital tympanic thermometer (First Temp®, Sherwood Davis & Geck, St Louis, USA) and ambient room temperature of the dialysis unit was also noted. Blood samples were collected before and after each session in lithium heparin and EDTA tubes, and biochemical analysis performed on a multichannel autoanalyser. Cardiac troponin-T (cTnT) analysis was performed using a third generation electrochemiluminescence assay (Roche diagnostics, Lewes, UK). Post dialysis cTnT values were corrected individually for haemoconcentration with reference to percentage change in haematocrit and blood volume using the formula:

$$\text{Adjusted cTnT} = \text{cTnT}_{post} \times \frac{BV_{post}\,(1 - Hct_{post})}{BV_{pre}\,(1 - Hct_{pre})}$$

where $cTnT_{post}$ equals post dialysis cTnT, Hct_{post} equals post dialysis haematocrit, Hct_{pre} equals pre dialysis haematocrit, BV_{post} equals end dialysis blood volume and BV_{pre} equals start dialysis blood volume. Single pool Kt/V_{urea} values were calculated from pre and post urea levels [157]. Pre dialysis blood tests were drawn immediately after insertion of access needles, and post levels were taken from the arterial line 10sec after reducing blood pump speed to 50ml/min. An investigator was present for the entirety of every dialysis session to record intradialytic symptoms. We also performed quality of life scoring for both types of dialysis using the validated Short Form (SF-36) questionnaire [158], and developed a simple questionnaire to systematically evaluate symptoms of cold (see

appendix 15.1). This latter questionnaire was formulated according to similar scoring tools [159] and the questions assessing severe symptoms of cold were weighted to score more heavily. The lowest score signifying no thermal symptoms was 6 and the highest score indicating severe symptoms of cold was 24.

The primary endpoint was the frequency of new LV regional wall motion abnormalities during HD_{37} and HD_{35} in relation to their effects on BP and systemic haemodynamics.

All patients gave informed consent prior to commencement, and ethical approval for the project was granted by Derbyshire Research Ethics Committee.

5.2.3 Echocardiography

Echocardiography and subsequent analysis were performed as described in chapters 3 and 4. Images were recorded prior to commencing dialysis (baseline), at 120min and 240min during dialysis and 30min after dialysis was finished (recovery). 10 regions of the left ventricle were assessed for the development of new RWMAs at each time point. We calculated mean SF for all ten segments (SF_{MEAN}) and for those segments that developed new RWMAs (SF_{WMA}). Peak stress was defined for each patient as the point during the first monitored dialysis session when most RWMAs were present (either 120min or 240min). When comparing dialysis modalities, the same time point was used in the second dialysis session.

5.2.4 Haemodialysis details

Haemodialysis was performed as described in chapter 3. For each session, net fluid removal was set on an individual basis according to ideal dry weight. However, if there was >20% difference in programmed UF volume from the first session, the second monitored session was rescheduled.

5.3 Statistical analysis

Results are expressed as mean ± SD if parametric or median (interquartile range, IQR) if non-parametric unless otherwise stated. Echocardiographic, BP and haemodynamic data were analysed using one-way analysis of variance (ANOVA) with a design for repeated measures and Bonferroni's test to correct for multiple comparisons. The frequencies of IDH and of new RWMAs occurring during each dialysis modality were compared using Poisson regression. For other data, either the paired *t*-test or Wilcoxon rank sum test was used depending on normality of the distribution. An alpha error at $P<0.05$ was judged to be significant.

5.4 Results

5.4.1 Blood pressure

During HD_{37}, mean systolic BP (SBP) was 141.6 ± 17mmHg, mean diastolic BP (DBP) was 69.4 ± 5mmHg and mean of the mean arterial pressure (MAP) was 92.6 ± 10mmHg. During HD_{35}, BP was significantly higher with mean SBP of 158.8 ± 14mmHg, mean DBP of 78.6 ± 4mmHg and mean MAP of 110.9 ± 7mmHg ($p<0.001$ for all comparisons). The lower mean BP with HD_{37} was the result of a fall in BP after the first hour of dialysis, whereas with HD_{35} BP was maintained until the last third of the treatment. These data are summarised in figure 5-a.

There were two episodes of symptomatic hypotension during HD_{37} as compared to one with HD_{35} (p=ns), all of which required administration of normal saline plus temporary cessation of UF. However, there was a significant difference in the number of asymptomatic IDH between the treatments, which occurred with a frequency of 0.4 episodes per session with HD_{35} as compared to a rate of 6.2 episodes per session with HD_{37} (OR 15.5, 95% CI 5.6 to 14.2).

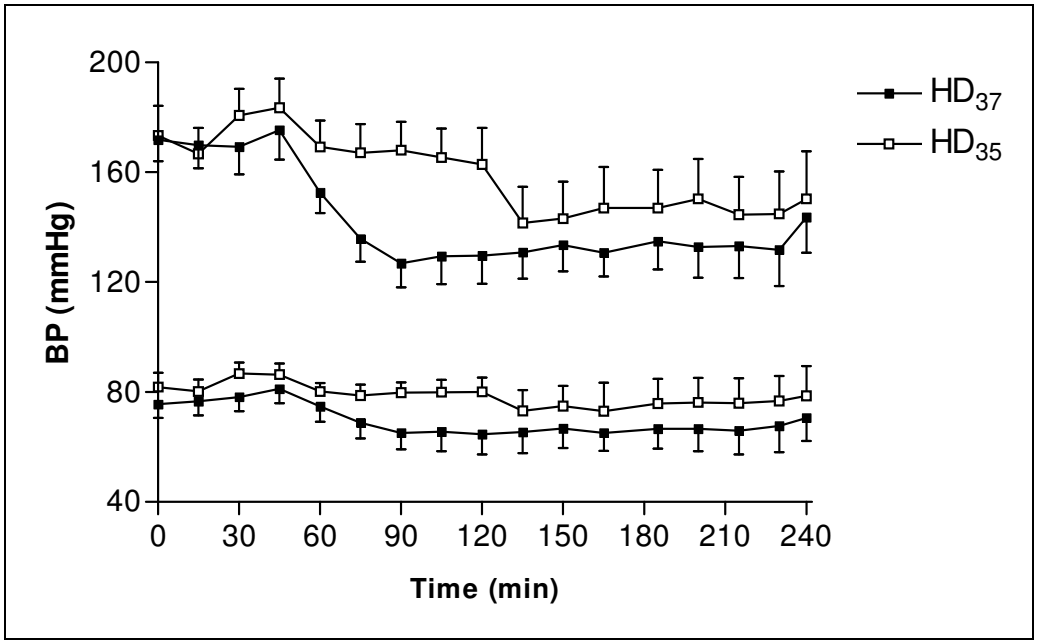


Figure 5-a.
Overall mean BP for HD_{37} and HD_{35}. BP was significantly higher during HD_{35} ($p<0.001$ for all comparisons).

5.4.2 Echocardiographic data

Throughout the study, all patients were in sinus rhythm and none had significant valvular disease or pulmonary hypertension. SF at baseline in all regions was compared on an individual basis for each type of dialysis; there were no significant differences in baseline SF in any of the patients.

A total of 49 new RWMAs occurred in nine patients during HD_{37}. In contrast, only 13 new RWMAs occurred in four patients during HD_{35} (OR 3.8, 95% CI 2.1 to 6.9). By 30min post dialysis, 24 (49%) of the affected areas with HD_{37} had recovered normal motion whilst with HD_{35} 8 (62%) of RWMAs had improved (OR 4.9, 95% CI 1.9 to 12.1). Therefore, with both types of dialysis but to a greater extent with HD_{37}, a significant proportion

of affected regions still had SF >20% less than baseline at 30min post dialysis. These data are summarised in figure 5-b.

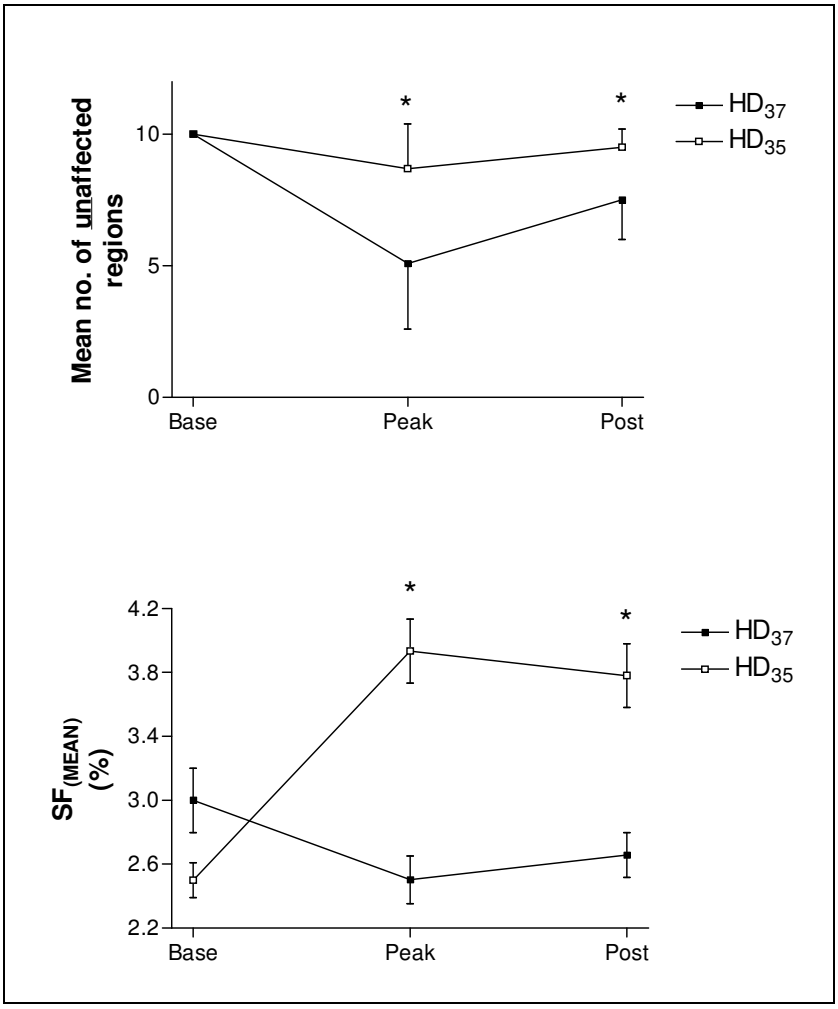


Figure 5-b.
i) Mean number of *un*affected LV regions during HD_{37} and HD_{35}. Data are expressed as mean ± SE. Comparison at peak stress, OR 3.8 (95% CI 2.1 to 6.9), in recovery OR 4.9 (95% CI 1.9 to 12.1).

ii) Overall mean regional LV function (SF) during HD_{37} and HD_{35}. Data are expressed as mean ± SE.
* $p < 0.001$ by ANOVA.

SF_{WMA} declined with both types of dialysis at peak stress before improving in recovery. There were no differences in SF_{WMA} between HD_{37} and HD_{35} at any of the time points, showing that the areas that did develop new RWMAs were affected to a similar magnitude. However, due to difference in the overall number of new RWMAs, SF_{MEAN} at peak stress was significantly lower with HD_{37} (2.5 ± 1.6%) as compared with HD_{35} (3.9 ± 1.9%, $p<0.001$). This pattern of lower SF_{MEAN} with HD_{37} was also seen in recovery ($p<0.001$). Complete SF_{WMA} and SF_{MEAN} data are shown in table 5-b, figure 5-b and figure 5-c.

	EF (%)	$SF_{(MEAN)}$ (%)	$SF_{(WMA)}$ (%)
HD_{37}			
Baseline	61 ± 14	3.0 ± 1.8	3.7 ± 2
Peak	**61 ± 10***	**2.5 ± 1.6****	1.7 ± 1.2
Recovery	60 ± 12	**2.7 ± 1.5****	2.8 ± 1.6
HD_{35}			
Baseline	57 ± 10	2.5 ± 1.2	3.2 ± 1.3
Peak	**72 ± 9***	**3.9 ± 1.9****	1.8 ± 0.9
Recovery	69 ± 11	**3.7 ± 1.9****	3.1 ± 1.7

* $p<0.05$ by ANOVA comparing HD_{37} and HD_{35}
** $p<0.001$ by ANOVA comparing HD_{37} and HD_{35}

Table 5-b.
Global (EF) and regional (SF) LV function during HD_{37} and HD_{35}. Baseline is before start of dialysis, peak stress is the point at which most RWMAs were present during dialysis, and recovery is 30min post dialysis.

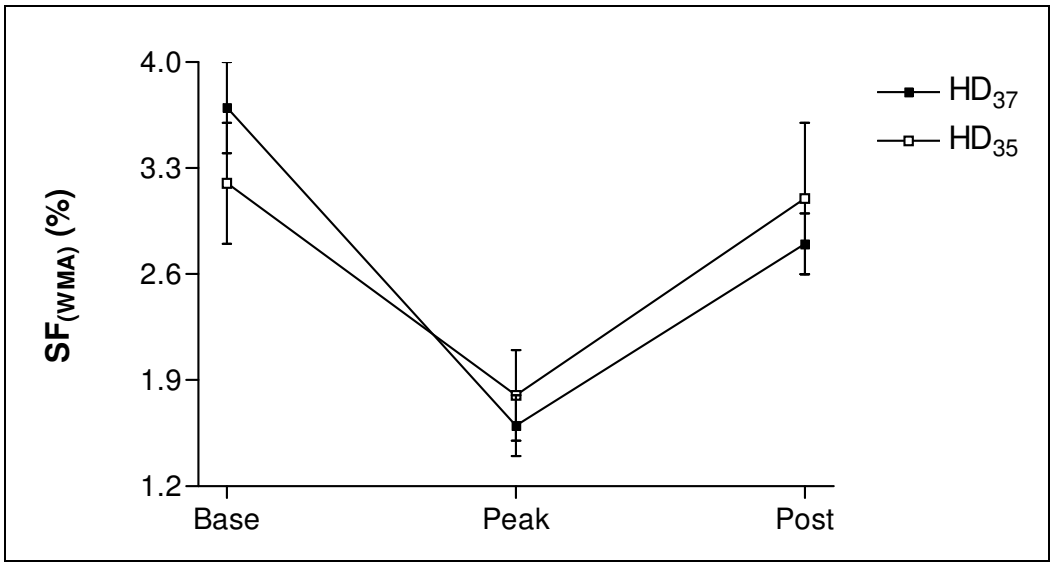


Figure 5-c.
Mean regional LV function (SF) in those regions that developed new RWMAs during HD_{37} and HD_{35}. Data are expressed as mean ± SE.

With HD_{35}, EF rose during dialysis and was higher than baseline at peak stress and in recovery. However, EF did not change during HD_{37} and therefore there was a significant difference in EF at peak stress when comparing dialysis modalities ($p<0.05$). Data for EF are shown in table 5-b and figure 5-d. We observed no differences in LV dimensions when comparing the two types of dialysis; these data are displayed in table 5-c.

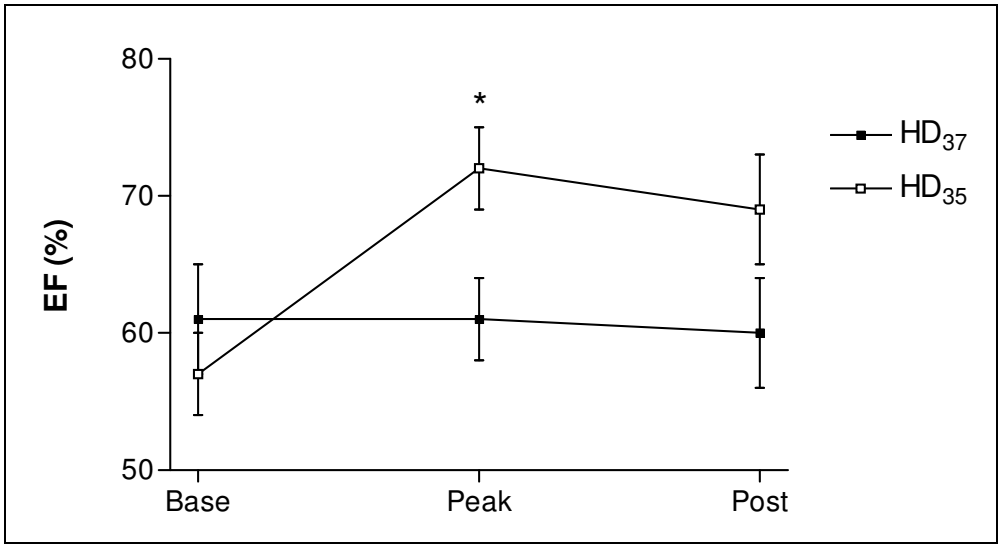


Figure 5-d.
Global systolic function (EF) during HD_{37} and HD_{35}. Data are expressed as mean ± SE.
* $p<0.05$ by ANOVA

	HD_{37}	**HD_{35}**
Pre dialysis		
LVDd (cm)	4.7 (4.3, 5.4)	5.0 (4.3, 5.5)
LVDs (cm)	3.2 (3.0, 3.8)	3.6 (3.0, 4.2)
120min		
LVDd (cm)	4.7 (4.1, 5.3)	4.5 (4.0, 5.6)
LVDs (cm)	3.1 (2.6, 3.9)	3.2 (2.7, 3.8)
240min		
LVDd (cm)	4.4 (3.9, 5.5)	4.8 (4.0, 5.5)
LVDs (cm)	2.8 (2.2, 4.0)	3.2 (2.8, 4.2)
30min post		
LVDd (cm)	4.9 (4.2, 5.5)	4.6 (4.4, 5.7)
LVDs (cm)	3.3 (2.9, 3.9)	3.4 (2.9, 4.0)

Table 5-c.
Echocardiographic measurements of cardiac dimensions before, during and after dialysis. LVDd = left ventricle diameter in diastole, LVDs = left ventricle diameter in systole. There were no significant differences in any of the dimensions when comparing the two dialysis modalities. Data are shown as median (interquartile range).

5.4.3 Haemodynamic data

Haemodynamic data are summarised in figure 5-e. SV declined throughout both dialysis modalities to a similar degree, with means of -19 ± 5% during HD_{37} and -23 ± 13% with HD_{35} (p=ns). Peripheral resistance (TPR) rose to a significantly greater extent with HD_{35}. Overall mean TPR for the entire HD_{35} dialysis session was +42 ± 18% above baseline as compared to a mean of +10 ± 8% during HD_{37} ($p<0.001$). Heart rate (HR) was lower with HD_{35} with a mean of 69 ± 2bpm representing a -4 ± 3% change from baseline. Mean HR with HD_{37} was 78 ± 2bpm, a change of +5 ± 3% from baseline ($p<0.05$). As a product of HR and SV, cardiac output (CO) was therefore lower during HD_{35} with an overall mean of -26 ± 14% as compared to a mean of -15 ± 5% with HD_{37} ($p<0.01$).

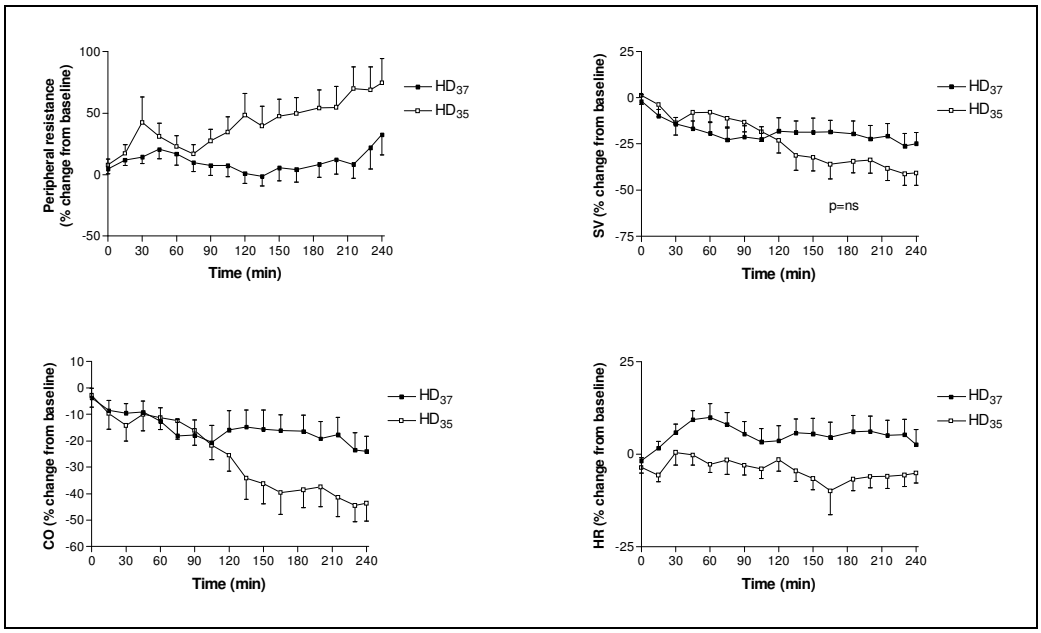


Figure 5-e.
Systemic haemodynamics during HD_{37} and HD_{35}. There was no difference in mean SV between the two dialysis modalities, but TPR was significantly higher during HD_{35}. HR was also lower with HD_{35} as was CO. Data are expressed as mean ± SE.

5.4.4 Thermal symptoms and quality of life assessments

Temperature score was higher (representing a greater sensation of cold) with HD_{35}, with a median of 12 (IQR 7 to 14) as compared to a median of 8 (IQR 6 to 12) with HD_{37} (p=0.01). Of the 10 patients, three experienced cold symptoms to a degree that made them uncomfortable during dialysis, two were able to detect the difference between modalities but did not feel uncomfortable or felt better with HD_{35}, and five were unable to differentiate between the treatments. During HD_{35}, the median temperature score for the patients that experienced unpleasant symptoms of cold was 14 (range 12 to 16) as compared to 10 (IQR 6 to 12) for those that tolerated the intervention (p=0.066). There were no obvious differences that distinguished the patients that did not tolerate HD_{35}; in particular, there were no correlations between temperature score and either pre dialysis body temperature or delta body temperature. In addition, there was no difference between the two types of dialysis in quality of life score as rated by the SF-36 questionnaire, with median values of 61 (IQR 39 to 78) with HD_{37} and 62 (IQR 50 to 73) with HD_{35} (p=ns).

There were no differences in body temperature pre dialysis but body temperature post dialysis was lower and delta body temperature was negative with HD_{35}. Ambient room temperature was also similar between the two types of dialysis and varied by less than 1^0C from the median for all study sessions. Complete temperature data are displayed in table 5-d.

	HD_{37}	HD_{35}
Temperature score	**8 (IQR 6 to 12)***	**12 (IQR 7 to 14)***
Body temp pre dialysis (^{0}C)	35.3 (IQR 34.9 to 36.3)	35.6 (IQR 35.1 to 36.6)
Body temp post dialysis (^{0}C)	**36.5 (IQR 35.3 to 36.3)****	**35.5 (IQR 35.0 to 35.9)****
Delta body temp (^{0}C)	**+0.7 (0.05 to 1.3)*****	**-0.6 (IQR-1.35 to 0.05)*****
Ambient room temp (^{0}C)	25.1 (24.3 to 25.5)	24.7 (IQR 24.5 to 25.4)

* p=0.01, **p=0.02, ***p<0.001 by Wilcoxon rank sum HD_{37} versus HD_{35}

Table 5-d.
Temperature data. Temperature score rates how cold a patient is feeling during the dialysis treatment, with the lowest score of 6 signifying no thermal symptoms and the highest score of 24 indicating severe symptoms of cold.

5.4.5 Fluid status and bioimpedance

Volume status of the patients was similar when the two dialysis modalities were compared. Equally there were no differences in body weight pre and post dialysis or in programmed ultrafiltration volume. These data are shown in table 5-e.

	HD_{37}	HD_{35}
Body weight pre dialysis (kg)	71.8 (60 to 86.4)	71.6 (60 to 87.3)
Body weight post dialysis (kg)	69.7 (59 to 84.4)	69.8 (59.3 to 84.7)
UF volume (ml)	2.0 (1.4 to 2.3)	2.1 (1.6 to 2.4)
Relative blood volume (%)	-4.1 (-8 to -2)	-6.3 (-10 to -4)
ECW (l) pre dialysis	14.5 (12.4 to 16.7)	14.4 (12.7 to 16.6)
ICW (l) pre dialysis	22 (17.9 to 24.3)	21.5 (18.4 to 24)
ECW/TBW	0.41 (0.4 to 0.41)	0.41 (0.4 to 0.42)

Table 5-e.
Bioimpedance data and pre and post dialysis body weights. Data are shown as median (interquartile range). There were no significant differences between HD_{37} and HD_{35} for any of the parameters.

5.4.6 Laboratory data

There were no differences in any of the biochemical parameters when the two types of dialysis were compared. In particular, Kt/V_{urea} was almost identical between HD_{37} and HD_{35}. In addition, cTnT levels were similar between the two modalities and did not change significantly post dialysis after correction for haemoconcentration. Laboratory data are shown in table 5-f.

	HD_{37}		**HD_{35}**	
	Pre dialysis	*Post dialysis*	*Pre dialysis*	*Post dialysis*
Haemoglobin (g/dl)	10.6 (9.6 to 12)	10.9 (9.7 to 12.1)	11.2 (10 to 12.6)	11.3 (10.1 to 12.9)
Bicarbonate (mmol/l)	23.5 (22 to 26)	28 (28 to 29.5)	24.5 (19.5 to 25.5)	28 (27 to 29)
Na^+ (mmol/l)	138 (135 to 140)	137 (136 to 138)	138 (136 to 140)	138 (137 to 138)
Corr. Ca^{2+} (mmol/l)	2.42 (2.19 to 2.53)	2.39 (2.25 to 2.45)	2.44 (2.12 to 2.55)	2.38 (2.22 to 2.52
Phosphate (mmol/l)	1.65 (1.24 to 1.92)	0.79 (0.6 to 0.92)	1.52 (1.26 to 2.03)	0.79 (0.67 to 0.91)
Albumin (g/l)	34.5 (32.5 to 37)	33.5 (32 to 40)	34 (33 to 38.5)	36.5 (33 to 39.5)
CRP (mg/l)	5 (4 to 16.5)	5 (5 to 16.5)	8.5 (4 to 16)	8 (4 to 17)
cTnT (µg/l)	0.05 (0.02 to 1.0)	0.05 (0.05 to 0.076)	0.05 (0.02 to 0.12)	0.06 (0.02 to 0.13)
PTH (ng/l)	290 (120 to 532)		302 (140 to 589	
Kt/V_{urea}	1.49 (1.2 to 1.73)		1.48 (1.1 to 1.73)	

Table 5-f.
Biochemical data. Data are shown as median (interquartile range). There were no significant differences between HD_{37} and HD_{35} for any of the parameters.

5.5 Discussion

This study shows that a significant number of new reversible LV regional wall motion abnormalities occur during standard dialysis, confirming the findings described in chapter 4. We have also demonstrated that by improving intradialytic BP and reducing IDH by cooling the dialysate, it is possible to achieve a significant reduction in the development of RWMAs.

There is an increasing body of evidence suggesting that subclinical myocardial ischaemia develops during haemodialysis. The results showing development of new RWMAs during dialysis from this current study and chapter 4 are similar, and together are strongly suggestive of subclinical ischaemia. In addition to the work from our centre, one study has demonstrated dialysis induced perfusion defects using single photon emission computed tomography (SPECT) [52] and there are 10 reports of silent intradialytic ST depression detected by Holter monitoring [28, 43-51]. Although initially there was concern that some ECG changes observed during dialysis were related to changes in electrolyte concentrations as opposed to myocardial hypoperfusion, the demonstration of dialysis induced ischaemia by electrocardiographic, echocardiographic and isotopic techniques certainly suggests that such findings are attributable to ischaemia.

In patients with coronary artery disease but without chronic kidney disease, transient myocardial ischaemia may lead to LV dysfunction that

can persist after the return of normal perfusion. This prolonged dysfunction is known as myocardial stunning [53]. Myocardial stunning has been demonstrated in humans after exercise and dobutamine stress in patients with coronary artery disease, and recent studies have provided evidence that repetitive episodes of ischaemia can be cumulative and lead to prolonged left ventricular dysfunction (myocardial hibernation) [53-59]. Myocardial stunning is therefore increasingly thought to be a causative mechanism for heart failure, with stunning and hibernation existing as part of a single spectrum. In our studies, a significant number of RWMAs persisted at 30min post dialysis when the conditions favouring the development of ischaemia during dialysis had been removed; this could be interpreted as preliminary evidence of dialysis induced myocardial stunning. Therefore, the occurrence of subclinical ischaemia in response to dialysis with sustained but reversible abnormalities in regional function could potentially contribute to the genesis of uraemic cardiac failure, particularly if the ischaemic insult is repeated up to three times per week. In support of this there is only circumstantial evidence. The fibrosis that characterises uraemic cardiomyopathy shares some common histological features with areas of hibernating myocardium in non-dialysis patients (harvested from affected areas during coronary artery bypass surgery) [160-162]. In addition, patients who receive a kidney transplant have significantly reduced all cause and cardiovascular death rates as compared to similar patients who remain on dialysis on the waiting list [163, 164]. In conjunction with this, it is also known that renal transplantation in patients with established heart failure improves left

ventricular (LV) ejection fraction and symptoms [165]. The reasons for this remain unclear and are generally attributed to reversal of the uraemic state. However, an alternative explanation is that some of this benefit seen following transplantation may be due to the avoidance of dialysis that has a negative impact on the myocardium. Importantly, a longer duration of dialysis before transplantation (greater than one year) reduces the benefit seen in terms of improvement in LV function, which is also consistent with the concept of repetitive ischaemic insults leading to more prolonged or even permanent reductions in myocardial performance [165]. However, our short term studies do not assess the long term sequelae of the presence of new RWMAs on systolic function, which at present remain speculative.

RWMAs developed to a much lesser degree during HD_{35}, implying less dialysis induced ischaemia. In addition, a greater proportion of affected regions had recovered by 30min post dialysis with HD_{35}. This latter finding may also suggest less of an ischaemic burden, as more severe episodes of myocardial hypoperfusion cause more prolonged reductions in regional LV function [166]. There was clear separation seen in terms of BP and haemodynamic response between HD_{37} and HD_{35}. BP was maintained at a higher overall level with less change throughout the HD_{35} dialysis session. In addition, there were fewer episodes of IDH. With no difference in SV, these differences were explained by a greater rise in TPR with HD_{35}, signifying vasoconstriction in response to the cooler temperature. These changes, including the lower HR with HD_{35}, are

consistent with the published studies [167, 168], but this favourable haemodynamic and BP response to HD_{35} may result in improved coronary artery perfusion during diastole and therefore explain why fewer RWMAs developed. It is possible that either the higher mean BP or the reduction in IDH may be responsible for the reduction in the incidence of RWMAs, although it is also conceivable that the effects of both of these factors are synergistic, with IDH that occurs at a lower mean BP potentially having a greater detrimental affect on myocardial perfusion. In summary, the results from this current study and in chapter 4 have shown that two different dialysis techniques that both improve the intradialytic BP profile reduce the incidence of new RWMAs in two different groups of patients.

EF increased during HD_{35} but remained unchanged during HD_{37}, resulting in a significant difference between the dialysis modalities at peak stress. The effect of haemodialysis on EF remains controversial, with different studies reporting increases, no change and also decreases [169-172]. The effect of dialysis upon EF is in part related to changes in volume status as are the changes in SV, so EF may increase whilst there is a concurrent decrease in SV. However, there is some evidence that the degree of cardiac disease may also influence the change in EF [171], possibly determining the degree to which myocardial contractility can be increased. In our study, in the absence of any differences in UF volume, fluid status or LV dimensions between the two types of dialysis, the greater number of RWMAs with HD_{37} could potentially explain why EF did

not increment in the same way as during HD_{35}. This is consistent with the work of Levy *et al* who also found an improvement in LV contractility with cooler dialysate [173], which is the only other study of which we are aware that examines LV function in response to cool dialysis.

Reducing the temperature of the dialysate improves IDH and our results show for the first time that it has a beneficial effect on intradialytic regional LV function. Furthermore, cooling the dialysate is possible on all dialysis monitors and is extremely simple to perform. However, concerns regarding unpleasant symptoms of cold and negative effects on small solute clearance due to increased peripheral sequestration have persisted. Although it is clear from the published literature and from this current study that there is no adverse affect on Kt/V_{urea} [167], the effects on patients' symptoms are less clear. In this study we found that the patient response to HD_{35} was heterogeneous, with three patients finding a dialysate temperature of 35^0C too cold (although no patients found it intolerable in the short term). The majority of patients were unable to detect a difference between the two types of dialysis and some patients who normally experience hot flushes with a dialysate temperature of 37^oC preferred HD_{35}. Our study design may have magnified any symptoms of cold by switching directly from 37^0C to 35^0C as opposed to a gradual reduction in temperature. However, there were no obvious features that predicted which patients did and did not tolerate the cooler dialysate; in particular tolerability was not predicted by pre dialysis body temperature. Therefore, a practical approach would be to gradually reduce dialysate

temperature in steps of 0.5^0C, stopping if the patient experiences excessive symptoms or when 35^0 is achieved [174]. This approach is necessary as there are no data concerning the optimal dialysate temperature to maximise potential benefits whilst avoiding excessive thermal symptoms. An alternative strategy is isothermic dialysis, in which a biofeedback device constantly adjusts dialysate temperature to keep patient body temperature constant. Isothermic dialysis has also been shown to reduce IDH effectively, and causes thermal symptoms in only 5% of treatments [175]. However, isothermic dialysis has not been evaluated in terms of its effects on regional LV function and has the disadvantage that it is less widely available as it requires specific dialysis monitors with a dedicated BTM® module (Fresenius, Bad Homburg, Germany).

cTnT is often elevated in dialysis patients and predicts mortality [61]. Furthermore, cTnT levels are higher in patients prone to IDH as compared to stable patients. However, there is continuing debate as to whether cTnT rises acutely following dialysis [63, 67]. In our current study we found no difference in cTnT levels between HD_{37} and HD_{35}, and also no acute rise in cTnT after correction for haemoconcentration. In addition, there were no correlations between cTnT levels and the frequency of RWMAs. However, all of the studies examining pre and post dialysis cTnT levels collected the post dialysis sample at the end of the session, but it is well recognised that plasma cTnT levels may only become elevated after 6 to 12 hours following an episode of ischaemia. Therefore,

although our findings in respect to cTnT do not refute that myocardial ischaemia develops in response to dialysis, they do mean that the development of RWMAs cannot be determined by measuring plasma cTnT levels in this way. It remains to be seen if cTnT levels prior to the subsequent dialysis session correlate with the frequency of RWMAs; one study found a significant increase in troponin I levels 44 hours post dialysis sessions complicated by IDH as compared to sessions in which patients were stable [78].

Our study does have some potential weaknesses. Patient numbers are relatively small and measurements were taken from only one dialysis session, but the results do replicate those of our previous study. There is some debate as to whether tympanic temperature accurately reflects body temperature, and although there are several studies supporting its accuracy we did not have the facility to measure blood line temperature [176, 177]. Finally, we did not perform coronary angiography upon these patients, so we cannot tell to what extent the degree of large vessel epicardial disease underlay our results.

In conclusion, we have confirmed our previous findings that reversible reductions in regional LV function occur in response to standard haemodialysis. We suggest that these are most likely to represent subclinical myocardial ischaemia and may be a potential aetiological factor in the development of cardiac dysfunction in this patient group. Reducing the temperature of the dialysate is an effective intervention to

lessen the development of RWMAs, and is also associated with improved haemodynamics and less IDH.

6. Results: the effects of acetate-free double chamber haemodiafiltration and standard dialysis on systemic haemodynamics and troponin-T levels

6.1 Introduction

Following the demonstration of left ventricular regional wall motion abnormalities during haemodialysis that were ameliorated by techniques that reduce IDH, we examined other novel modifications of the dialysis method that are purported to improve haemodynamic stability. These studies are described in the following two chapters.

In this chapter, we aimed to investigate whether an acetate free dialysis technique is capable of abrogating the changes in systemic haemodynamics and in biomarkers of myocardial damage seen with conventional haemodialysis. Historically, acetate was used as the sole buffer in haemodialysis. However, it was recognised that acetate was an aetiological factor in IDH, and switching from acetate to bicarbonate as the principle buffer improved cardiovascular stability and reduced intradialytic symptoms [5]. However, standard bicarbonate based dialysis is not acetate free and can still result in significant transfer of acetate to the patient, which can make up as much as 25-49% of the buffer load [113, 114]. Paired haemodiafiltration (PHF, Bellco, Mirandola, Italy) is a novel online convective technique that has been designed to be used with

dialysate that is completely acetate free. In place of acetate, the dialysate concentrate contains hydrochloric acid to prevent calcium precipitation (this is converted to water and sodium chloride during online dialysate preparation) and there is no requirement for a sterile bicarbonate infusion.

In addition to its potential haemodynamic effects, acetate has also been cited as a possible pro-inflammatory stimulus [178]. Inflammation has also been linked to cardiovascular risk in the dialysis population so as a secondary endpoint we also sought to evaluate the effects of acetate free PHF upon the systemic inflammatory response. We decided not to examine the effect of acetate on the genesis of myocardial stunning until we had elucidated whether PHF had real benefits in terms of reducing IDH and improving systemic haemodynamics during dialysis.

6.2 Subjects and Methods

6.2.1 Patients

We recruited 12 patients on chronic haemodialysis for a prospective randomised crossover study. Two patients were female and all had been on dialysis for more than six months. 10 patients had native arteriovenous fistulae and two dialysed via tunnelled internal jugular catheters. All but one patient was anuric. Remaining baseline patient information is shown in table 6-a.

A mixture of hypotension-prone and hypotension-resistant patients were recruited. Six patients were defined as 'unstable on dialysis'; they had episodes of IDH in more than 30% of dialysis sessions in the month before recruitment to the study. IDH was defined as systolic blood pressure (SBP) ≤ 100mmHg even in the absence of symptoms, or a fall in SBP > 10% of the pre dialysis reading in association with any of the classical symptoms of hypotension (headaches, cramps, light-headedness). The remaining six patients were defined as 'stable on dialysis'.

Patients were excluded if they had haemoglobin (Hb) <10g/dl, or if they had significant co-morbidity which, in the opinion of the investigator, would make completion of the study unlikely.

6.2.2 Study protocol

All patients gave informed consent prior to commencement, and ethical approval for the project was granted by Derbyshire Research Ethics Committee. Upon entry to the study, patients had their dry weight confirmed with reference to clinical examination, serial blood pressure readings and relative blood volume monitoring. Following this, dry weight and anti-hypertensive medications remained unchanged for the duration of the study. Patients were then randomised to group A or B. Group A patients were commenced on standard thrice weekly HD, whereas group B patients started thrice weekly PHF treatment. Both groups underwent 1 week of stabilisation before one of the dialysis sessions during the

second week was monitored. At the end of the second week, patients then crossed over to the other dialysis modality thereby acting as their own controls. After a further week of stabilisation, patients underwent a second monitored session.

Patient	Age	Months on dialysis	Epo (U/wk)	Anti-hypertensive medication	Cause of ESRF	Vascular disease?	IHD?
1	58	52	25000		Obstructive uropathy	N	N
2	81	18	8000		Unknown	Y	N
3	66	51	14000	Lisinopril 5mg od	Unknown	N	N
4	66	19	20000		Anti-GBM disease	N	N
5	74	35	8000	Nifedipine 60mg od, Doxazosin 6mg od	FSGS	Y	N
6	80	34	16000		Diabetic nephropathy	Y	Y
7	41	67	2000	Lisinopril 5mg od	APKD	Y	Y
8	72	62	25000		Lupus nephritis	N	N
9	64	53	12000		APKD	N	Y*
10	78	28	7000	Diltiazem 120mg bd	Renovascular disease	Y	Y
11	62	8	9000	Lisinopril 20mg od, Nifedipine 60mg od, Moxonidine 200mcg od	Diabetic nephropathy	Y	N
12	74	47	30000		Obstructive uropathy	N	N
Mean ± SD	**68 ± 11.2**	**39.5 ± 18.7**	**14667 ± 8669**				

Table 6-a.
Baseline characteristics of randomised patients. Epo = epoetin. All patients were being administered intravenous Eprex®. Epo doses and anti-hypertensive medications were left unchanged for the duration of the study. ESRF = end stage renal failure, FSGS = focal segmental glomerulosclerosis, APKD = adult polycystic kidney disease. IHD = ischemic heart disease, and refers to a diagnosis of angina or previous myocardial infarction.
* Coronary angiogram result for patient 9. Mild disease of LAD, good RCA, recannulised an occluded branch of LAD.

For each monitored dialysis treatment, non-invasive haemodynamic monitoring was undertaken using a Finometer (full description in chapter 3). The finger cuff was left in place for the entire session, and the non-fistula arm was used. To obtain baseline values, monitoring was commenced 30min prior to commencement of dialysis. Body temperature was recorded before and after each session using a digital tympanic thermometer (First Temp®, Sherwood Davis & Geck, St Louis, USA). Blood samples were collected before and after each session in lithium heparin and EDTA tubes, and biochemical analysis performed on a multichannel autoanalyser. Cardiac troponin-T (cTnT) analysis was performed using a third generation electrochemiluminescence assay (Roche diagnostics, Lewes, UK). Post dialysis cTnT values were corrected individually for haemoconcentration with reference to percentage change in haematocrit and blood volume using the formula:

$$\text{Adjusted cTnT} = \text{cTnT}_{post} \times \frac{BV_{post}\,(1 - Hct_{post})}{BV_{pre}\,(1 - Hct_{pre})}$$

where $cTnT_{post}$ equals post dialysis cTnT, Hct_{post} equals post dialysis haematocrit, Hct_{pre} equals pre dialysis haematocrit, BV_{post} equals end dialysis blood volume and BV_{pre} equals start dialysis blood volume. Single pool Kt/V_{urea} values were calculated from pre and post urea levels [157]. In addition, blood samples were collected pre and post dialysis in EDTA tubes for measurement of plasma C-reactive protein (CRP), tumour necrosis factor (TNFα) and interleukin-6 (IL6). Samples were centrifuged

at 3500rpm for 15min to separate the plasma, which was immediately frozen at -80^{0}C until time of analysis. CRP, TNFα and IL6 were subsequently measured using commercially available enzyme linked immunosorbent assays (ELISA, described in chapter 3). Pre dialysis blood tests were drawn immediately after insertion of access needles, and post levels were taken from the arterial line 10sec after reducing blood pump speed to 50ml/min. An investigator was present for the entirety of every dialysis session to record intradialytic symptoms.

Primary endpoints were percentage change in blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) in response to HD and PHF. Secondary endpoints were changes in cTnT in response to the two dialysis modalities.

6.2.3 Bicarbonate Dialysis and Paired Haemodiafiltration

All dialysis treatments were performed using Formula 2000® monitors (Bellco, Mirandola, Italy). HD was performed using low-flux polysulphone dialysers either 1.8m^2 or 2.0m^2 as per individual patients' usual prescription (LOPS® 18/20, Braun Medical Ltd, Sheffield, UK. PHF is a novel on-line HDF technique and was performed using Diapes® polyethersulphone double chamber dialysers consisting of a combined 1.9m^2 dialyser and 0.7m^2 ultrafilter (Bellco, Mirandola, Italy). This arrangement allows reinfusion to occur inside the dialyser, as previously described [117]. PHF was employed in predilution mode with the

ultrapure infusion rate set at 10l/hr. For both treatments, dialysate contained sodium 138mmol/l, potassium 1mmol/l, calcium 1.5mmol/l, magnesium 0.5mmol/l, bicarbonate 32mmol/l and glucose 1g/l. For HD treatments, dialysate contained acetate 3mmol/l whereas for acetate free PHF, the dialysate concentrate contained hydrochloric acid at 3g/l. Ultrapure water was used for dialysate preparation. For HD, sodium conductivity was set at 13.6mS/cm. To ensure equivalent sodium removal during PHF, during the stabilising week intradialytic sodium levels were monitored using a bedside analyser (AVL analyser®, Roche diagnostics, Lewes, UK) and conductivity set to ensure identical end dialysis plasma sodium levels. Other aspects of dialysis treatments did not differ between groups. All treatments were of four hours duration and anti-coagulation was achieved with unfractionated heparin. Dialysate flow was 500ml/min and dialysate temperature was set at 36^0C. For each session, net fluid removal was set on an individual basis according to dry weight. Blood pump speed varied between 250ml/min and 450ml/min depending on patients' vascular access but each individual patient had the same blood flow for their two monitored sessions.

6.2.4 Troponin analysis in the general dialysis population

To supplement the cTnT data, we recruited a further 54 patients to obtain cTnT, creatinine kinase (CK) and creatinine kinase MB (CK-MB) profiles in a representative group of our centre's haemodialysis population. All of these patients had been established on dialysis for more than six months. 40 were defined as 'stable' and the remaining 14 'unstable' as per the

above definition. Less than 10% in each group had documented ischaemic heart disease. A single pre-dialysis blood sample was collected in each of the 54 patients. Blood collection and analysis was performed as above.

6.3 Statistical analysis

Results are expressed as the mean ± SD, or for non-parametric data as the median with the interquartile range (IQR) within parentheses. Data for cTnT underwent logarithmic transformation prior to analysis due to the distribution of the data points. For statistical analysis the paired *t*-test for parametric data and Wilcoxon analysis for non-parametric data were used. An alpha error at $P<0.05$ was judged to be significant.

6.4 Results

6.4.1 Blood pressure

Mean BP was higher during HD as compared to PHF. During HD, mean systolic BP (SBP) for the entire session was 145.5 ± 8.0mmHg, mean diastolic BP (DBP) was 80.8 ± 3.5mmHg and mean of the mean arterial pressure (MAP) was 104.1 ± 5.2mmHg. During PHF, mean SBP for the entire session was 7.7mmHg lower at 137.8 ± 5.3 mmHg ($p<0.0001$), mean DBP was 1.6mmHg lower at 79.2 ± 1.9mmHg ($p=0.005$) and mean MAP was 3.6mmHg lower at 100.5 ± 2.9mmHg ($p<0.0001$). As is apparent from the wider ranges and larger SDs, there was more variation in BP during HD. Blood pressure data are summarised in figure 6-a.

The total number of IDH episodes during HD was 37, as compared with 23 episodes during PHF. There were relatively few symptomatic episodes of IDH - two during both HD and PHF. The remaining IDH episodes were asymptomatic SBP recordings <100mmHg. The difference in mean number of IDH episodes between HD and PHF did not reach statistical significance. For the asymptomatic episodes no corrective action was taken. For two of the four symptomatic episodes, UF was temporarily stopped and 200ml of saline or online reinfusate were delivered (depending on modality). For the other two episodes, symptoms resolved with temporary cessation of UF.

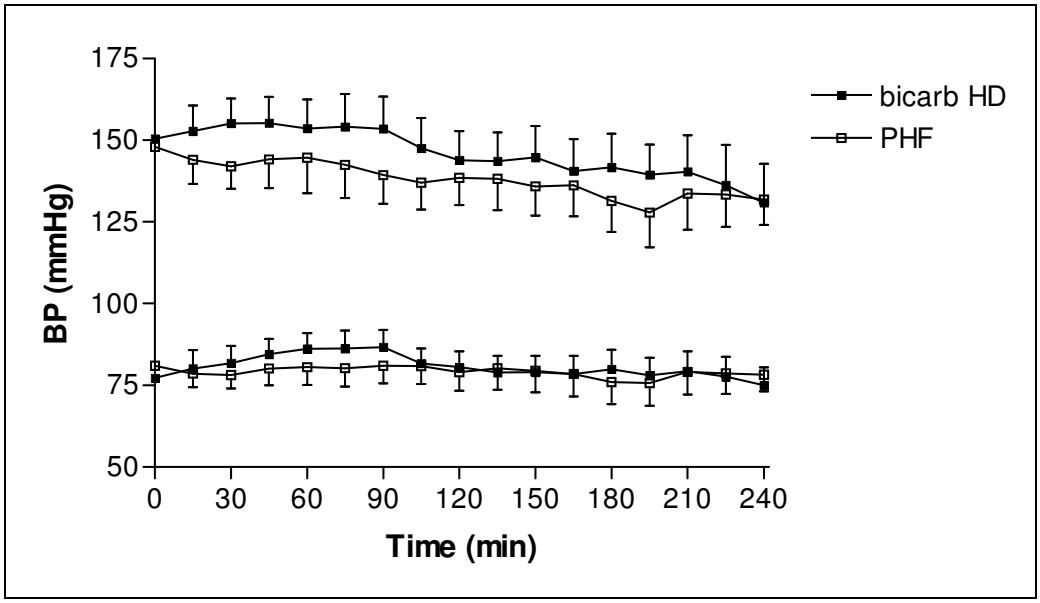


Figure 6-a.
Mean BP for HD and PHF. During PHF, BP was lower (SBP p<0.0001, MAP p<0.0001, DBP p=0.005) but there was less variation in BP. BP is seen to fall in the last 15min of HD but is maintained during the same period of PHF. For clarity, only SBP and DBP are shown. Data are expressed as mean ± SEM.

6.4.2 Haemodynamic data

Haemodynamic and BP measurements at baseline were compared to ensure repeatability of measurement technique and conditions. There were no significant differences in any of the parameters between HD and PHF.

HR did not differ between HD and PHF treatments, with mean values for the entire session of +2.1 ± 3.2 % and +2.9 ± 3.9 % respectively (p=0.199). During both treatments there was a trend for HR to increase slightly.

SV progressively declined during both treatments relative to baseline, but to a greater extent during HD. Mean SV for the entire HD session was -24.8 ± 8.4% and mean SV during PHF was -18.5 ± 8.6% (p=0.003). SV at the end of HD had fallen by -47.9% of baseline, and by -30.9% of baseline at the end PHF. As a product of HR and SV, CO showed similar changes. During HD, CO declined by -37.4% from baseline, with a mean value for the entire session of -24.8 ± 6.6%. During PHF, CO declined by -24.6% from baseline and the mean value was significantly higher at -15.2 ± 6.3% (p<0.0001).

TPR rose progressively during both treatments. However, in light of the changes in CO, TPR rose to a greater degree during HD. TPR rose by 71% from baseline during HD, with a mean value for the entire session of +39.3 ± 14.5%. During PHF, TPR rose by 47.9% with a mean value of +19.4 ± 10.3%. The difference in mean TPR values was statistically significant (p<0.0001). All haemodynamic data are summarised in figure 6-b.

The above data pertains to the population as a whole. Examining haemodynamic and blood pressure data for individual patients, 9/12 demonstrated similar changes although two patients exhibited a greater increase in HR during PHF as compared with HD. One patient demonstrated no differences between HD and PHF. The remaining two patients exhibited opposite findings to the overall data, with greater decline in CO and a greater rise in TPR during PHF as compared with HD.

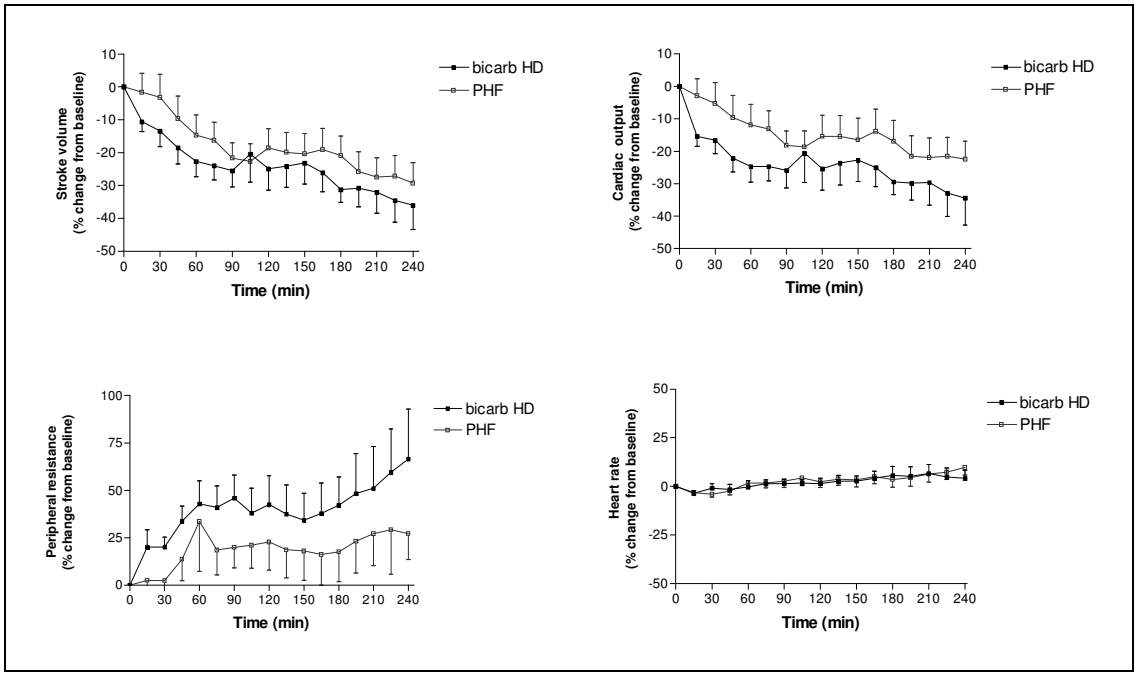


Figure 6-b.
Haemodynamic changes during HD and PHF. Data are shown as mean ± SEM.

6.4.3 Biochemical results

There were significant differences in cTnT between the two treatments. Pre PHF median cTnT levels were lower at 0.03 (IQR 0.03 to 0.16)µg/l as compared with HD with a median of 0.05 (IQR 0.03 to 0.12)µg/l ($p=0.031$). Post HD median cTnT levels (adjusted for haemoconcentration) rose to 0.06 (IQR 0.035 to 0.17)µg/l, whereas adjusted post PHF median cTnT levels fell to 0.02 (IQR 0.01 to 0.07)µg/l ($p<0.0001$). There was also a statistically significant difference between median pre and adjusted post values for HD ($p=0.01$) and for PHF ($p=0.0008$). cTnT data are summarised in figure 6-c.

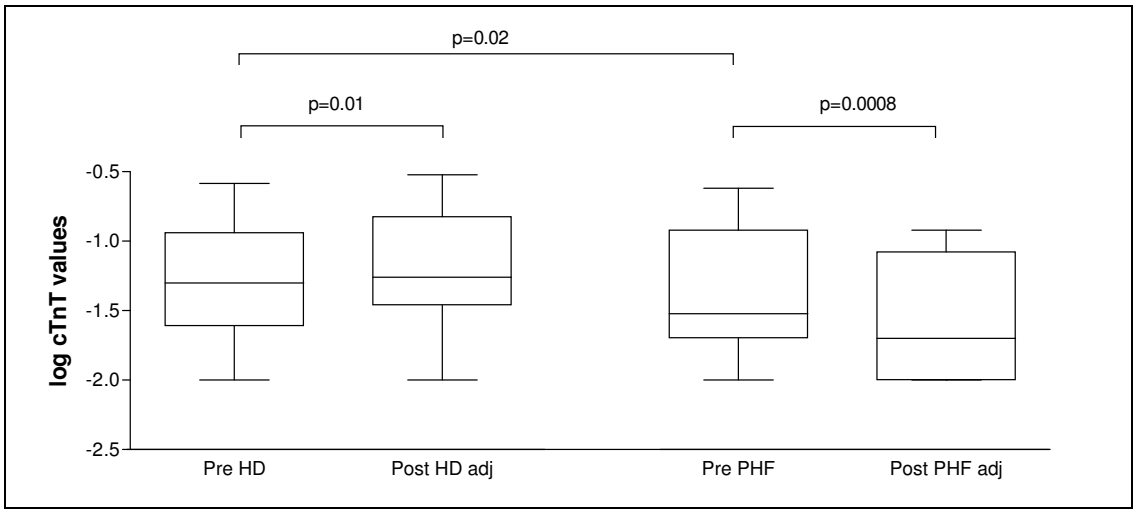


Figure 6-c.
Cardiac troponin T levels pre and post HD and PHF. Post levels are corrected for hemoconcentration.

The cTnT data for the general dialysis population is summarised in table 6-b. Pre dialysis median serum cTnT was significantly higher in the unstable group of patients, but there were no significant differences between the median CK or CK-MB levels.

	Stable	**Unstable**	***p*-value**
Age (years)	56 ± 14	59 ± 16	0.26
Pre dialysis cTnT (μg/l)	0.01 (0.01 to 0.04)	0.1 (0.05 to 0.8)	<0.0001
Pre dialysis CK (iu/l)	84	98	0.28
Pre dialysis CK MB (iu/l)	0.5	1.2	0.12

Table 6-b.
General dialysis population pre-dialysis cTnT, CK and CK-MB levels, with patients divided into 'stable' or 'unstable' on dialysis. Biochemical data are expressed as medians with IQR within parentheses.

Other biochemical results from pre and post blood samples are shown in table 6-c. Most blood tests were similar between the two treatments, including pre and post plasma sodium levels. Equally, there were no differences between dialysis treatments for mean intradialytic sodium levels at 1 and 3 hours (p=0.53 and 0.49 respectively). Median serum bicarbonate levels were 3.0mmol/l lower pre PHF as compared with pre HD levels (p=0.031) but post treatment levels were similar between the two.

	Pre HD	**Pre PHF**	***p*-value**	**Post HD**	**Post PHF**	***p*-value**
Hb (g/dl)	10.9 (10.4-12)	10.7 (9.5-11.8)	0.77	11.3 (10.7-12.9)	11.8 (10.8-12.9)	0.47
HCO_3 (mmol/l)	25 (23.5-27)	22 (20-24)	**0.016**	29 (26.5-31)	27 (25-29)	0.156
Na (mmol/l)	138 (138-141)	139 (138-141)	0.625	137 (135-139)	137 (136.5-139)	0.33
Urea (mg/dl)	63.3 (48.2-76.5)	61.9 (47.1-75.9)	0.47	19.8 (13.4-19.9)	21.3 (13.4-24.6)	0.365
Creatinine (mg/dl)	9.6 (7.5-10.2)	9.2 (7.8-10.3)	0.97	3.6 (2.9-4.2)	3.7 (3.2-4.8)	0.831
Adj calcium (mmol/l)	2.43 (2.36-2.51)	2.48 (2.36-2.55)	0.148	2.35 (2.33-2.42)	2.29 (2.28-2.44)	0.16
$P0_4$ (mmol/l)	1.81 (1.39-2.03)	1.62 (1.37-1.88)	0.73	0.78 (0.64-0.87)	0.75 (0.65-0.89)	0.13
Albumin (g/dl)	3.3 (3.1-3.7)	3.4 (3.3-3.5)	0.90	3.8 (3.6-4.0)	3.7 (3.2-4.0)	0.44
PTH (ng/l)	236 (138-382)	173 (122-310)	0.637			

Table 6-c.
Pre and post biochemical and haematological results for HD and PHF. Values shown are medians with the interquartile range within parentheses. The majority of results were similar between treatments. There was a significantly lower serum bicarbonate level pre PHF as compared to pre HD, although post levels were compatible.

6.4.4 Cytokine and CRP levels

There were no differences between any of the measured cytokines when comparing HD and PHF, either before or after dialysis. CRP and albumin levels were also similar between modalities. TNFα tended to rise following HD (+1.9 ± 3.3pg/ml) and fall following PHF (-0.9 ± 2.6pg/ml), so delta TNF was different between the two modalities ($p<0.05$). This may reflect greater clearance due to the convective element of PHF. IL6 and CRP were strongly correlated ($r=0.54$, $p<0.0001$), but there were no correlations between TNFα and either CRP or IL6. These data are shown in table 6-d.

	IL6 (pg/ml)	**TNFα (pg/ml)**	**CRP (mg/l)**	**Albumin (g/l)**
Pre HD	11.2 (7.5 – 14.8)	1.1 (0 – 2.7)	9.2 (3.7 – 14.9)	33 (30.5 – 36.5)
Pre PHF	9.9 (9.4 – 14.5)	2.7 (0 – 5.2)	10.6 (4.0 – 36)	34 (32.5 – 35)
Post HD	10.2 (7.7 – 16.9)	2.9 (0 – 2.7)	8.2 (4.3 – 15.8)	38 (36 – 40)
Post PHF	10.6 (7.4 – 14.5)	1.6 (0 – 1.8)	11.2 (3.4 – 37)	36 (32 – 39.5)

Table 6-d.
Levels of cytokines (TNFα, IL6), CRP and albumin before and after standard dialysis and acetate free PHF. There were no significant differences in any of the values.

6.4.5 Dialysis details

Pre-dialysis body weight was 76.2 ± 12.6kg for HD and 76.3 ± 12.7 for PHF (p=0.93). There were no differences between the two treatments in relative blood volume reduction, ultrafiltration (UF) rate, single-pool Kt/V or change in body temperature. Fluid removal and reduction in plasma sodium were also the same for each modality. In all patients, the prescribed dry weight was achieved. These data are shown in table 6-e.

During the stabilising week of PHF, dialysate sodium conductivity was tailored to deliver the same end dialysis plasma sodium level as HD. In predilution mode, this resulted in PHF sodium conductivity being set 2mS/cm higher than HD in all patients.

	HD		**PHF**		***p*-value**
	Mean ± SD	*Range*	*Mean ± SD*	*Range*	
Weight loss (% of body wgt)	1.9 ± 0.7	0.6 to 2.8	1.9 ± 0.8	0.4 to 3.1	0.8
Delta RBV (%)	-9.24 ± 7.56	-21 to -1.1	-9.46 ± 7.59	-22.1 to 2.6	0.94
UF rate (l/hr)	0.42 ± 0.17	0.22 to 0.67	0.45 ± 0.17	0.1 to 0.65	0.75
Delta serum Na (mmol/l)	-2.17 ± 1.95	-5.0 to 1.0	-2.10 ± 2.62	-6.0 to 4.0	0.94
Delta patient temperature (^{0}C)	0.18 ± 0.69	-0.8 to 1.5	0.28 ± 0.49	-0.7 to 1.0	0.69
Kt/V	1.38 ± 0.32	0.67 to 1.88	1.37 ± 0.28	0.81 to 1.77	0.91

Table 6-e.
Comparison of dialysis parameters between HD and PHF. RBV = relative blood volume reduction, UF = ultrafiltration. Kt/V_{urea} was a single pool value.

6.5 Discussion

Acetate transfer is significant during bicarbonate-based HD and may be a factor in the aetiology of IDH. This study demonstrates that acetate free PHF is associated with improved haemodynamics, a lower BP but with a trend towards less IDH and lower cTnT levels as compared to HD.

The haemodynamic response to PHF was characterised by attenuation of the characteristic decline in SV and CO seen with HD, and as a result a smaller increase in TPR was observed. These differences were present despite identical fluid removal, relative blood volume change and pre, intra and post dialysis plasma sodium levels. It remains contentious as to whether acetate transfer during dialysis causes myocardial depression - although several studies do show that acetate causes a reduction in myocardial contractility, there are other studies that do not confirm this [109-112]. Acetate may directly affect myocardial contractility [113, 114], although it is also possible that increased endothelial NO production stimulated by acetate affects the baroreflex arc [112, 179]. In terms of the findings of our study, it is possible that the lack of acetate transfer during PHF treatments resulted in less suppression of myocardial contractility and therefore a smaller decline in SV and CO.

An alternative explanation for our results would be that the improved haemodynamics seen with PHF are due to the comparison of a predominantly convective with a diffusive technique. However, there is

accumulating evidence that the acute benefit of convective therapies on improved stability during dialysis is due to increased patient cooling, which is mediated by a greater rise in TPR. When the therapies are matched for extracorporeal energy balance, low flux dialysis is equivalent in terms of IDH frequency and changes in systemic haemodynamics to both pre and post dilution HDF [93, 94]. These results also show that differences in solute clearance cannot explain the improved haemodynamics of HDF. In our study, we appear to have avoided significantly greater cooling during PHF by reducing the dialysate temperature during HD. Change in patients' body temperature did not differ between the two modalities, and TPR was lower during PHF. However, one potential weakness of our study is that we did not have access to thermal energy measurement equipment to formally document energy transfer during dialysis treatments. Other suggested mechanisms to explain improved stability with convective therapies include differences in sodium removal and improved biocompatibility. In our study, treatments were matched for changes in plasma sodium, and the membranes and lines were of sufficient similarity to make a difference in biocompatibility unlikely. Therefore, it seems more likely that the lack of acetate as opposed to the difference in convection explains our results, although the design of this study cannot exclude other, as yet undiscovered, advantages of convective therapies.

Vasodilatation, another well-recognised effect of acetate did not seem to be predominant as TPR was higher during HD. The higher TPR seen with

HD may have occurred in response to the greater fall in SV and CO, suggesting that a greater degree of vasoconstriction was required during HD to prevent hypotension. These observations were unexpected, as many studies have shown that acetate can cause vasodilatation, although most of this work is in respect to acetate dialysis where acetate transfer would be higher as compared to standard bicarbonate HD. If acetate is responsible for the observed differences in our study, then this might suggest that the myocardial depressant effects of acetate are the predominant influence at the levels experienced during bicarbonate dialysis.

Overall BP was lower during PHF, but was preserved with fewer fluctuations as compared to HD. This was especially evident in the last 15min of the treatments when BP during HD dropped but during the same period of PHF was maintained. In addition, there was a trend towards fewer episodes of IDH during PHF treatments. This study was not powered to detect a significant difference in IDH episodes and it may be that this trend towards less IDH would have been statistically significant if participant numbers were larger or more sessions studied. In support of this, acetate free biofiltration (AFB), a technique that employs base free dialysate and a post-dilution infusion of ultra sterile bicarbonate, has been shown to reduce IDH as compared to standard dialysis [112]. Furthermore, one longer-term study showed AFB to provide improved control of pre-dialysis BP that is consistent with our findings of lower blood pressure during PHF, but without increased instability [116]. The

results from our study show that the higher BP with HD was due to an excessive rise in TPR (greater than that required to compensate for the larger fall in CO), but the mechanisms behind this remain obscure.

We observed significant differences between HD and PHF in serum cTnT levels both pre and post dialysis. Levels were lower pre PHF as compared to HD, and after PHF the levels fell further. Following HD, cTnT levels rose, and one explanation for this is that the BP fluctuations and change in volume status seen during HD may be sufficient to induce subclinical myocardial cell damage. This is supported by our finding of higher cTnT levels in IDH prone patients in our general dialysis population, who are more at risk of myocardial hypoperfusion. These results differ from those reported in chapter 5 when cTnT did not rise following dialysis, even in the presence of echocardiographic evidence of ischaemia. Therefore, because cTnT may take up to 12 hours to rise following an ischaemic insult, measuring levels immediately following dialysis may only intermittently detect acute, dialysis induced rises in cTnT. However, if dialysis does cause subclinical myocardial ischaemia, then this may have the potential to impair cardiac performance. The lower pre dialysis cTnT levels (possibly signifying reduced overall release) and the improved myocardial performance may indicate that this effect is less with PHF.

Alternatively, it is possible that the fall in cTnT levels with PHF was due to increased clearance across the high flux membrane. High flux membranes clear molecules up to 15kDa by diffusion and can clear larger

molecules up to 25kDa by convection. However, cTnT is a charged molecule that is 38kDa in size, and some individual patients demonstrated as much as a 50% reduction in cTnT levels. It has been shown that smaller cTnT fragments (8 to 25kDa) are also present in the serum of dialysis patients [180], and these might be cleared significantly by PHF. However, recent work has shown that these fragments are not responsible for the elevated readings seen in many dialysis patients when the current third generation assay is used [64]. Therefore, to determine the mechanism of the reduced cTnT levels seen with PHF, it would be necessary to compare pre and post dialysis cTnT levels with PHF versus a standard haemodiafiltration technique that provides similar clearance.

Pre PHF bicarbonate levels were noted to be slightly lower than pre HD levels, although there was no difference in post dialysis levels. This may reflect the fact that acetate will continue to be converted to bicarbonate in the immediate post dialysis period, and as a result, serum bicarbonate levels can initially continue to rise. In contrast, the post dialysis serum bicarbonate with acetate free dialysis represents the maximum level achieved. Our results suggest that bicarbonate conductivity should be set 3-4 mS/cm higher for PHF than HD to achieve equivalent overall acid-base control. Sodium balance during predilution haemodiafiltration (HDF) remains a matter of debate, with different authors reaching different conclusions. We took a pragmatic approach to ensure the dialysis modalities were matched in terms of change in plasma sodium concentrations but did not perform detailed sodium balance studies.

However, our findings of slightly higher conductivity settings during PHF have been previously reported during predilution HDF [181]. We can only speculate as to possible causes, but this may be due to large infusion volumes added before the dialyser leading to a reduction in the Donnan effect. Therefore, despite the relative hypotonicity of the reinfusate, the ultrafiltrable sodium fraction may have been increased.

We did not find any significant differences in inflammatory effects between standard dialysis (using a biocompatible membrane and ultrapure dialysate) and acetate free PHF, with similar levels of CRP and cytokines with both treatments. These results are similar to one other report comparing these two treatments [182], and suggest that the levels of acetate transfer during standard bicarbonate dialysis are insufficient to stimulate an inflammatory response. The fall in TNFα following PHF may represent greater clearance of this molecule as compared to standard dialysis, either due to convection or to the higher flux membrane.

In conclusion this study demonstrates that acetate-free PHF is associated with a lower BP without increased instability and significantly less deterioration in systemic haemodynamics as compared to cool temperature low-flux bicarbonate-based dialysis. We suggest that one possible explanation for this is the absence of acetate that may be important in maintaining myocardial contractility. The rise in troponin with standard dialysis and the higher readings seen in IDH prone patients provide further evidence that subclinical myocardial damage may occur

during the procedure. PHF may be associated with a reduction in myocardial cell injury, increased clearance of cTnT across a high flux membrane or a combination of both. However, there was no evidence that PHF reduced systemic inflammation. This study provides initial evidence that PHF may be a superior treatment in selected patient groups. However, given the relative expense and complexity of this treatment compared to standard dialysis and lack of clear separation in terms of IDH between the treatments, it is remains doubtful that PHF would be the most efficient technique to reduce dialysis induced myocardial stunning.

7. Results: a comparison of progressive dialysate conductivity reduction with Diacontrol® and standard dialysis

7.1 Introduction

Empirical reduction of dialysate sodium to below plasma water sodium enhances sodium removal by diffusion, and may lead to a reduction in total body sodium. Some studies, including previous work from our centre have shown that progressively reducing dialysate sodium results in significant improvements in pre and post dialysis blood pressure, matched by a fall in IDWGs (figure 7-a, [102]). This approach leads to dialysis with lower UF rates, which may in turn be important in avoiding IDH and therefore myocardial hypoperfusion during dialysis. The drawback of this approach is that in the early period of dialysis an excessive reduction in plasma tonicity may result, leading to rapid falls in circulating volume with resultant symptoms and hypotension. Therefore, in an ideal situation dialysate sodium should be as low as possible, providing this can be achieved without excessive intradialytic symptoms.

In an attempt to achieve this, a novel biofeedback dialysis technique has been developed (Diacontrol®, DC described in chapter 1) that returns a patient to a prescribed plasma sodium level by the end of dialysis. It is programmed to avoid rapid drops in plasma tonicity early in dialysis and

therefore may confer a benefit in terms of reducing IDH (figure 1-e), although clinical evaluation of DC remains limited.

Within a protocol of progressive dialysate conductivity reduction, we sought to further investigate whether using DC would lead to improved haemodynamic tolerability and less IDH as compared to conventional dialysis (with a fixed dialysate sodium), thereby allowing maximisation of potential benefits of sodium reduction.

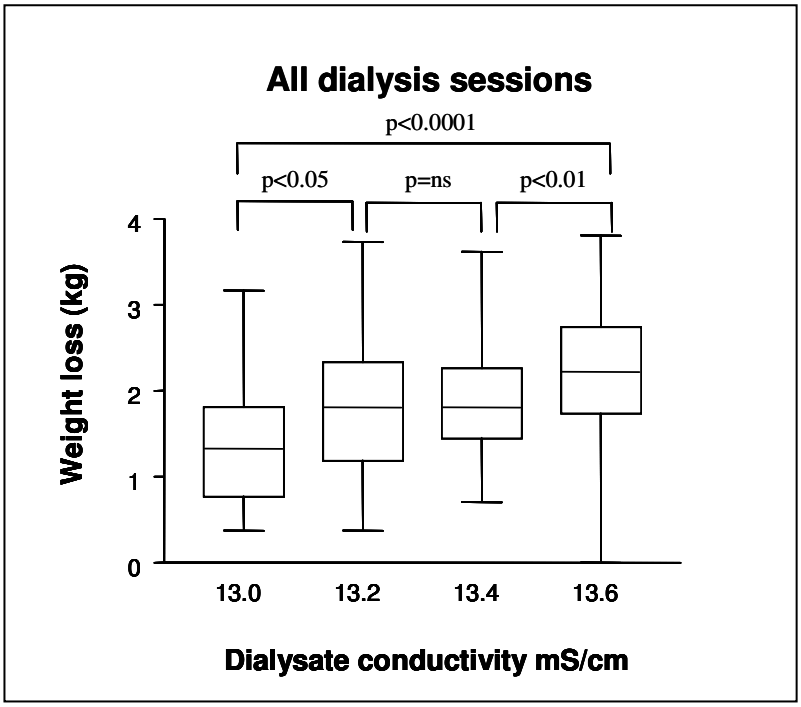


Figure 7-a.
Interdialytic weight gains progressively fall as dialysate conductivity is reduced [102].

7.2 Subjects and Methods

7.2.1 Patients

We recruited 10 patients on chronic haemodialysis for a prospective randomised single-blind crossover study. Patients were characterised by a low incidence of IDH, defined as less than three episodes of IDH in the month before recruitment. Three patients were female and all had been on dialysis for more than six months. Eight patients had native arteriovenous fistulae and two dialysed via tunnelled internal jugular catheters. All but one patient was anuric, and the remaining patient had a residual 24-hour urine volume of only 300ml. Seven patients were not taking anti-hypertensive medication. At the outset of the study, mean haemoglobin was 11.7 ± 0.7g/dl (range 10.3 – 12.6) and serum albumin was 36.3 ± 3g/l (range 32 – 42). Remaining baseline patient information is shown in table 7-a.

Patients were excluded if they had experienced three or more episodes of IDH, if their target weight had been altered in the month before recruitment or if they were treated with any modality other than standard bicarbonate-based haemodialysis. All patients gave informed consent prior to commencement, and ethical approval for the project was granted by Derbyshire Research Ethics Committee.

Patient	Age (years)	Months on dialysis	Anti-hypertensive medication	Cause of ESRF
1	69	17	Lisinopril 20mg od	APKD
2	42	240		Reflux nephropathy
3	66	6		Vasculitis
4	66	72		TIN
5	55	6	Nifedipine 60mg od Ramipril 2.5mg od	Unknown
6	73	6	Lisinopril 2.5mg od Atenolol 50mg od Amlodipine 10mg od	Unknown
7	76	67		Anti-GBM disease
8	65	81		APKD
9	79	38		ARVD
10	74	33		Obstructive uropathy
Mean ± SD	66.5 ± 11	57 ± 71		

Table 7-a.
Patient characteristics. ESRF = end stage renal failure; APKD = adult polycystic kidney disease, ARVD = atherosclerotic renovascular disease, TIN = tubulo-interstitial nephritis.

7.2.2 Study protocol

Upon entry to the study, patients had their dry weight confirmed with reference to clinical examination and serial blood pressure readings. Standard low sodium diet (<6g per day) was reinforced with additional dietetic interactions, but no additional dietary interventions were performed. Patients were then randomised to group A or B. Group A patients were commenced on standard thrice-weekly HD and group B were started on DC. After each two week period (six dialysis treatments) conductivity settings were progressively reduced as shown in figure 7-b. For HD, initial fixed dialysate conductivity was 13.6mS/cm ($HD_{13.6}$), reducing to 13.4mS/cm ($HD_{13.4}$) then to 13.2mS/cm ($HD_{13.2}$). We have previously demonstrated the average end dialysis plasma conductivities obtained at these settings [102]. Therefore, initial prescribed end dialysis plasma conductivity with DC was 13.7 ($DC_{13.7}$), reducing to 13.6mS/cm

($DC_{13.6}$) and then 13.5mS/cm ($DC_{13.5}$). 13.5mS/cm is the lowest target conductivity allowed by DC when inputting the prescription into the dialysis monitor due to software programming. At the end of the sixth week, patients returned to their usual dialysis prescription for two weeks washout. At the start of the ninth week patients crossed over to the other dialysis modality thereby acting as their own controls, and the same conductivity reduction was performed. If intradialytic symptoms or IDH attributable to a reduction in conductivity developed, patients were returned to the last conductivity level at which they has been stable. Study duration was 14 weeks and data were collected from a total of 360 dialysis sessions.

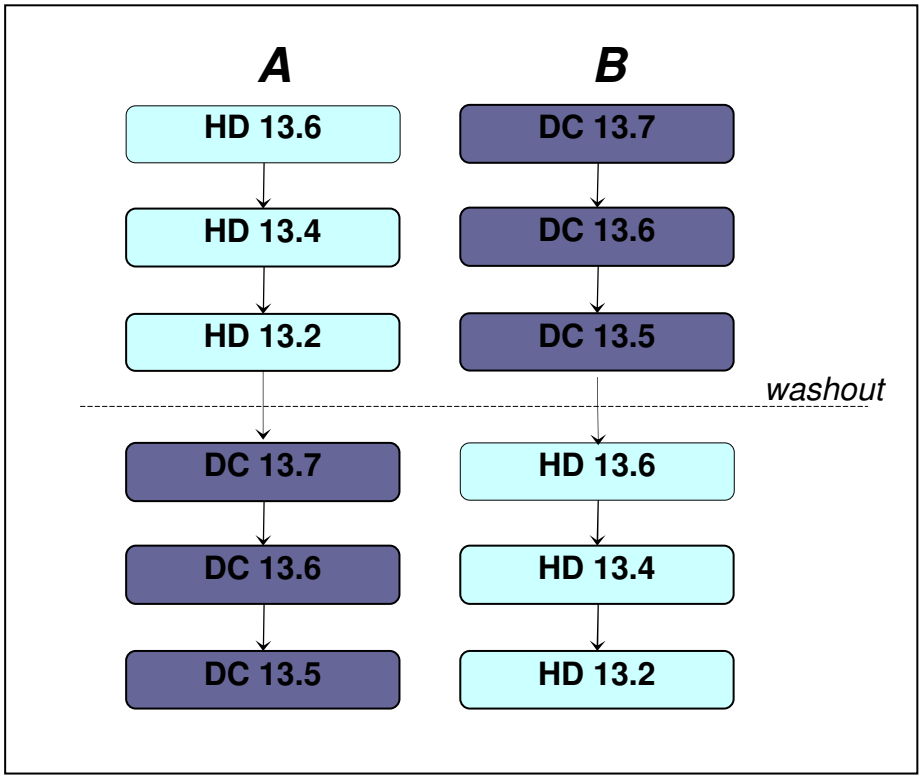


Figure 7-b.
Flow diagram of study design.

7.2.3 Dialysis details

Dialysis was performed as described in chapter 3. Technical aspects and validation of Diascan® and Diacontrol® (DC) have been described previously [104-106, 108]. DC is a biofeedback system that uses single pool kinetic modelling, and in response to plasma conductivity (measured by ionic dialysance, Diascan®) automatically adjusts dialysate conductivity throughout the dialysis session to achieve the prescribed plasma conductivity by the end of dialysis.

7.2.4 Data collection

For every treatment dialysis run data were automatically collected and downloaded to a database (Exalis®, Gambro-Hospal, Mirandola, Italy). This included pre, post and intradialytic BP, start and end plasma conductivities, dialysate conductivity (during DC a time averaged mean was subsequently calculated), ionic mass balance (IMB) and relative blood volume (RBV). Conductivities and IMB were estimated by Diascan® every 15min and RBV continuously measured using an optical measure of haemoglobin concentration (Hemoscan®, Gambro-Hospal, Mirandola, Italy). An investigator interviewed patients at each dialysis session to monitor symptoms and episodes of IDH, defined as systolic blood pressure (SBP) ≤ 100mmHg even in the absence of symptoms, or a fall in SBP > 10% of the pre dialysis reading in association with any of the classical symptoms of hypotension (headaches, cramps, lightheadedness). In addition, each patient completed a validated thirst

score on a weekly basis (see appendix 15.2). Absence of thirst is represented by a minimum score of 7 and most thirsty represented by a maximum score of 35.

During the second week of each conductivity setting, both before and after a mid-week dialysis session, patients had segmental multi-frequency bioimpedance measurements using tactile electrodes (InBody® BS20, Seoul, Korea) to assess intracellular (ICW) and extracellular water that was then corrected for body weight (ECW). Before and after the same dialysis session blood samples were collected in lithium heparin and EDTA tubes. Biochemical analysis of the blood samples was performed on a multichannel autoanalyser. Plasma sodium concentration was determined by indirect ionometry, and the diffusible concentration was obtained by using a correction factor of 0.94 [183]. Single pool Kt/V_{urea} values were calculated from pre and post urea levels according to Daugirdas II.

At the highest and lowest conductivity settings with each dialysis modality (weeks 2, 6, 10 and 14) patients had ambulatory BP monitors fitted (OSCAR2, SunTech Medical, Eynsham, UK) for the first 24 hour period immediately following dialysis. The initial reading was taken at the same time as the post dialysis BP. This monitor has been shown to meet the accuracy criteria of the European Society of Hypertension International Protocol.

7.3 Statistical analysis

Two separate analyses were planned to take account of the fact that individual patients display varying pre dialysis sodium and conductivity levels, and therefore empirical reduction of fixed dialysate conductivity will result in differing end dialysate plasma conductivity in different patients. Therefore, a direct comparison between HD and DC may compare dialysis sessions with different sodium balance. The first analysis ('conductivity reduction') was designed to compare the different variables within each dialysis modality at the different conductivity settings and all treatment sessions were included.

The second analysis was designed to compare HD with DC. For each individual, data were paired and analysed when end dialysis plasma conductivity was equal for both dialysis modalities. Pairs will be referred to as DC_H or HD_H corresponding to those with end dialysis plasma conductivity of 13.7mS/cm, DC_M or HD_M corresponding to those pairs with end dialysis plasma conductivity of 13.6mS/cm, and DC_L or HD_L corresponding to those pairs with end dialysis plasma conductivity of 13.5mS/cm.

Results are expressed as mean ± SD unless otherwise stated. Data were analysed using one-way ANOVA with Bonferroni's test to correct for multiple comparisons after confirmation of a normal distribution and equal variance. The IDH rate for each setting was compared using Poisson regression. An alpha error at $P<0.05$ was judged to be significant.

7.4 Results

Table 7-b shows the number of patients at each level of fixed dialysate conductivity that had end dialysis plasma conductivity equal to each setting of DC. 10 pairs of data were excluded from the second analysis; with HD end dialysate plasma conductivity was <13.5mS/cm in nine cases, and was >13.7 mS/cm in one case.

	$DC_{13.7}$	$DC_{13.6}$	$DC_{13.5}$
$HD_{13.6}$	**6**	2	0
$HD_{13.4}$	1	**5**	2
$HD_{13.2}$	0	0	**4**

Table 7-b.
Table to demonstrate number of patients at each level of fixed dialysate conductivity (HD) that had end dialysis plasma conductivity equal to each setting of Diacontrol® (DC).

7.4.1 BP and intradialytic symptoms

BP data are shown in table 7-c. BP at the outset of the study was largely well controlled, and during conductivity reduction with both HD and DC there were no significant changes in pre, intra or post dialysis BP. Equally there was no difference in IDH frequency between the conductivity settings, with rates of 0.14, 0.28 and 0.22 events/session at $HD_{13.6}$, $HD_{13.4}$ and $HD_{13.2}$ respectively and IDH rates of 0.29, 0.3 and 0.34 events/session with $DC_{13.7}$, $DC_{13.6}$, and $DC_{13.5}$ (p=ns for all). Reduction of conductivity was well tolerated during both types of dialysis, and only one of the ten patients experienced unacceptable symptom levels (muscle

cramps) during $HD_{13.2}$ for which the dialysate conductivity was returned to 13.4mS/cm.

	HD			**DC**	
Pre-dialysis	SBP	DBP	**Pre-dialysis**	SBP	DBP
HD 13.6	140.5 ± 20.2	76.8 ± 15.3	DC 13.7	146.4 ± 20.3	80.1 ± 13.2
HD 13.4	143.6 ± 19.5	79.1 ± 17.0	DC 13.6	143.1 ± 16.4	78.3 ± 14.0
HD 13.2	138.4 ± 22.6	75.8 ± 11.2	DC 13.5	141.8 ± 24.1	76.5 ± 10.6
Intra-dialysis			**Intra-dialysis**		
HD 13.6	126.5 ± 16.5	70.7 ± 9.9	DC 13.7	126.9 ± 17.6	70 ± 10.4
HD 13.4	125.1 ± 18.2	70.9 ± 10.2	DC 13.6	124 ± 16.4	69.9 ± 9.9
HD 13.2	127.8 ± 19.1	68.5 ± 10.0	DC 13.5	123.5 ± 16.7	68 ± 8.9
Post-dialysis			**Post-dialysis**		
HD 13.6	126.7 ± 18.6	71.3 ± 12.0	DC 13.7	133.6 ± 26.6	70.4 ± 12.7
HD 13.4	128.8 ± 23.8	69.6 ± 13.1	DC 13.6	124.2 ± 18.9	68.8 ± 13.4
HD 13.2	126.9 ± 18.5	68.9 ± 11.9	DC 13.5	125 ± 18.5	72.5 ± 16.7

Table 7-c.
Blood pressure during conductivity reduction with fixed dialysate conductivity (HD) and Diacontrol® (DC). Comparisons were made only within each modality (i.e. HD and DC were not directly compared) and $p>0.05$ for all.

There were few differences in pre, intra or post dialytic BP between HD and DC when matched for end dialysis plasma conductivity. The only difference was seen in post dialysis SBP when comparing HD_L that had a mean of 130.5 ± 25mmHg with DC_L with a mean of 118 ± 17mmHg

(p<0.05). Equally there were no differences in IDH frequency, with rates of 0.2 events/session and 0.4 events/session for HD_H and DC_H, 0.2 events/session and 0.1 events/session for HD_M and DC_M respectively and 0.3 events/session and 0.6 events/session for HD_L and DC_L respectively (p=ns for all). BP data for matched sessions are shown in table 7-d.

	Pre SBP	**Post SBP**	**Pre DBP**	**Post DBP**	**Intra-dialysis SBP**	**Intra-dialysis DBP**
HD_H	145 ± 20	133 ± 27	80 ± 16	76 ± 16	129 ± 22	72 ± 15
DC_H	150 ± 21	139 ± 18	83 ± 19	75 ± 13	132 ± 19	73 ± 11
HD_M	145 ± 24	131 ± 29	81 ± 10	71 ± 14	132 ± 20	76 ± 12
DC_M	151 ± 23	130 ± 22	82 ± 16	75 ± 21	134 ± 26	76 ± 17
HD_L	141 ± 17	**130 ± 23***	74 ± 16	70 ± 16	130 ± 18	70 ± 11
HD_L	142 ± 22	**118 ± 17***	75 ± 10	67 ± 19	124 ± 19	65 ± 11

Table 7-d.
BP data for those sessions when end dialysis plasma conductivity was matched for HD and DC. * p<0.05 by ANOVA.

7.4.2 Ambulatory BP

One patient did not tolerate ABPM and therefore only nine patients were included in this part of the analysis. ABPM data are summarised in figure 7-c and table 7-e. Again, baseline BP was low at the outset. Overall 24hour, daytime and night time BP did appear lower when comparing $HD_{13.6}$ to $HD_{13.2}$ and when comparing $DC_{13.7}$ to $DC_{13.5}$; however, none of these differences reached statistical significance (p=ns).

The number of patients displaying night time dipping (defined as a reduction in daytime SBP of >10%) did not change with conductivity reduction. Three patients displayed dipping during $HD_{13.6}$, $HD_{13.2}$ and $DC_{13.7}$ and four patients displayed dipping during $DC_{13.5}$. Also, there were no differences between night-day SBP ratios at the different conductivity settings.

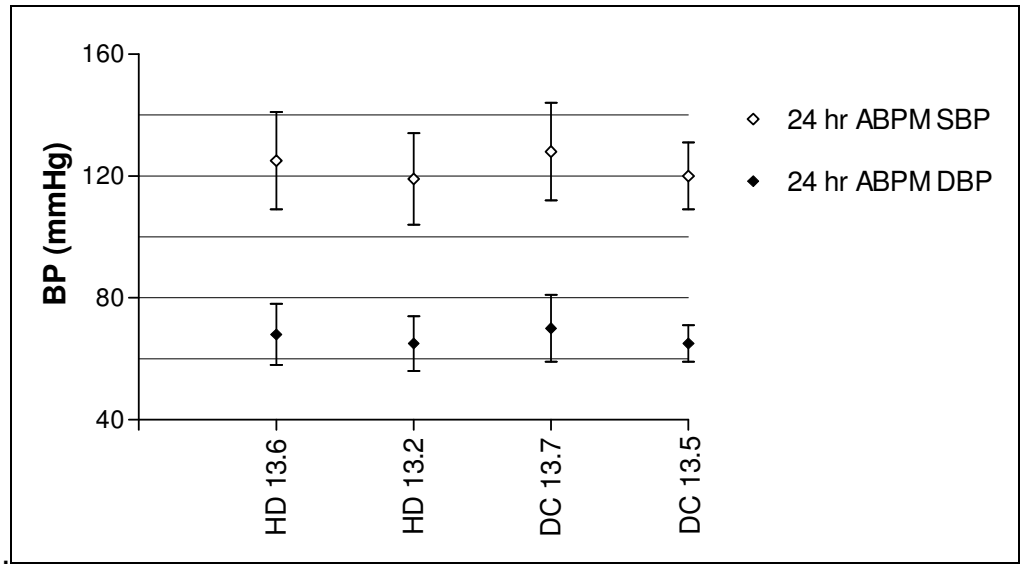


Figure 7-c.
Overall interdialytic ambulatory BP data.

	$HD_{13.6}$	$HD_{13.2}$	$DC_{13.7}$	$DC_{13.5}$
Overall 24hr SBP	125 ± 16	119 ± 15	128 ± 16	120 ± 11
Daytime SBP	126 ± 17	121 ± 14	131 ± 16	123 ± 10
Night time SBP	119 ± 14	115 ± 16	119 ± 17	116 ± 17
Overall 24hr DBP	68 ± 10	65 ± 9	70 ± 11	65 ± 6
Daytime DBP	69 ± 10	67 ± 9	71 ± 11	66 ± 6
Night time DBP	64 ± 8	62 ± 10	67 ± 10	62 ± 8

Table 7-e.
Interdialytic ambulatory BP data. There were no differences when comparing ABPM results for any of the dialysis settings.

7.4.3 Sodium balance

During conductivity reduction with HD, start dialysis plasma conductivity was significantly lower with $HD_{13.2}$ (13.8 ± 0.28mS/cm) as compared with $HD_{13.6}$ (14.0 ± 0.23mS/cm, $p<0.05$). This was also reflected in pre-dialysis plasma sodium concentration, which was 138.3 ± 2.6mmol/l with $HD_{13.6}$ as compared to 135.9 ± 2.1mmol/l with $HD_{13.2}$ ($p<0.05$). During conductivity reduction with DC, there was a non-significant trend for start dialysis plasma conductivity to fall as conductivity was reduced. Start dialysis plasma conductivity was 14.0 ± 0.27mS/cm with $DC_{13.7}$, 14.0 ± 0.21mS/cm with $DC_{13.6}$ and 13.9 ± 0.25mS/cm with $DC_{13.5}$ (p=ns). However, there was a difference between pre-dialysis plasma sodium concentration with $DC_{13.7}$ (139 ± 2.2mmol/l) and $DC_{13.5}$ (136.6 ± 2.0mmol/l, $p<0.05$).

End dialysis plasma conductivity fell during conductivity reduction with both types of dialysis. With $HD_{13.6}$, $HD_{13.4}$ and $HD_{13.2}$, end dialysis plasma conductivity was 13.7 ± 0.1mS/cm, 13.6 ± 0.2mS/cm and 13.4 ± 0.14mS/cm respectively ($p<0.001$ for each comparison). Diacontrol successfully achieved prescribed end dialysis plasma conductivity with a mean difference of 0.019 ± 0.03mS/cm between prescribed and achieved values (p=ns). End dialysis plasma conductivity was significantly lower following $HD_{13.2}$ as compared to $DC_{13.5}$ ($p<0.001$), but there were no differences between $HD_{13.6}$ and $DC_{13.7}$, or between $HD_{13.4}$ and $DC_{13.6}$

(p=ns). Start and end dialysis plasma conductivity data are summarised in figure 7-d.

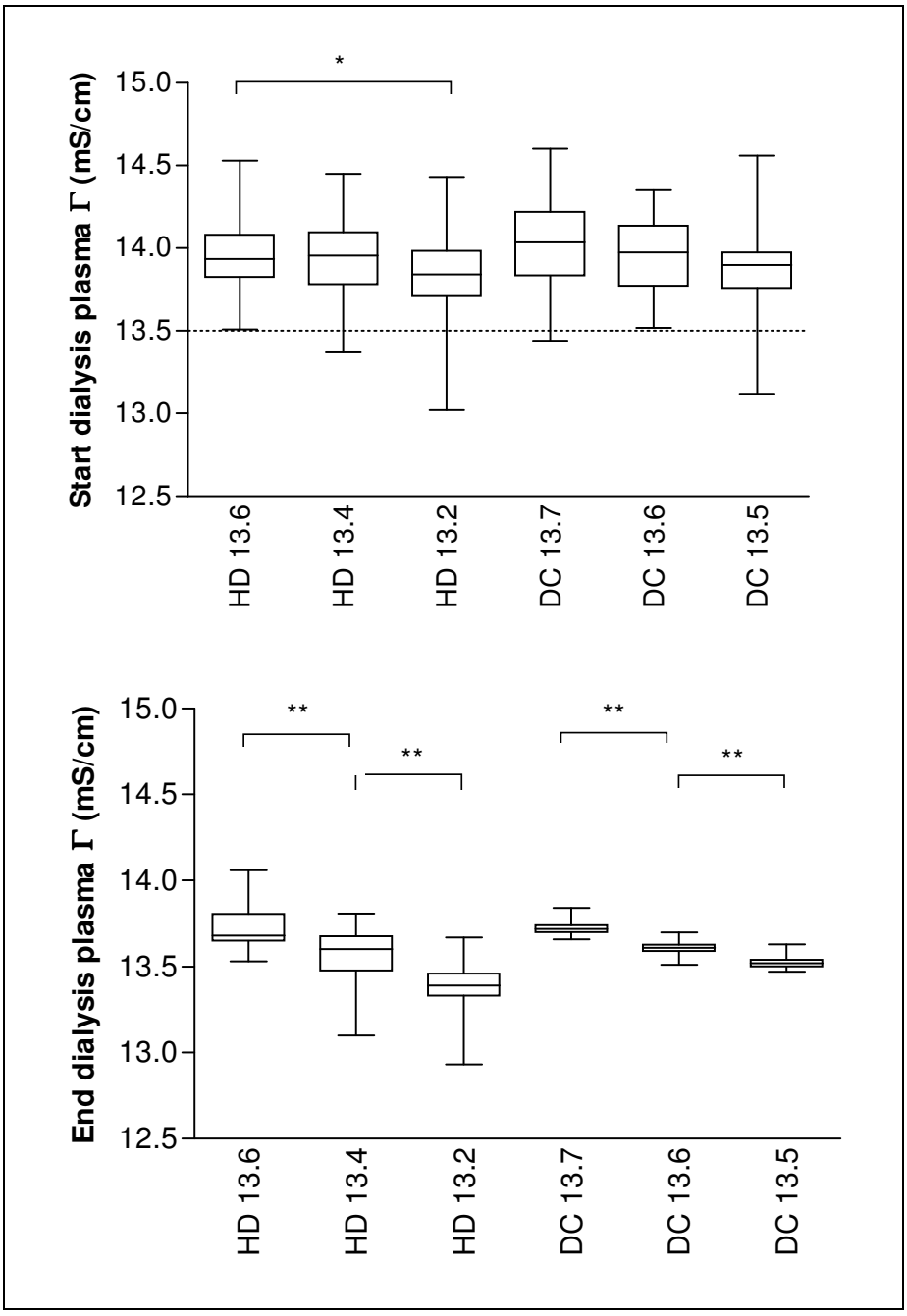


Figure 7-d.
Pre and post dialysis plasma conductivity (Γ) data for HD and DC. *p<0.05 by ANOVA, ** p<0.001 by ANOVA. There were no statistically significant differences between start conductivity at different DC settings. The dotted line in the start dialysis plasma conductivity graph shows that a proportion of patients have start dialysis plasma conductivity less than 13.5mS/cm, the lowest allowed target with DC.

Post-dialysis mean plasma sodium concentration was 137.3 ± 1.0mmol/l with $HD_{13.6}$, which was significantly higher than with $HD_{13.4}$ (135.9 ± 1.3mmol/l, $p<0.05$) or $HD_{13.2}$ (135.7 ± 1.0mmol/l, $p<0.05$). However, the trend for post-dialysis plasma sodium to fall with conductivity reduction with DC did not reach statistical significance. Mean post dialysis plasma sodium concentrations were 137.5 ± 1.2mmol/l with $DC_{13.7}$, 136.5 ± 1.9mmol/l with $DC_{13.6}$ and 136.8 ± 3 with $DC_{13.5}$. Equally the differences when comparing $HD_{13.6}$ and $DC_{13.7}$, $HD_{13.4}$ and $DC_{13.6}$ or $HD_{13.2}$ and $DC_{13.5}$ did not reach significance, although the trend was for lower post dialysis plasma sodium concentrations with fixed dialysate conductivity in the latter two comparisons.

Start dialysis plasma conductivities were also very similar when modalities were matched for end dialysis plasma conductivity. Mean start dialysis plasma conductivities for HD_H and DC_H were 14.0 ± 0.2mS/cm and 14.0 ± 0.2mS/cm respectively, for HD_M and DC_M were 13.9 ± 0.2mS/cm and 14.0 0.3mS/cm respectively and for HD_L and DC_L were 14.0 ± 0.2mS/cm and 14.0 ± 0.3mS/cm respectively (p=ns for all). This was reflected in similar pre and post dialysis plasma sodium concentrations.

There were no differences in IMB during either conductivity reduction or when modalities were matched for end dialysis plasma conductivity. IMB

values at the different conductivity settings are shown in tables 7-f and 7-g.

Thirst score		**Thirst score**	
$HD_{13.6}$	10.8 ± 3	$DC_{13.7}$	10.3 ± 2.5
$HD_{13.4}$	10.5 ± 4.5	$DC_{13.6}$	9.8 ± 2.1
$HD_{13.2}$	9 ± 1.4	$DC_{13.5}$	9.6 ± 2.0
Relative blood vol. (%)		**Relative blood vol. (%)**	
$HD_{13.6}$	-5.8 ± 4.4	$DC_{13.7}$	-5.9 ± 4.7
$HD_{13.4}$	-5.8 ± 3.6	$DC_{13.6}$	-6.7 ± 3.6
$HD_{13.2}$	-5.7 ± 3.5	$DC_{13.5}$	-5.2 ± 4.4
Ionic mass balance (mmol)		**Ionic mass balance (mmol)**	
$HD_{13.6}$	331 ± 117	$DC_{13.7}$	390 ± 173
$HD_{13.4}$	369 ± 106	$DC_{13.6}$	411 ± 139
$HD_{13.2}$	347 ± 125	$DC_{13.5}$	398 ± 125
Kt/V_{urea}		**Kt/V_{urea}**	
$HD_{13.6}$	1.4 ± 0.3	$DC_{13.7}$	1.4 ± 0.2
$HD_{13.4}$	1.4 ± 0.2	$DC_{13.6}$	1.5 ± 0.3
$HD_{13.2}$	1.4 ± 0.2	$DC_{13.5}$	1.5 ± 0.2

Table 7-f.
Dialysis details for HD and DC during conductivity reduction. Comparisons were made only within each modality (i.e. HD and DC were not directly compared) and $p>0.05$ for all.

7.4.4 Volume status

With conductivity reduction during HD, there was a reduction in pre-dialysis ECW and a trend towards a reduction in IDWG. Mean ECW was 0.22 ± 0.04l/kg during $HD_{13.6}$ as compared to 0.21 ± 0.09l/kg during $HD_{13.2}$ ($p<0.05$). Mean weight gain was 2.0 ± 0.7kg during $HD_{13.6}$ as compared with 1.9 ± 0.6kg during $HD_{13.2}$ ($p=0.06$). However, during conductivity reduction with DC, these differences were not apparent. Weight gain and ECW were 1.90 ± 0.8kg and 0.21 ± 0.04l/kg during

$DC_{13.7}$. During $DC_{13.5}$ mean weight gain was 2.0 ± 0.8kg and pre dialysis ECW was 0.21 ± 0.04l/kg (p=ns). There were no differences in pre dialysis ICW, post-dialysis weight or in thirst score in any of the comparisons. Post dialysis BIA measurements were found to be inaccurate and displayed a large degree of variation.

When HD and DC were matched for end dialysis plasma conductivity, there were no differences in IDWG, post dialysis weight, thirst score or pre-dialysis ECW at any of the three end dialysis conductivity levels. These data are shown in table 7-g.

7.4.5 Dialysis details

There were no differences during conductivity reduction with either modality in relative blood volume or Kt/V. Equally, there were no differences in any of these outcomes when HD and DC were matched for end dialysis plasma conductivity. These data during conductivity reduction are displayed in table 7-f and for the matched analysis in table 7-g.

	HD_H	DC_H	HD_M	DC_M	HD_L	DC_L
Thirst score	11 ± 4	10 ± 2	11 ± 5	10 ± 2	9 ± 1	9 ± 2
Rel. blood vol. (%)	-4.2 ± 3	-4.8 ± 5	-5.9 ± 4	-7.9 ± 4	-7.3 ± 4	-4.4 ± 6
ECW pre (l/kg)	0.22 ± 0.03	0.21 ± 0.03	0.22 ± 0.04	0.21 ± 0.04	0.21 ± 0.04	0.20 ± 0.04
IMB (mmol/l)	324 ± 83	350 ± 170	344 ± 124	428 ± 165	414 ± 108	447 ± 158
Kt/V_{urea}	1.5 ± 0.3	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.5 ± 0.3

Table 7-g.
Dialysis details for those sessions when end dialysis plasma conductivity was matched for HD and DC. ECW = extracellular water corrected for body weight, measured before dialysis. There were no differences in any of the parameters between the paired groups.

7.5 Discussion

We have demonstrated that in stable haemodialysis patients, empirical reduction of dialysate sodium is safe, practicable and does not significantly reduce dialysis tolerability. Using this approach, reduction of end dialysis plasma conductivity with biofeedback dialysis has no significant short-term clinical benefit over fixed dialysate conductivity.

Although it is intuitive that reducing dialysate sodium will have beneficial effects on thirst, IDWG and blood pressure, available data on this issue are conflicting. Several studies show that pre dialysis BP is improved as dialysate conductivity is reduced [97, 100, 102] and one study suggests an improvement in interdialytic ambulatory BP [99]. Other studies however found that sodium reduction does not affect BP [98, 101]. Equally, it is controversial whether dialysate sodium reduction reduces IDWG [98-100, 102, 103] or improves thirst [98, 102, 103]. However, the majority of the observed benefit in these studies was in those patients who were hypertensive at the outset, or had large IDWG [97, 98, 100, 103]. Patients in our study demonstrated good baseline BP control and low IDWG. Equally, average systolic ambulatory BP at the outset was over 15mmHg less than the study by Farmer *et al* in which conductivity reduction improved ABPM readings [99]. This may explain why we did not demonstrate any significant change in these parameters with conductivity reduction. However, conductivity reduction was well tolerated in our

patients, did not increase IDH rates and was practicable in the setting of a busy outpatient haemodialysis unit.

Plasma osmolality, which is largely determined by plasma sodium concentration, is the major stimulus to drink and therefore reducing the plasma conductivity and sodium concentration should theoretically improve thirst. Although other studies have reported an improvement in thirst scores with conductivity reduction [98, 102], another study reported a paradoxical increase [103]. However, in the latter study higher overall thirst scores were due to increased thirst on dialysis days, and thirst scores were lower in the interdialytic period, with resultant lower weight gains. We did not demonstrate any statistically significant change in thirst score as conductivity was reduced. However, in our patients thirst score at the outset was remarkably low, which may reflect good compliance with dietary sodium restriction and also may explain why we failed to demonstrate a significant impact of conductivity reduction on IDWG. This is similar to the results of Kooman *et al* who enforced an extremely strict dietary sodium restriction, and found that conductivity reduction did not lead to any reductions in BP or IDWG [101].

DC is a biofeedback system that returns a patient to a prescribed plasma conductivity by the end of every dialysis session. Our results further confirm that DC accurately achieves the prescribed conductivity, even at levels <14.0mS/cm. By doing so, DC will completely remove the

interdialytic sodium load independently of variations in pre dialysis plasma sodium. In the long term this should prevent chronic sodium and therefore fluid overload. Initial work suggested that DC may also provide short-term benefit by improving haemodynamic stability during dialysis. It was postulated that this was due to the avoidance of rapid reductions in plasma tonicity and therefore circulating volume as shown in figure 1-e [106, 107]. However, when matched for end dialysis plasma conductivity, we observed no differences in pre, post or intra dialytic BP, or in IDH frequency between the HD and DC. When so matched, sodium balance appeared similar between modalities as manifest by equal pre and post dialysis plasma conductivity and sodium concentrations, similar IMB, equal post dialysis weights and IDWG. In one of the previous studies that reported improved intradialytic stability with DC, it was not clear if standard dialysis and DC had equal sodium balance, and in the other study in which sodium balance was matched, the authors demonstrated a smaller fall in BP during DC but IDH frequency was not altered. Other studies have, like us, failed to demonstrate any advantage in intradialytic stability [108, 184, 185]. Furthermore, we did not observe a difference in relative blood volume between HD and DC when end dialysis plasma conductivity was matched.

When comparing HD and DC at the lower conductivity settings, we found the range of DC to be limited. The lowest target plasma conductivity setting allowable with DC is 13.5mS/cm, which may be higher than initial plasma conductivity in a proportion of patients. Therefore intra-dialytic

sodium loading can still occur with DC in those patients with low pre dialysis plasma sodium concentrations [184]. Certainly significantly lower end dialysis plasma conductivities and sodium concentrations were achieved with a fixed dialysate conductivity of 13.2mS/cm as compared to $DC_{13.5}$ without a reduction in dialysis tolerability. This may explain why ECW was reduced during conductivity reduction with HD but not with DC. Furthermore, we have previously shown that fixed dialysate conductivity can be safely set as low as 13.0mS/cm, and although only tolerated by approximately 40% of stable patients, maximal benefits in terms of BP and IDWG were seen in this group [102]. Therefore, within a strategy aiming to reduce total body sodium, DC may be a less effective tool than dialysis with fixed dialysate sodium [96, 184].

During conductivity reduction with HD, we observed a progressive fall in pre dialysis plasma sodium concentrations and in start dialysis plasma conductivity, whereas with DC we saw a fall in plasma sodium but without a fall in plasma conductivity. Start dialysis plasma conductivity is measured after the commencement of dialysis in contrast to plasma sodium for which the blood is drawn prior to the treatment commencing. With HD, the largest plasma dialysate concentration gradient for sodium is likely to occur early in the treatment and therefore rapid falls in plasma tonicity can occur. In the early stages of DC, dialysate sodium tends to be high to prevent this. Therefore, the progressive reduction in pre dialysis plasma sodium concentrations (with stable weight and IDWG) reflects

both modalities reducing the sodium pool, despite the lack of change in start dialysis plasma conductivity with DC.

Our study does have some limitations. As the first study to compare HD and DC with a strategy of dialysate sodium reduction, we selected stable patients. We may well have observed greater benefits of conductivity reduction if hypertensive patients or those with large IDWG were studied. In addition, it is possible that if DC does have any short-term benefits over fixed dialysate sodium, they may only be apparent in such patients. Conductivity reduction was performed every two weeks, and it is conceivable that greater benefit may have been seen if patients remained at each setting for longer periods, as BP can lag behind changes in volume status [186].

In conclusion, we have shown that empirical reduction of dialysate sodium in stable patients is practicable and well tolerated. In this setting, DC did not appear to have any short tem clinical advantage over fixed dialysate sodium, and indeed was not able to reduce plasma conductivity to as great a degree. Further work is required to compare the effects of HD and DC during conductivity reduction in those patients with large IDWGs or uncontrolled hypertension. Also, it is not yet clear if DC has any impact on long-term patient outcomes in either stable or unstable populations.

8. Results: a comparison of the effects of hypertonic glucose-based peritoneal dialysate and Icodextrin on blood pressure and systemic haemodynamics

8.1 Introduction

Little is known about the haemodynamic response to CAPD. The next three chapters describe studies designed to characterise this, and also to examine the varying effects of the different commercially available dialysate solutions.

It is becoming increasingly recognised that glucose has limitations as the osmotic agent in peritoneal dialysate. Glucose and glucose degradation products (GDPs) are toxic to the peritoneum [12], and glucose is also systemically absorbed leading to hyperinsulinaemia plus weight gain [13]. This has driven the development of other dialysate solutions, including Icodextrin (Extraneal®), which is a large molecular weight glucose polymer that is not systemically absorbed. Icodextrin reduces systemic glucose absorption and achieves equivalent ultrafiltration as compared with hypertonic glucose fluids during prolonged intraperitoneal dwells [187].

This chapter describes an initial study to examine the hypotheses that CAPD is associated with significant haemodynamic change, and that 1.36% glucose, 3.86% glucose and Icodextrin containing-dialysate fluids exert differing haemodynamic effects.

8.2 Subjects and methods

8.2.1 Patients

We recruited eight patients on CAPD for a prospective crossover study. Baseline characteristics and CAPD prescriptions are shown in table 8-a. All patients had been on CAPD for more than six months (mean 40.5 months, range 18-73). All had a weekly Kt/V of greater than 2.0 of which less than 50% was provided by residual renal function. One patient was anuric.

Patients were eligible only if their blood pressure had been stable (BP <140/85 mmHg with no changes in anti-hypertensive medications) over the four weeks prior to recruitment, and if less than 50% of their PD regime was made up of 3.86% glucose solution or Icodextrin. Patients were excluded if they had severe peripheral vascular disease, of if they had an arterio-venous fistula or renal transplant in situ.

All patients underwent clinical examination prior to commencing the study to ensure that they were at their optimal weight. All patients underwent standard peritoneal equilibration testing (PET) and assessment of dialysis adequacy (Adequest 2.0® program, Baxter Healthcare, Norfolk, UK).

Patient	Sex	Age	PD regime	Cause of ESRF	Transporter status	Epo dose (mcg/wk)	Antihypertensive drugs
1	M	70	LLLL	Type 2 diabetes	LA	0	Lisinopril 10mg od
2	F	60	LLL I *	Type 2 diabetes	LA	10	
3	M	57	LLLLL	Malignant hypertension	HA	10	Lisinopril 40mg od
4	M	77	LLLN	Unknown	HA	0	
5	F	66	LLL I *	Type 2 diabetes	HA	10	Amlodipine 10mg
6	M	77	LLL I	ARVD	HA	30	Diltiazem 300mg od, Moxonidine 0.3mg od, Lisinopril 2.5mg od
7	M	74	LLLLL	TIN	LA	40	
8	M	83	LLL I	IgA nephropathy	HA	20	Doxazosin 4mg od, Felodipine 10mg od
Mean ± SD		70.5 ± 9.0				15 ± 14.1	

Table 8-a.
Baseline patient characteristics.
L= 1.36% glucose, I=Icodextrin, N=Nutrineal. 4 patients were using Icodextrin regularly as their nighttime dwell, where as 4 patients were Icodextrin naïve. * denotes patients that used 3.86% glucose dialysate regularly three times per week. All other patients used 3.86% glucose on an as required basis. TIN = tubulointerstitial nephritis, ARVD = atherosclerotic renovascular disease. Transporter status refers to peritoneal membrane transporter characteristics, with LA = low average transporter and HA = high average transporter. Epo = erythropoeitin; all patients were self-administering darbepoetin alfa. Anti-hypertensive medications were left unchanged for the duration of the study.

8.2.2 Study protocol

All patients gave informed consent prior to commencement, and ethical approval for the project was granted by Derbyshire Research Ethics Committee. Patients were asked to attend for two study days (A and B), the order of which was randomly determined. For each investigatory session, patients were admitted to a clinical investigations unit where CAPD was performed. All fluids were manufactured by Baxter Healthcare (Norfolk, UK) and were warmed to 37^0C before instillation. Non-invasive haemodynamic monitoring was undertaken using a Finometer (chapter 3), which was fitted for the entirety of each session. To obtain baseline values, monitoring commenced 30min prior to draining the nighttime dwell. On day A, patients underwent CAPD with 2.5L of low osmolar Physioneal® (bicarbonate/lactate-based pH neutral fluid containing 1.36% glucose) followed by high osmolar Physioneal® (3.86% glucose). Dwell times were 150min and each drain/dwell cycle was planned to last approximately three hours, although this was not absolute due to variable draining times of different patients. On day B, 2.5L Icodextrin was substituted for the 3.86% Physioneal® but the protocol was otherwise identical. There was at least a week's washout period between the two study days. Patients were allowed a light breakfast two hours before the first CAPD exchange, and were supplied with a standardised midday meal that was consumed one hour before the second CAPD exchange. Blood samples were collected before and after each session in lithium heparin and EDTA tubes, and biochemical analysis performed on a multichannel autoanalyser. The volume and electrolyte concentration of

the peritoneal waste fluid was also assessed. Primary endpoints were percentage change in blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) in response to different PD dialysate fluids.

8.3 Statistical analysis

Results are expressed as mean ± SD. For BP and haemodynamic data, the mean refers to the complete dwell period, and these data were compared using one-way ANOVA with a design for repeated measures and Bonferroni's test to correct for multiple comparisons. For other data, the paired *t*-test was used after significant deviations from a normal distribution were excluded with the Kolmogorov-Smirnov test. Correlation coefficients were calculated using Pearson's test. An alpha error at $P<0.05$ was judged to be significant.

8.4 Results

8.4.1 Blood pressure during dwell periods

We found BP to be significantly higher during 3.86% glucose dwells as compared to both 1.36% glucose and Icodextrin dwells ($p<0.001$). The mean systolic BP (SBP) for the entire dwell with 1.36% glucose was 149.8 ± 4mmHg, the mean diastolic BP (DBP) 86.6 ± 3mmHg and the mean of the mean arterial pressure (MAP) 109.5 ± 3mmHg. During the 3.86% glucose dwells all three BP parameters were higher; the mean SBP for the entire dwell was 159.9 ± 6mmHg ($p<0.001$), mean DBP was 93.9 ± 4mmHg ($p<0.001$) and mean MAP was 117.5 ± 5mmHg ($p<0.001$). During the Icodextrin dwells, SBP and MAP did not differ significantly from the 1.36% glucose dwells with means of 150.9 ± 7mmHg ($p=0.44$) and 108.0 ± 4mmHg ($p=0.14$) respectively. However, mean DBP was 2.4 ± 1mmHg lower during the Icodextrin dwells at 84.3 ± 2mmHg ($p=0.003$). Comparing mean BP during 3.86% glucose dwells with Icodextrin dwells, readings were significantly higher during the former for SBP, DBP and MAP ($p<0.001$ for each). BP data are summarised in figure 8-a.

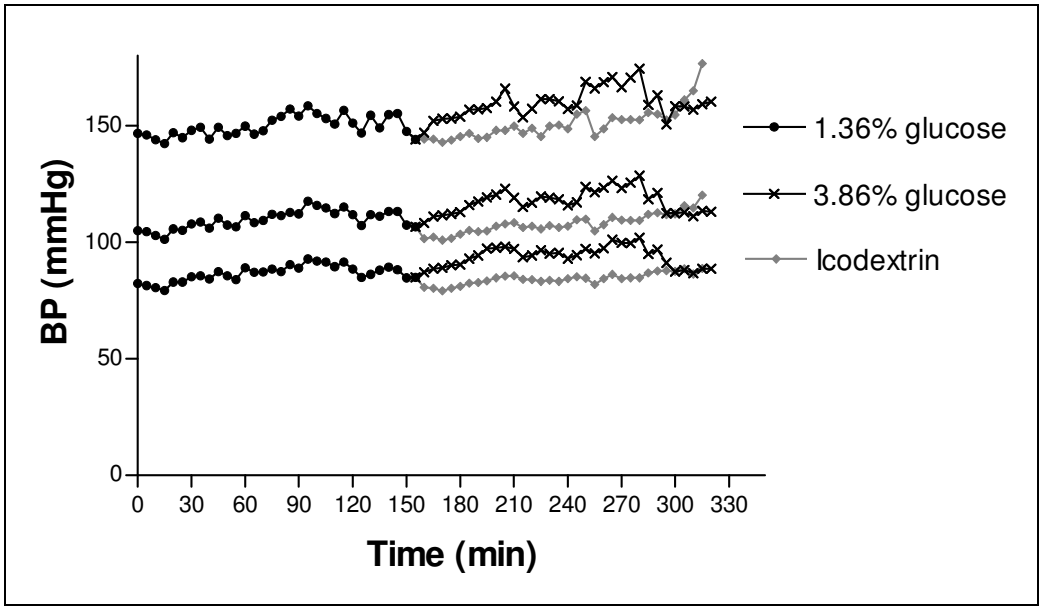


Figure 8-a.
Mean BP changes during CAPD dwells with different fluids. BP rises marginally during each dwell, but is significantly higher during 3.86% glucose dwell as compared to both 1.36% glucose and Icodextrin dwells ($p < 0.001$).

8.4.2 Haemodynamic data during dwell periods

We also found significant differences in HR between 3.86% glucose dwells and both the 1.36% glucose and Icodextrin dwells ($p<0.001$). Mean HR for the entire 1.36% glucose and Icodextrin dwell periods were not significantly different at -4.3 ± 2% and -4.2 ± 1% respectively ($p=0.74$). The mean HR during 3.86% glucose dwell was -2.3 ± 3% and this was significantly greater than either the 1.36% glucose ($p<0.01$) or Icodextrin dwell periods ($p<0.01$). These data are summarised in figure 8-b.

SV and CO were also found to be significantly higher during 3.86% glucose dwells ($p<0.001$). During 1.36% glucose dwell, mean SV for the

whole period was -5.7 ± 6%. After an initial increase, SV during the Icodextrin dwell was stable with a mean for the entire period of -8.1 ± 4%, which was not statistically different from 1.36% glucose dwell (p=ns). During 3.86% glucose dwell, mean SV for whole period was -1.2 ± 7%, which was significantly higher than both 1.36% glucose ($p<0.001$) and Icodextrin dwell periods ($p<0.001$).

As the product of SV and HR, CO showed similar changes. Mean CO for whole dwell period with 1.36% glucose was -10.5 ± 7%. CO increased initially for both Icodextrin and 3.86% glucose fluids, but to a much greater extent with 3.86% glucose. For the Icodextrin dwell the mean for the entire period was -12.6 ± 4%. The mean CO for 3.86% glucose dwell was -4.2 ± 8%, which was significantly higher than 1.36% glucose ($p<0.001$) and Icodextrin dwells ($p<0.001$).

TPR increased progressively during all three dwell phases at a similar rate and by a similar magnitude. Mean TPR for the entire dwell period was +25.7 ± 13% for 1.36% glucose, +30.6 ± 16% for 3.86% glucose and +28.1 ± 11% for Icodextrin. There were no significant differences between these mean values (p=ns). All haemodynamic data are summarised in figure 8-b.

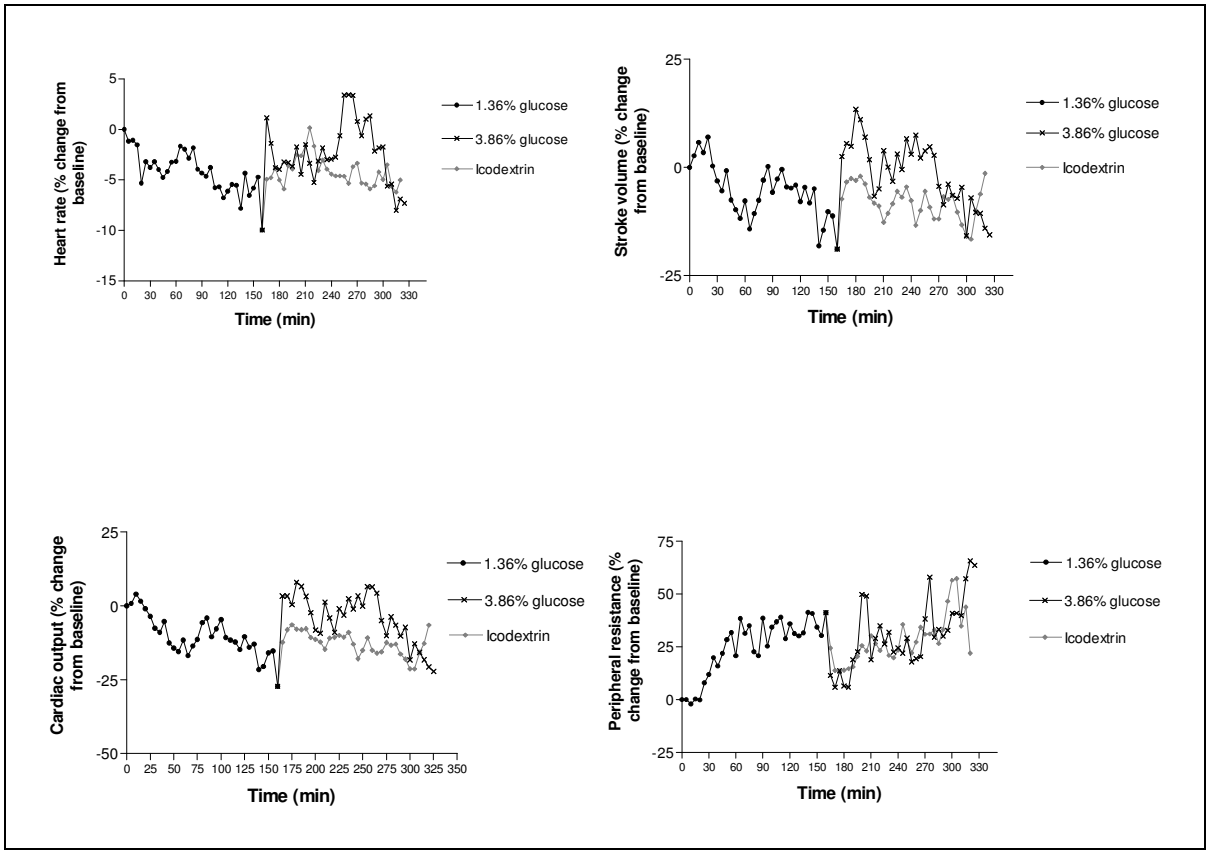


Figure 8-b.
Haemodynamic changes during CAPD dwells. TPR during all three dwells was similar; however, a higher HR ($p<0.001$), SV ($p<0.001$) and therefore CO ($p<0.001$) were observed during 3.86% glucose dwells.

The haemodynamic and BP patterns described above are population means. Analysing patients individually, 7/8 exhibited similar patterns for BP. 2/3 of the diabetic patients were insulin treated and they did not display a higher SV and CO during 3.86% glucose dwell, as compared to the non-insulin treated patient who did behave in a similar overall fashion. No insulin was administered during the study periods.

8.4.3 Ultrafiltration volumes and sodium removal

The mean ultrafiltration (UF) volume during the 3.86% glucose dwell was 500 ± 290ml. This value was significantly higher than both Icodextrin dwell with a mean of 243.8 ± 111ml (p=0.028) and the 1.36% glucose dwell with a mean of 143.8 ± 96.3ml (p=0.001). The difference between the mean UF volumes with Icodextrin versus 1.36% glucose was of borderline statistical significance (p=0.057). There was no correlation between BP and UF volume. These data are summarised in figure 8-c.

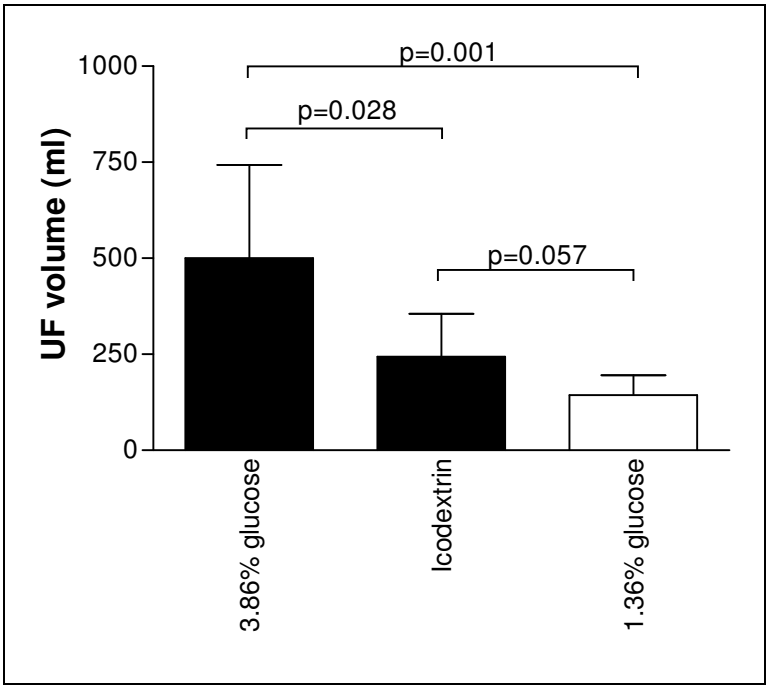


Figure 8-c.
Mean UF volumes for CAPD dwells with different fluids. Mean UF volume is significantly greater after 3.86% glucose dwell as compared to both icodextrin and 1.36% glucose dwells. Icodextrin does result in a greater UF volume as compared to 1.36% glucose dwell although this is only of borderline statistical significance. The modest UF volume seen with the icodextrin is likely to be due to the relatively short dwell time.

There were no significant differences in sodium loss between Icodextrin and 3.86% glucose dwells (p=ns), with means of -10.8 ± 14mmol and -12.5 ± 31mmol respectively. During 1.36% glucose dwell, sodium was retained with a mean value of +5.7 ± 12mmol. This value was significantly lower than Icodextrin dwell (p<0.01), but of borderline significance compared with 3.86% glucose dwell (p=0.055).

8.4.4 Peritoneal transport characteristics and urea kinetic modelling

All patients had a weekly Kt/V of greater than 2.0 (mean 2.5, range 2.0 to 3.4). Mean weekly creatinine clearance was 78.1l/wk (range 61.8 to 115.5) with mean residual renal function 3.2ml/min (range 0 to 7.7). There were no correlations between BP and residual renal function, weekly creatinine clearance or Kt/V. Individuals' overall haemodynamic response did not differ depending on peritoneal membrane transporter characteristics (shown in table 8-a), nor did this influence the speed in which the haemodynamic response occurred following instillation of dialysate.

8.5 Discussion

Although CAPD is generally regarded as being better tolerated than haemodialysis from a cardiovascular point of view, this study demonstrates that CAPD is associated with significant haemodynamic changes. We have also shown that there are disparities in the haemodynamic effects of different dialysate fluids, with significant differences between 3.86% glucose dialysate and both 1.36% glucose and Icodextrin. To our knowledge, there are no previous reports describing similar changes.

Mean BP was higher during the 3.86% glucose dwells, as were HR, SV and therefore CO. The higher BP during the 3.86% glucose dwells was present despite larger UF volumes and greater sodium removal. It is known that glucose, systemically absorbed from peritoneal dialysate leads to hyperinsulinaemia [13], and it is possible that this has direct effects on myocardial function and BP. Certainly hyperinsulinaemia has been described as causing a rise in SV and CO coupled to a fall in TPR in healthy subjects [131, 188] whilst an increase in aortic systolic BP in response to insulin infusion has also been reported [189]. The fact that there were no significant differences in TPR between dialysate fluid types suggests that the higher BP seen with 3.86% glucose was due to increased myocardial performance. This would be consistent with work by Khoury, who has shown that a glucose/insulin/potassium infusion can

stimulate an increase in myocardial performance in patients with left ventricular dysfunction [190].

In association with the slight rise in BP, TPR rose throughout each dwell phase. The mechanisms behind this rise in TPR remain unexplained, but one explanation for this would be mesenteric vasoconstriction in response to the cooling effect of instilling dialysate. Haemodialysis patients experiencing progressive UF demonstrate a rise in TPR in association with an increase in peripheral sympathetic nerve activity [81], so an alternative explanation would be that UF and the subsequent volume contraction cause a compensatory rise in TPR [191].

In our study, when comparing 1.36% glucose and Icodextrin, we observed differences with a lower DBP and trend towards lower SV during Icodextrin dwells. With regard to the relative amount of glucose in the two fluids these differences may be less than expected, although some authors have found minimal effects on systemic haemodynamics with lower amounts of glucose and insulin. This raises the possibility of 'dose-response' or 'threshold' effects and that the amount of glucose absorbed is important in determining the magnitude of hyperinsulinaemia and haemodynamic response.

Three of our study patients were type II diabetics, two on insulin and one on sulphonylurea treatment. These patients were not analysed separately

due to small numbers, but the patient on sulphonylurea treatment appeared to behave in a similar fashion to non-diabetics. The two patients on insulin treatment however did not display higher SV and CO during 3.86% glucose dwells. It is possible that the patient on sulphonylurea treatment had some degree of preserved residual insulin secretion to explain the changes in haemodynamics, although without further work measuring glucose and insulin levels in addition to haemodynamics this is conjecture.

Other than the difference in glucose content, Icodextrin contains lactate as a buffer whereas Physioneal® contains bicarbonate/lactate mix and has a neutral pH. It is therefore possible that the difference in lactate content or in intraperitoneal pH might be responsible for some of the observed difference in haemodynamic effects. However, this seems unlikely because there were still significant differences between 1.36% and 3.86% Physioneal® which both have identical buffer. Furthermore, there are no changes in systemic pH as a result of using either buffer system [192]. Another difference between the fluid types is the lower amounts of glucose degradation products (GDPs) in Icodextrin. Despite an extensive literature search no published work on the potential haemodynamic effects of GDPs was found.

In conclusion, these data demonstrate that CAPD is associated with significant haemodynamic disturbance. We have also demonstrated that BP, HR, SV and CO are significantly higher during 3.86% glucose dwells compared to both 1.36% glucose and Icodextrin dwells. BP

measurements taken during 3.86% glucose dwells may therefore not be representative of overall BP. However, the design of this initial study did not allow elucidation of the mechanisms underlying the diverse responses to the differing dialysate types.

9. Results: the systemic haemodynamic and metabolic effects of hypertonic glucose and amino-acid based peritoneal dialysis fluids

9.1 Introduction

Our initial study showed that BP rose during CAPD with 3.86% glucose dialysate due to higher HR, SV and CO (chapter 8); however, it was not possible to determine the mechanisms underlying these changes. One possible explanation was that the changes were due to the effects of greater systemic absorption of glucose from the 3.86% glucose solution, leading to hyperglycaemia and hyperinsulinaemia. Certainly, both hyperglycaemia and hyperinsulinaemia are recognised to elevate BP and exert independent effects on systemic haemodynamics [129, 130]. Alternatively, the greater UF and therefore intraperitoneal volumes with 3.86% glucose may have increased venous return and therefore cardiac filling. In addition, the fluids differed not only in glucose content but also in buffer type and in the amount of glucose degradation products (GDPs).

We therefore performed a further study to address the limitations of the previous study. We sought to re-examine the acute effects of CAPD on BP and haemodynamics in a different group of fasted, non-diabetic patients. We also wanted to relate BP and haemodynamic changes to the degree of systemic absorption of glucose and subsequent

hyperinsulinaemia. In addition, we planned to examine whether any haemodynamic changes observed were due to differences in cardiac filling as assessed by echocardiography. Finally, we wanted to confirm the differential haemodynamic effects of a glucose sparing regime, this time using an alternative glucose free solution (Nutrineal®, which contains 1.1% amino acids as the osmotic agent).

9.2 Methods

9.2.1 Patients

We recruited ten non-diabetic patients on CAPD for a prospective crossover study. Baseline characteristics and CAPD prescriptions are shown in table 9-a. All patients had been on CAPD for more than six months (mean 26 ± 26months, range 6 to 89).

Patients were eligible only if their blood pressure had been stable (BP <140/85 mmHg with no changes in anti-hypertensive medications) over the four weeks prior to recruitment, and if less than 50% of their PD regime was made up of 3.86% glucose solution or Icodextrin. Patients were excluded if they had diabetes mellitus, severe peripheral vascular disease, of if they had an arterio-venous fistula or renal transplant in situ.

All patients underwent clinical examination prior to commencing the study to ensure that they were at their optimal weight, and had standard peritoneal equilibration testing (PET) and assessment of dialysis adequacy (Adequest 2.0® program, Baxter Healthcare, Norfolk, UK).

Patient	**Sex**	**Age**	**PD regime**	**Cause of ESRF**	**Transporter status**	**Epo dose (mcg/wk)**	**Antihypertensive drugs**
1	F	63	LLLL*	Chronic pyelonephritis	HA	30.0	
2	M	59	LLLHI	Malignant hypertension	HA	10.0	Lisinopril 40mg od, Nifedipine 90mg od, Doxazosin 16mg od
3	F	62	LLL	Chronic pyelonephritis	LA	10.0	Doxazosin 4mg od, Atenolol 50mg od
4	M	33	LLLL*	MCGN	HA	30.0	Lisinopril 20mg od, Amlodipine 10mg od
5	M	68	LLLI	Chronic GN (type undefined)	HA	7.5	
6	M	39	LLLL*	IgA nephropathy	LA	30.0	Lisinopril 20mg od
7	F	52	LLL	Myeloma	HA	10.0	
8	M	66	LLLI	Unknown	HA	0.0	Nifedipine 90mg od
Mean ± SD		55 ± 13				16 ± 12	
Excluded patients							
9	*F*	*55*	*LLLL*	*Chronic TIN*	*L*	*10*	*Amlodipine 10mg od*
10	*M*	*62*	*LLL*	*Unknown*	*LA*	*0*	*Lisinopril 10mg od*

Table 9-a.
Baseline characteristics of study patients. L = low osmolar (1.36%) glucose, H = high osmolar (3.86%) glucose, I = Icodextrin. * denotes patients who used 3.86% glucose on a regular basis, all other patients used 3.86% glucose on an as required basis. MCGN = membrano proliferative glomerulonephritis, TIN = tubulointerstitial nephritis. Transporter status – HA = high average, LA = low average, L = low. Epo = erythropoietin, all patients were taking subcutaneous Aranesp®. Patients 9 and 10 did not complete the study and were excluded from the analysis.

9.2.2 Study protocol

Patients attended for three study days (A, B and C), the order of which was randomly determined. For each investigatory session, patients were admitted to a clinical investigations unit where CAPD was performed. All fluids were manufactured by Baxter Healthcare (Norfolk, UK) and were warmed to 37^0C before instillation. Non-invasive haemodynamic monitoring was undertaken using a Finometer (described in chapter 3) that was fitted for the entirety of each session. To obtain baseline values, monitoring commenced 30min prior to draining the night-time dwell.

On day A, conventional bioincompatible fluids (PDF_{CONV}) were used (Dianeal®, lactate buffered, acidic pH). Patients underwent CAPD with 2.5L of 1.36% glucose solution followed by fluid containing 3.86% glucose. On day B, patients received dialysis with pH neutral bicarbonate/lactate buffered fluid low in GDPs (Physioneal®, PDF_{BIO}); patients again received 1.36% glucose followed by 3.86% glucose based solutions. On day C, patients had PDF_{CONV} with 1.36% glucose followed by 1.1% amino acid solution (PDF_{AA} - Nutrineal®, lactate buffer, acidic pH, no GDPs). Dwell times were 120min and each drain/dwell cycle was planned to last approximately 2.5 hours, although this was not absolute due to variable draining times of different patients. There was at least a week's washout period between the study days. Patients were fasted from the midnight before and throughout the study period.

M-mode echocardiography was performed using commercially available equipment at the start and end of each dwell period to measure left

ventricular dimensions (1.5-3.6 MHz 3S probe, Vivid 3®, GE medical systems, Sonigen, Germany). The volume of the peritoneal waste fluid from each dwell was also recorded. Blood samples were collected before and after each session in lithium heparin and EDTA tubes, and biochemical analysis performed on a multichannel autoanalyser. In addition, blood samples were collected at baseline and then at 10min, 30min and 60min of each dwell phase for analysis of plasma glucose (fluoride oxidase tubes) and at 10min and 60min for measurement of insulin (EDTA tubes). Samples for insulin analysis were centrifuged at 3500rpm for 15min to separate the plasma, which was immediately frozen at -80^{0}C. Insulin was subsequently measured using a commercially available enzyme-linked immunosorbent assay kit as described in chapter 3 (Insulin ELISA, DRG diagnostics, Marburg, Germany). Insulin resistance was assessed by calculating the homeostasis model of assessment index (HOMA-IR) using the following equation [193]:

HOMA IR = Baseline glucose (mmol/l) x baseline insulin (µ/IU/l)/22.5

in which a value > 3.8 indicates insulin resistance. This measure has been validated in renal patients [194].

Primary endpoints were percentage change in blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) in relation to plasma glucose and insulin levels and cardiac dimensions.

All patients gave informed consent prior to commencement, and ethical approval for the project was granted by Derbyshire Research Ethics Committee.

9.3 Statistical analysis

Results are expressed as mean ± SD unless otherwise stated. For BP and haemodynamic data, the mean refers to the complete dwell period. After demonstration of a normal distribution, all data were compared using one-way ANOVA with a design for repeated measures and Bonferroni's test to correct for multiple comparisons. An alpha error at $P<0.05$ was judged to be significant.

9.4 Results

Two patients did not complete the study – one became ill after attending for only the first study day, and the second patient had an inaccurate Finometer trace (intermittent detection of pulse wave resulting in unacceptable data quality). These patients were excluded from the analysis. Analysis of baseline measurements showed that there were no differences in BP or in any of the haemodynamic variables between the three different study days.

9.4.1 Blood pressure, PDF_{CONV} versus PDF_{AA}

BP was higher during 3.86% PDF_{CONV} dwells as compared to PDF_{AA}. During 3.86% PDF_{CONV}, mean systolic BP (SBP) for the entire dwell was 163.5 ± 5mmHg, mean diastolic BP (DBP) was 92.8 ± 3mmHg and mean arterial pressure (MAP) 118.9 ± 3mmHg, as compared to a mean SBP of 158.9 ± 6mmHg ($p<0.01$), DBP of 87.1 ± 6 ($p<0.001$) and MAP of 113.2 ± 5mmHg ($p<0.001$) during PDF_{AA} dwells. The higher overall BP with 3.86% PDF_{CONV} was due to differences in first half of the dwell, and systolic readings were similar during the last third of the dwell. These data are summarised in figure 9-a. No differences were observed when comparing BP during 1.36% PDF_{CONV} and PDF_{AA} dwells (BP data for 1.36% PDF_{CONV} dwells are shown in table 9-b).

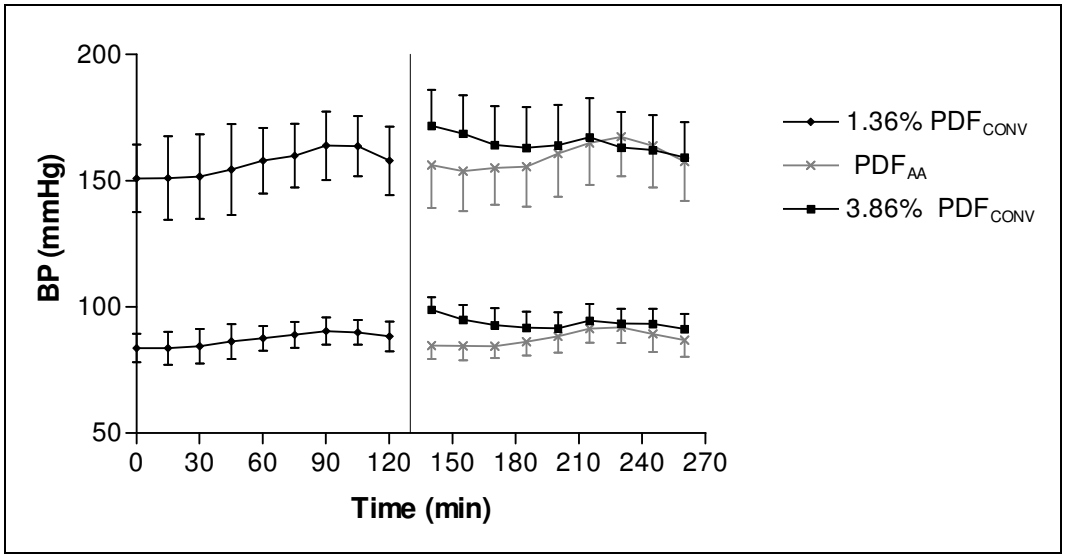


Figure 9-a.
BP during PDF_{CONV} and PDF_{AA} dwells. BP was significantly higher during 3.86% PDF_{CONV} as compared to either 1.36% PDF_{CONV} or PDF_{AA} ($p<0.001$ for all comparisons). 1.36% PDF_{CONV} data points are combined value for both days as values were extremely similar. Data are shown as mean ± SEM.

9.4.2 Blood pressure, PDF_{CONV} versus PDF_{BIO}

BP was similar when comparing PDF_{CONV} and PDF_{BIO} during both 1.36% and 3.86% dwells. These data are shown in table 9-b. However, when comparing 1.36% and 3.86% glucose dwells, BP was significantly higher with 3.86% glucose for both PDF_{CONV} and PDF_{BIO} ($p<0.001$ for all comparisons). BP tended to decline throughout 3.86% PDF_{CONV} dwells, which was in contrast to the pattern seen with all other fluids where BP rose progressively. These data are summarised in figure 9-b.

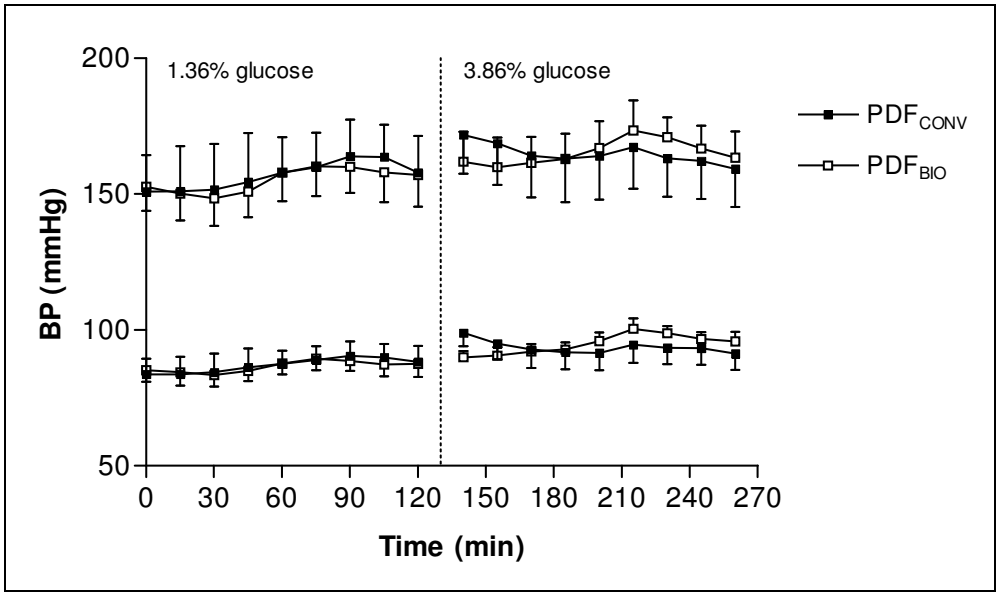


Figure 9-b.
BP during PDF_{CONV} and PDF_{BIO} dwells. There were no differences in mean BP when comparing the two fluid types, but mean BP was higher when comparing 3.86% glucose fluids with the corresponding 1.36% glucose fluid ($p<0.001$ for both comparisons). Data are shown as mean ± SEM.

	1.36% PDF_{CONV}	**1.36% PDF_{BIO}**	**3.86% PDF_{CONV}**	**3.86% PDF_{BIO}**
SBP (mmHg)	157.3 ± 5	155.3 ± 5	163.5 ± 5	164.7 ± 6
DBP (mmHg)	87.2 ± 2	86.6 ± 2	92.8 ± 3	94.4 ± 4
MAP (mmHg)	113.1 ± 4	113.4 ± 3	118.9 ± 3	121.1 ± 5

Table 9-b.
Blood pressure during 1.36% and 3.86% PDF_{CONV} and PDF_{BIO} dwells. There were no differences when comparing fluid types, but BP was significantly higher when comparing 3.86% to 1.36% ($p<0.001$ for all comparisons).

9.4.3 Haemodynamics, PDF_{CONV} versus PDF_{AA}

Mean HR for the entire dwell period was 69 ± 8bpm with 3.86% PDF_{CONV}, which reflected a rise of +2 ± 5% from baseline. This was not significantly different from a mean HR of 71 ± 13bpm with PDF_{AA} (+5 ± 6% from baseline, p=ns). However, SV and CO were both higher during PDF_{CONV} dwells. SV for entire dwell period was -9 ± 4% from baseline with 3.86% PDF_{CONV} and was -20 ± 7% with PDF_{AA} ($p<0.001$). CO for entire dwell was -7 ± 5% beneath baseline with 3.86% PDF_{CONV} as compared with -15 ± 6% with PDF_{AA} ($p<0.001$). TPR also differed significantly between 3.86% PDF_{CONV} and PDF_{AA}. With the former, TPR rose to a mean of +12 ± 8% above baseline for the entire dwell period, and with PDF_{AA} mean TPR was +25 ± 11% above baseline ($p<0.001$). These data are summarised in figure 9-c.

9.4.4 Haemodynamics, PDF_{CONV} versus PDF_{BIO}

There were no differences in any of the haemodynamic variables when comparing PDF_{CONV} and PDF_{BIO} during 1.36% glucose dwells. These data are shown in table 9-c. However, as compared to 3.86% PDF_{BIO}, TPR was significantly lower with 3.86% PDF_{CONV}, with a mean for the entire 3.86% PDF_{BIO} dwell of +31 ± 18% from baseline ($p<0.001$). As a result, SV and CO were therefore higher during 3.86% PDF_{CONV}; mean values during the PDF_{BIO} dwell were -17 ± 8% ($p<0.05$) and -13 ± 6% ($p<0.01$) respectively. These data are summarised in figure 9-d.

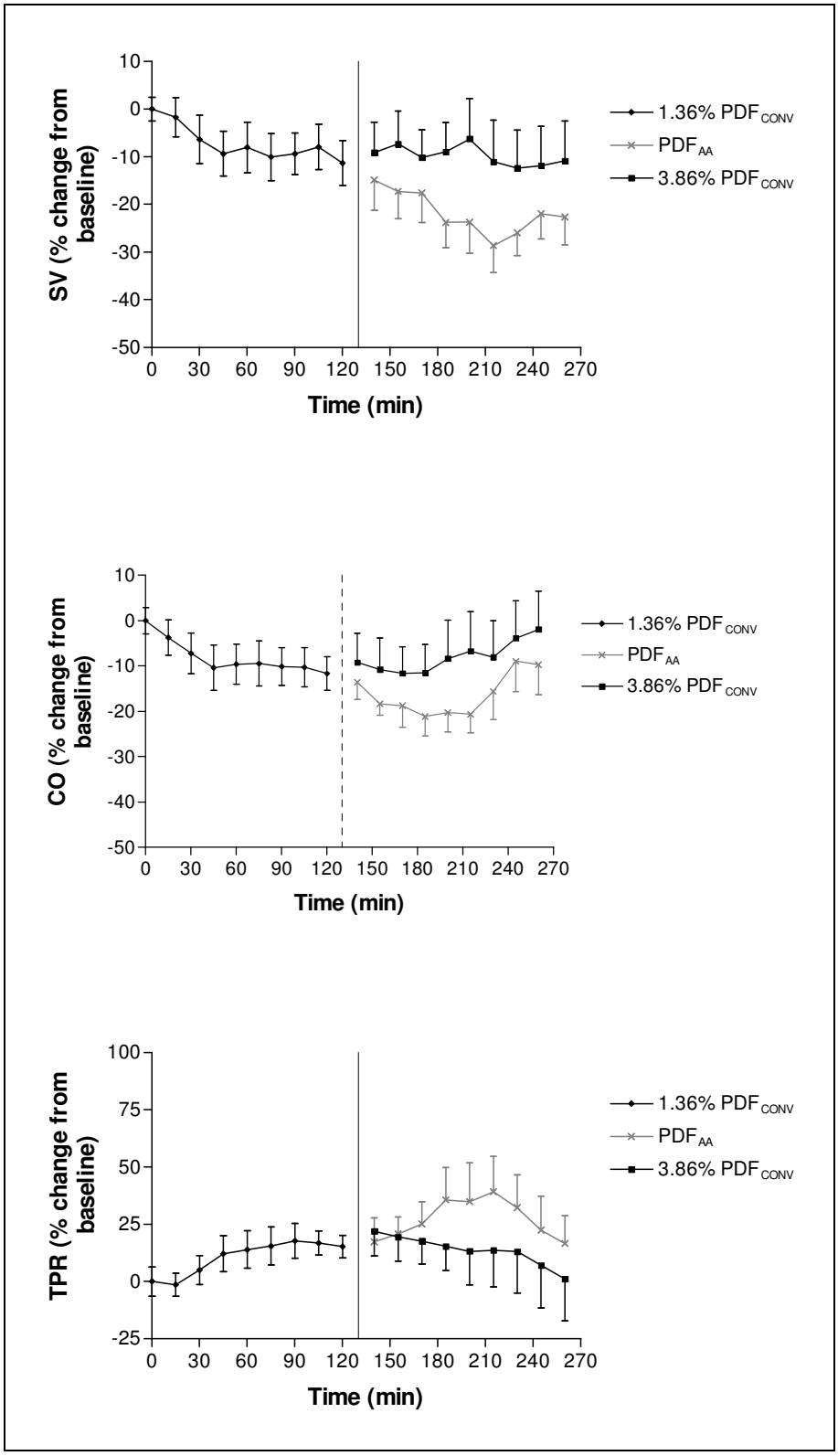


Figure 9-c.
Haemodynamics during PDF_{CONV} and PDF_{AA} dwells. SV and CO were significantly higher during PDF_{CONV} dwells and TPR was significantly lower, as compared to PDF_{AA}. 1.36% PDF_{CONV} data points are combined value for both days as values were extremely similar. Data are shown as mean ± SEM.

Mirroring the trend for BP to increase during dwell periods, TPR also increased throughout all dwell periods (although TPR did fall during the last 30min of PDF_{AA} dwell). The exception was during 3.86% PDF_{CONV} dwell, when TPR progressively fell, which resulted in a smaller decline in CO as compared to the other fluid types.

	1.36% PDF_{CONV}	**1.36% PDF_{BIO}**
HR (% change from baseline)	-1 ± 1	-1 ± 1
SV (% change from baseline)	-7 ± 3	-8 ± 3
CO (% change from baseline)	-9 ± 3	-9 ± 3
TPR (% change from baseline)	+11 ± 7	+9 ± 8

Table 9-c.
Systemic haemodynamics during 1.36% PDF_{CONV} and PDF_{BIO} dwells. HR = heart rate, SV = stroke volume, CO = cardiac output, TPR = total peripheral resistance. All data are shown as percentage change from baseline. There were no differences in any of the variables when compared between fluid types.

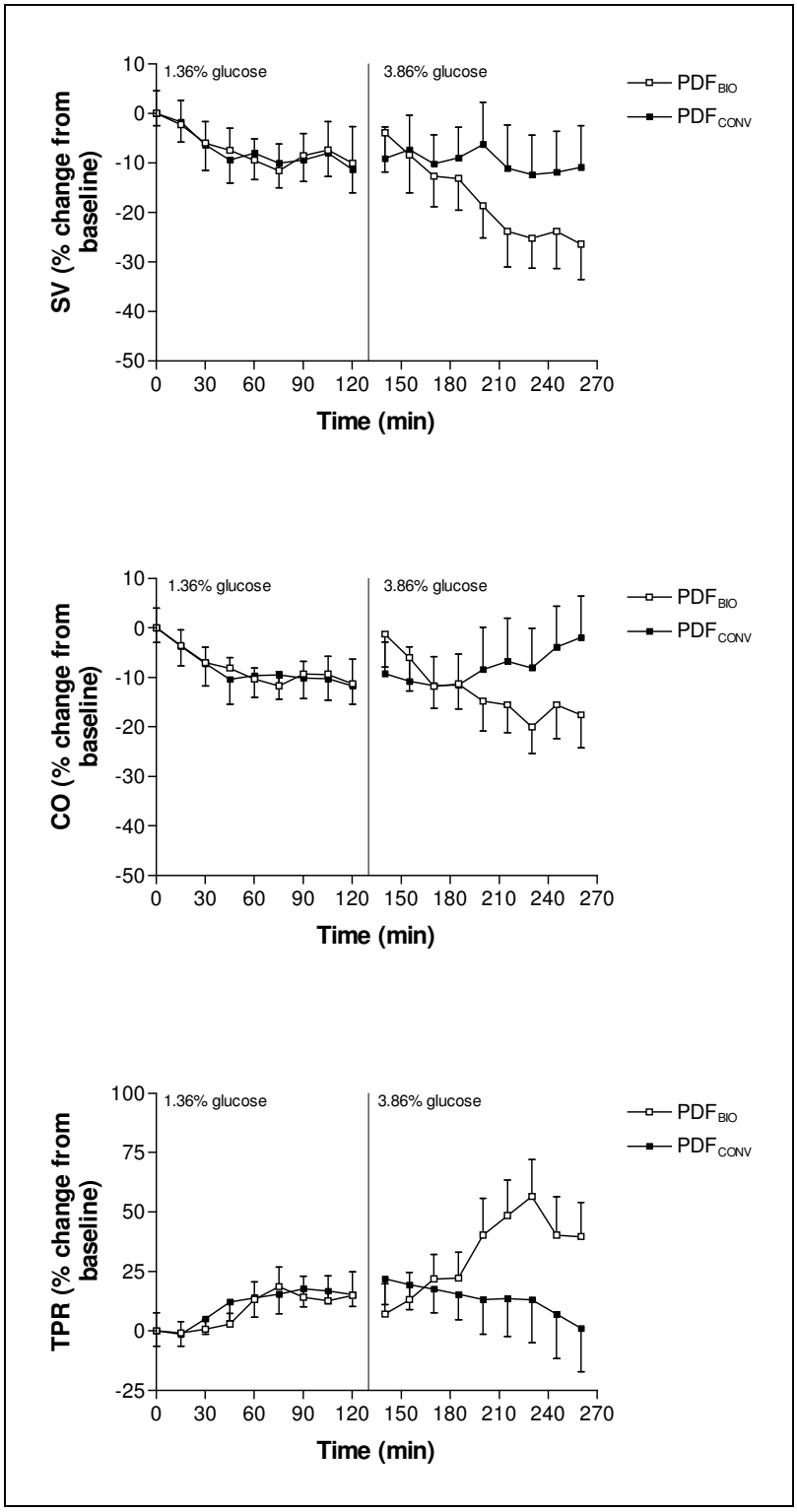


Figure 9-d.
Haemodynamics during PDF_{CONV} and PDF_{BIO} dwells. Parameters were very similar during 1.36% glucose dwells. SV and CO were significantly higher during 3.86% PDF_{CONV} dwells and TPR was significantly lower, as compared to 3.86% PDF_{BIO}. Data are shown as mean ± SEM.

9.4.5 Plasma glucose and insulin levels

Mean fasting glucose at baseline was 5.4 ± 1.1mmol/l. Plasma glucose did not change significantly during 1.36% PDF_{CONV}, 1.36% PDF_{BIO} or PDF_{AA} dwells. Highest mean glucose was 5.8 ± 0.7mmol/l during 1.36% PDF_{CONV} dwells, 5.6 ± 0.7mmol/l during 1.36% PDF_{BIO} dwells and 5.1 ± 0.6mmol/l PDF_{AA} dwells (p=ns for all comparisons). However, during 3.86% glucose dwells, plasma glucose rose significantly. Glucose was significantly higher than baseline by 10min during 3.86% PDF_{CONV} and PDF_{BIO} dwells, with mean levels of 7.5 ± 1.3mmol/l ($p<0.01$ versus baseline) and 7.7 ± 1.1mmol/l ($p<0.001$ versus baseline) respectively. By 60min, mean plasma glucose had risen to 9.1 ± 2.1mmol/l during 3.86% PDF_{CONV} and 9.1 ± 1.9mmol/l during 3.86% PDF_{BIO}. These values were significantly higher than baseline ($p<0.001$), and higher than the peak levels during PDF_{AA} and 1.36% glucose dwells ($p<0.001$ for all comparisons).

A similar pattern was seen with plasma insulin levels. Mean fasting plasma insulin was 16.9 ± 6.7µIU/ml, and did not change significantly during 1.36% PDF_{CONV} (plasma insulin 18.1 ± 6.3µIU/ml at 60min), 1.36% PDF_{BIO} dwells (18.43 ± 6.7µIU/ml at 60min) or PDF_{AA} dwells (16.7 ± 6.1µIU/ml at 60min, p=ns for all comparisons). However, insulin did rise significantly in response to the hyperglycaemia of the 3.86% glucose dwells. Mean plasma insulin was 22.9 ± 8.8µIU/ml at 10min (p=ns versus baseline) and 31.6 ± 14.4µIU/ml at 60min during 3.86% PDF_{CONV} dwells ($p<0.05$ versus baseline). During 3.86% PDF_{BIO}, mean plasma insulin

was 29.0 ± 11.1µIU/ml at 10min (p=ns versus baseline) and 35.6 ± 16.2µIU/ml at 60min (p<0.01 versus baseline). For PDF_{CONV} and PDF_{BIO}, both 10min and 60min plasma insulin levels were significantly higher than the corresponding values for PDF_{AA} (p<0.05 for 10min comparison, p<0.001 for 60min comparison PDF_{CONV} versus PDF_{AA}, p<0.01 for 60min comparison PDF_{BIO} versus PDF_{AA}). Plasma glucose and insulin data are summarised in figure 9-e.

At baseline, there were only two fasting glucose readings of greater than 7.0mmol/l, and in both these patients readings were less than 7.0mmol/l on the other two study days. Mean HOMA IR was 4.6 ± 2.7 and four patients had values suggestive of insulin resistance (>3.8).

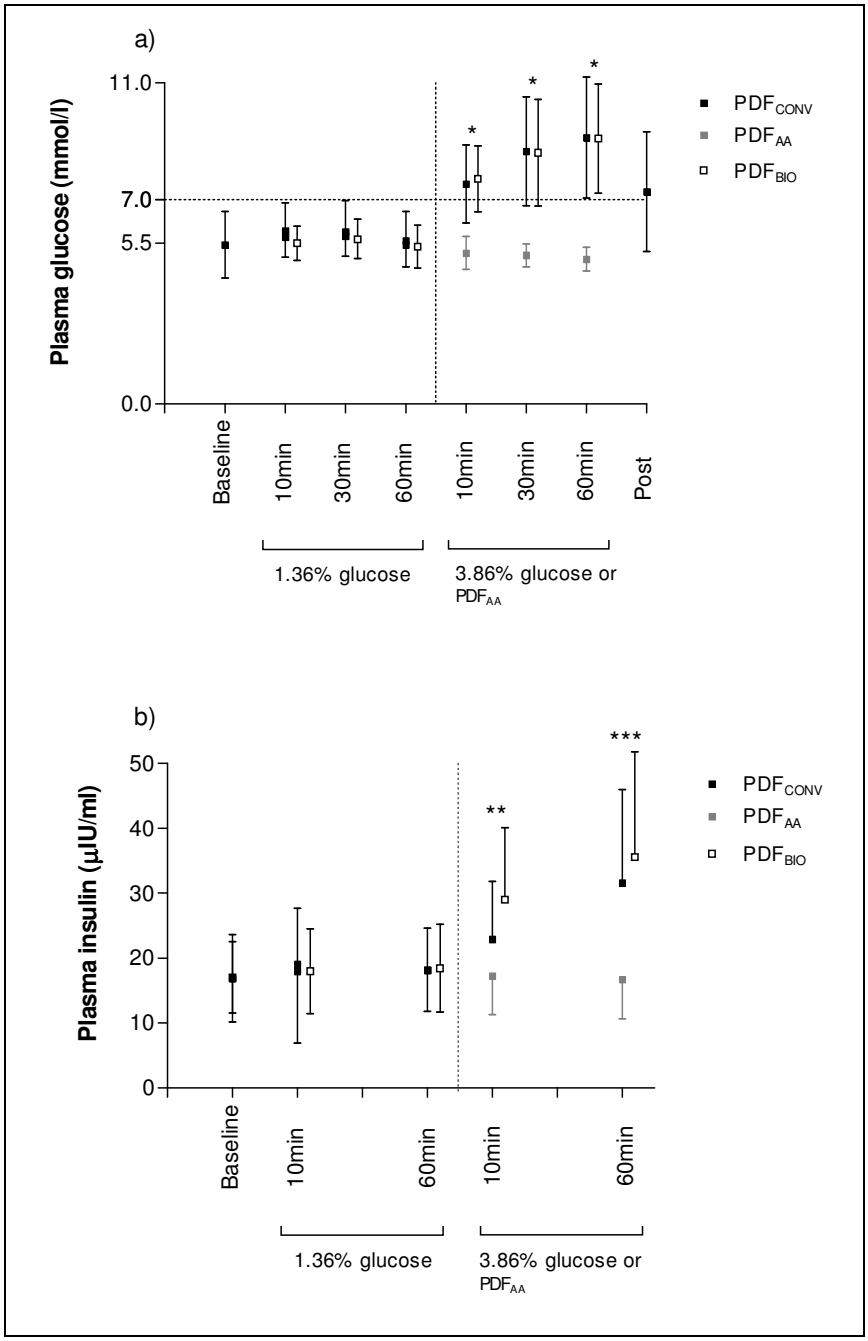


Figure 9-e.
Plasma glucose (a) and insulin (b) levels during PDF_{CONV}, PDF_{BIO} and PDF_{AA} dwells. Plasma glucose and insulin rose significantly during 3.86% glucose dwells as compared to both baseline and the corresponding values during PDF_{AA} dwells. The two data points for 1.36% PDF_{CONV} represent values for PDF_{CONV} and PDF_{AA} study days. Data are shown as mean ± SD. * $p<0.001$ versus baseline, corresponding measurements during 1.36% glucose and PDF_{AA} by ANOVA. ** $p<0.05$ versus $PDF_{AA.}$ *** $p<0.05$ versus baseline, $p<0.001$ PDF_{CONV} versus PDF_{AA} and $p<0.01$ PDF_{BIO} versus PDF_{AA}

9.4.6 Ultrafiltration volumes

UF volumes were similar during PDF_{AA}, 1.36% PDF_{CONV} and 1.36% PDF_{BIO} dwells, with means of 177 ± 167ml, 116 ± 129ml and 134 ± 138ml respectively (p=ns for all comparisons). UF volumes however were significantly higher with 3.86% glucose dwells, with means of 736 ± 180ml with PDF_{CONV} and 649 ± 232 with PDF_{BIO} ($p<0.001$ for all comparisons versus 1.36% glucose and $p<0.05$ for all comparisons versus PDF_{AA}). There was no difference when comparing UF volumes between 3.86% glucose PDF_{CONV} and PDF_{BIO} (p=ns). These data are shown in figure 9-f.

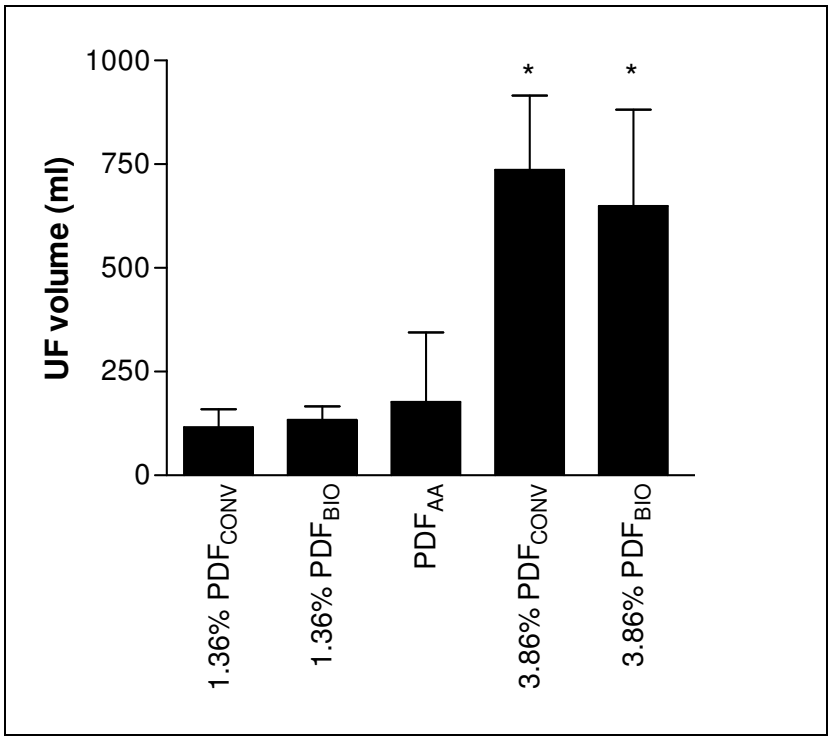


Figure 9-f.
Ultrafiltration volumes during PDF_{CONV}, PDF_{BIO} and PDF_{AA} dwells. 1.36% PDF_{CONV} data represent the combined value for both days as values were extremely similar. Data are shown as mean ± SD. * $p<0.001$ versus 1.36% glucose, $p<0.05$ versus PDF_{AA} by ANOVA.

9.4.7 Echocardiographic measurements

At the outset of the study, only two patients had LV hypertrophy (defined as LVMI >51g/$m^{2.7}$ or interventricular septal thickness >1.1cm) and all had normal ejection fractions. Despite the differences in ultrafiltration volume (and therefore in intraperitoneal volume during the dwell periods) there were no significant differences in LV dimensions throughout the study period. Equally, ejection fraction remained constant throughout all dwell periods. These data are shown in table 9-d.

	LVDd start (cm)	**LVDd end (cm)**	**LVDs start (cm)**	**LVDs end (cm)**	**EF start (%)**	**EF end (%)**
1.36% PDF_{CONV}	4.0 ± 0.6	4.1 ± 0.5	2.4 ± 0.6	2.4 ± 0.5	72 ± 9	72 ± 9
1.36% PDF_{BIO}	3.8 ± 0.6	4.1 ± 0.5	2.3 ± 0.5	2.4 ± 0.4	70 ± 8	73 ± 6
3.86% PDF_{CONV}	4.2 ± 0.5	4.1 ± 0.4	2.3 ± 0.3	2.2 ± 0.4	76 ± 5	77 ± 8
3.86% PDF_{BIO}	4.0 ± 0.5	4.0 ± 0.6	2.2 ± 0.4	2.2 ± 0.6	75 ± 8	75 ± 9
PDF_{AA}	3.9 ± 0.5	4.1 ± 0.5	2.3 ± 0.5	2.2 ± 0.5	73 ± 9	77 ± 12

Table 9-d.
Echocardiographic measurements of left ventricular (LV) diameter. LVDd = LV diameter in diastole, LVDs = LV diameter in systole, EF = ejection fraction, start = start of dwell immediately after fluid instillation, end = end of dwell immediately before fluid is drained out.

9.4.8 Biochemical parameters

Biochemical data from the start and end of each study session are shown in table 9-e. There were no differences in any of the variables. In particular, mean post PDF_{CONV} bicarbonate was 28.3 ± 6mmol/l and did not differ from either post PDF_{BIO} bicarbonate (29.8 ± 5mmol/l) or post PDF_{AA} bicarbonate (mean of 27.4 ± 5mmol/l, p=ns for both comparisons).

	Pre PDF_{CONV}	**Post PDF_{CONV}**	**Pre PDF_{BIO}**	**Post PDF_{BIO}**	**Pre PDF_{AA}**	**Post PDF_{AA}**
Haemoglobin (g/dl)	12.6 ± 1	12.4 ± 1	12.4 ± 1	12.2 ± 2	12.9 ± 1	12.3 ± 1
Bicarbonate (mmol/l)	26.3 ± 5	28.3 ± 6	26.4 ± 5	29.8 ± 5	26.2 ± 6	27.4 ± 5
Sodium (mmol/l)	139 ± 4	140 ± 2	138 ± 4	140 ± 3	139 ± 4	139 ± 3
Potassium (mmol/l)	4.3 ± 0.5	3.9 ± 0.5	4.4 ± 0.6	4.0 ± 0.6	4.3 ± 0.5	4.1 ± 0.6
Corr calcium (mmol/l)	2.47 ± 0.1	2.47 ± 0.1	2.45 ± 0.2	2.53 ± 0.1	2.47 ± 0.2	2.47 ± 0.2
Phosphate (mmol/l)	1.5 ± 0.4	1.3 ± 0.5	1.6 ± 0.5	1.4 ± 0.5	1.7 ± 0.4	1.4 ± 0.3
Albumin (g/dl)	33 ± 3	33 ± 3	31 ± 3	31 ± 3	32 ± 4	31 ± 4
PTH (ng/l)	356 ± 244		352 ± 234		371 ± 232	
CRP (mg/l)	4 ± 4	4 ± 4	7 ± 11	8 ± 14	5 ± 6	4 ± 6

Table 9-e.
Biochemical parameters for each of the study days. PTH = parathormone, CRP = C reactive protein. There were no differences in any of the parameters when comparing PDF_{CONV} and PDF_{AA}, or PDF_{CONV} and PDF_{BIO}.

9.4.9 Peritoneal transport characteristics and urea kinetic modelling

All patients had a weekly Kt/V_{urea} of over 2.0 (mean 2.57 ± 0.5, range 2.09 to 3.46). Mean weekly creatinine clearance was 94 ± 19l/wk (68 to 127) and residual renal function was 4.8 ± 3ml/min (0.2 to 10.2). Peritoneal transport characteristics for each patient are listed in table 9-a. Transport characteristics did not appear to affect the haemodynamic response to the different fluid types, or the magnitude or rate of change in plasma glucose during the 3.86% glucose dwells.

9.5 Discussion

This study confirms that hypertonic glucose dialysate results in an acute elevation of BP as compared to low osmolar glucose or amino acid containing solutions. The elevation in BP was associated with hyperglycaemia and hyperinsulinaemia, but was not due to differences in cardiac filling. We also observed differences between the haemodynamic response to conventional and biocompatible hypertonic glucose solutions.

In accordance with results from chapter 8 in which we demonstrated higher BP with 3.86% glucose as compared to Icodextrin, results from this study also show a rise in BP with hypertonic glucose solutions as compared with either 1.36% glucose or PDF_{AA}, the latter being entirely glucose free. This rise in BP occurred with both hypertonic PDF_{BIO} and

PDF_{CONV} and did not appear dependent on buffer type. The higher BP also occurred despite the largest UF volumes, but the absence of change in LV diameters indicates the effects on BP were not caused by increased cardiac filling due to the larger intraperitoneal volume. Equally, the stable LV dimensions argue against the study patients being volume overloaded at baseline, in which situation an improvement in cardiac function may be seen in response to ultrafiltration. Therefore, the hyperglycaemia and hyperinsulinaemia demonstrated in our study during 3.86% glucose dwells seem the most likely cause of the rise in BP.

BP has been shown to rise in response to hyperglycaemia in several studies in both non diabetic and diabetic patients, while glucose and insulin exert independent effects on systemic haemodynamics [129, 130, 195, 196]. Marfen et al demonstrated a rise in BP during hyperglycaemia, during both normal and elevated insulin levels [130]. BP was returned to baseline with a glutathione infusion, suggesting the direct effect of hyperglycaemia on haemodynamics may be mediated by free radical production leading to reductions in nitric oxide. Equally, hyperinsulinaemia during euglycaemic clamp studies has been shown to cause elevations in HR, SV and CO whilst also causing a fall in TPR [131]. These changes would be consistent with those observed when comparing the haemodynamic response to PDF_{AA} and PDF_{CONV}, with SV and CO significantly higher and TPR lower in the presence of hyperinsulinaemia during 3.86% PDF_{CONV}.

Despite a similar rise in BP, the underlying haemodynamic response differed between PDF_{CONV} and PDF_{BIO} 3.86% solutions. These two fluids produced equal changes in plasma insulin and glucose, similar UF volumes and the fluids were warmed to exactly the same temperature before instillation. Although the fluids differ in buffer, there was no significant difference in plasma bicarbonate at the end of the study period. In addition, there were no differences between 1.36% PDF_{CONV} and PDF_{BIO}, so it seems unlikely that the buffer type alone exerted a significant effect on systemic haemodynamics. However, as the metabolism of glucose and lactate are linked in both skeletal muscle and hepatocytes [197], it is possible to speculate that the difference in buffer type resulted in differences in subsequent glucose metabolism. Alternatively, PDF_{BIO} does contain significantly fewer glucose degradation products (GDPs) as compared to PDF_{CONV}. The amount of GDPs generated during heat sterilisation depends on both dialysate pH and the amount of glucose present, and therefore 3.86% PDF_{CONV} contains more GDPs than 1.36% PDF_{CONV} [11]. This may explain why 1.36% PDF_{BIO} and PDF_{CONV} behaved in a similar fashion, but why 3.86% fluids differed. One report describes mesenteric artery vasodilatation in the rat in response to acidic buffered, 4.25% glucose fluid with high GDP content, and abrogation of this effect with similar fluid low in GDPs [198]. These data would be consistent with our observation of lower TPR during 3.86% PDF_{CONV} dwells. Although very difficult to isolate from biological fluids, indirect measurements suggest that systemic absorption of GDPs occurs during CAPD with conventional solutions [124, 125]. However, there are

no available data on whether or not GDPs can affect systemic haemodynamics.

In short, the haemodynamic response during CAPD is affected by multiple different factors. In addition to the possible effects of hyperglycaemia and hyperinsulinaemia, cooling due to the dialysate fluid plus the effects of ultrafiltration are likely explanations for the rise in TPR and BP throughout the dwell periods (seen with all fluids except 3.86% PDF_{CONV}). In addition, autonomic function and baroreflex sensitivity can be altered by all of the above factors. Therefore, it is only possible to speculate as to the exact cause of the haemodynamic changes that we observed, particularly when comparing PDF_{CONV} and PDF_{BIO}.

In contrast to the findings in chapter 8, we did not observe significant differences in HR between the fluid types during this current study. Only one patient was taking a rate limiting drug (atenolol) so it may be that our patients, as with many dialysis patients, had impaired autonomic function [41]. However, this was not formally assessed.

We observed a relatively high degree of insulin resistance in our patients at baseline. This, in combination with the large amount of glucose delivered during 3.86% dwells explains the magnitude of hyperglycaemia seen in some of these non-diabetic patients. Patients were fasted throughout, yet plasma glucose exceeded 11mmol/l in three patients. These data are consistent with other work that shows patients on CAPD have a higher prevalence of insulin resistance and therefore display

higher plasma glucose levels in response to a glucose load compared with normals [13, 199]. Furthermore, the hyperinsulinaemia seen in response to glucose may be abnormally prolonged in CAPD patients [199]. Although not assessed in our short term study, the frequent use of hypertonic glucose solutions resulting in repeated excursions of glucose and insulin to outside of their normal ranges may well have the potential to exert negative long term metabolic effects. Certainly, several large prospective studies in non-diabetics have shown that a hyperglycaemic response to a glucose load is a strong predictor of cardiovascular death. [122, 123]. This may be important in the context of the extremely high cardiovascular mortality rates in dialysis patients that are not explained by conventional risk factors alone. Crucially, this may be modifiable as preliminary data show that a PD regime employing Icodextrin to reduce glucose exposure can improve insulin resistance after a period of nine months [13].

In conclusion, we have demonstrated that the hyperglycaemia and hyperinsulinaemia observed during CAPD with hypertonic glucose dialysate is associated with an acute rise in BP. CAPD with 1.1% amino acid solution did not cause any such derangements. In addition, a differing haemodynamic response to conventional and newer biocompatible PD solutions was observed, the cause of which is at present unclear. We suggest that these adverse haemodynamic and metabolic effects may have the potential to negatively impact on cardiovascular outcomes. As cardiovascular risk reduction is key to

ensuring optimal outcome in CAPD patients, manipulation of these haemodynamic and metabolic consequences by using low glucose, biocompatible fluids should be the subject of longer term, outcome-based studies.

10. Results: the acute effects of automated peritoneal dialysis on systemic haemodynamics

10.1 Introduction

Automated peritoneal dialysis (APD) is an increasingly utilised modality in the treatment of CKD stage 5. It is often used in preference to continuous ambulatory peritoneal dialysis (CAPD) to improve dialysis adequacy or for lifestyle reasons. To date, there are no published data examining the haemodynamic effects of APD.

Residual renal function (RRF) is crucial in maintaining adequacy in many peritoneal dialysis patients, and also in helping maintain fluid and electrolyte balance. In addition, maintenance of RRF has been shown to affect survival [134, 135]. Debate continues as to whether APD increases the rate of decline of RRF as compared to CAPD, and the published literature in this area is quite discrepant [136-138, 200-204]. Some authors have postulated that APD causes greater shifts in osmotic load and exerts more intensive ultrafiltration as compared to CAPD, and this in turn may potentially cause adverse haemodynamic effects [136, 139]. Other work demonstrates that episodes of dehydration are associated with greater decline of RRF in CAPD patients, analogous to the association of episodes of intradialytic hypotension (IDH) with an increased loss of RRF in HD patients [203].

Following our demonstration of significant haemodynamic changes in response to CAPD, we undertook a study to examine the BP and haemodynamic response to APD.

10.2 Subjects and Methods

10.2.1 Patients

The same eight patients as described in chapter 8 were studied. Baseline characteristics, RRF, transporter status and CAPD prescriptions are shown in table 8-a.

10.2.2 Study protocol

All patients gave informed consent prior to commencement and ethical approval for the project was granted by Derbyshire Research Ethics Committee. Patients were admitted to a clinical investigations unit where APD was performed using Homechoice™ APD machines (Baxter Healthcare, Norfolk, UK). APD was carried out during daylight hours and patients were awake throughout. Non-invasive haemodynamic monitoring was undertaken using a Finometer that was fitted for the entirety of the investigatory session (described in chapter 3). To obtain baseline values, monitoring started 30min prior to commencing APD. The APD prescription stipulated a treatment time of four hours, consisting of three

drain/instillation cycles each using 2.5L of dialysate fluid. The dwell time for each cycle was 76mins. 1.36% glucose-based Physioneal® (bicarbonate/lactate-based pH neutral fluid, Baxter Healthcare, Norfolk, UK) was used for the first and third exchanges and 3.86% glucose Physioneal® for the second exchange. The APD prescription ensured that 1.36% glucose and 3.86% glucose fluids did not mix in the reservoir bag. Dialysate fluid was warmed by the Homechoice™ machine prior to instillation. Due to the physical constraints of continuous monitoring, patients remained semi-recumbent throughout.

Blood samples were collected before and after each session in lithium heparin and EDTA tubes, and biochemical analysis performed on a multichannel autoanalyser. Peritoneal waste fluid was also assessed (volume, electrolyte concentration). Primary endpoints were percentage change in blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) in response to the APD cycle.

10.3 Statistical analysis

Results are expressed as mean ± SD (where given, values in parentheses represent the range). BP and haemodynamic data were compared using one-way ANOVA with a design for repeated measures and Bonferroni's test to correct for multiple comparisons. For other data, the paired *t*-test was used after significant deviations from a normal

distribution were excluded with the Kolmogorov-Smirnov test. Correlation coefficients were calculated using Pearson's test. An alpha error at $P<0.05$ was judged to be significant.

10.4 Results

10.4.1 Blood pressure and heart rate

For the entire study period, overall mean systolic BP (SBP) was 148 ± 5mmHg, mean diastolic BP (DBP) was 87 ± 3mmHg and mean of the mean arterial pressure (MAP) was 108 ± 5mmHg. Initial BP was not significantly different from end BP (for SBP p=0.32, for DBP p=0.87, and for MAP p=0.97). There were no statistically significant differences between the mean BP values for the dwell periods with 1.36% and 3.86% glucose. Mean SBP during 1.36% glucose dwell was 149 ± 3mmHg, mean DBP 87 ± 1mmHg and mean MAP 108 ± 2mmHg. Mean SBP during 3.86% glucose dwell was 149 ± 6mmHg, mean DBP 89 ± 4mmHg and mean MAP 110.7 ± 4.7mmHg. As can be seen from the larger SDs and ranges, BP varied more during the 3.86% dwell, falling at 20min (SBP fell by 14mmHg) and then plateauing. BP data are summarised in figure 10-a.

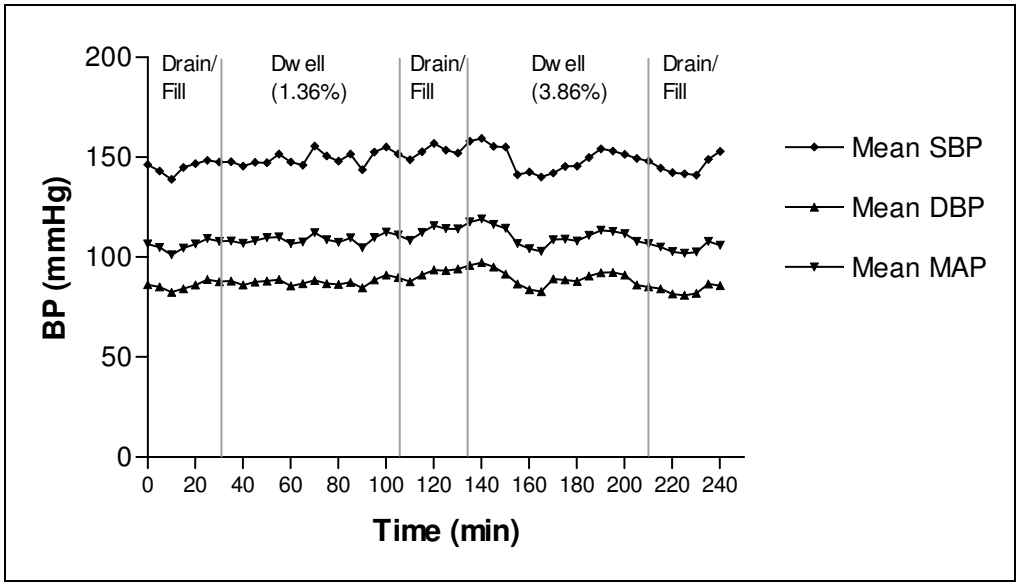


Figure 10-a.
Mean BP data for entire study period. All 3 BP parameters drop during the 1st and 3rd drainage periods and then rise upon dialysate fluid instillation. This is not seen in the 2nd drain/fill period. There is no significant difference between mean BP values at the start and end of the investigatory period.

Mean SBP, DBP and MAP dropped during drainage and rose on instillation of dialysate in two of the three drain/fill periods. The largest magnitude of change occurred during the third drain/fill period, where mean SBP fell from 150mmHg to 142mmHg during drainage (p=0.029), before rising to 148mmHg on instillation. Mean DBP fell from 85mmHg to 80mmHg (p=0.01) before climbing to 86mmHg, while mean MAP fell from 107mmHg to 102mmHg (p=0.01) and then rose to 108mmHg. The falls in BP parameters during the first and second drainage periods were smaller and did not reach statistical significance.

10.4.2 Haemodynamic parameters

Throughout the entire study period, HR remained relatively stable. There was no significant difference between mean HR at the start and end of the study period (p=0.28). Mean percentage change from baseline was -1.9 ± 2%. Data for HR are summarised in figure 10-b.

Throughout the study period, SV and CO both fell. The fall in SV to -21.1% from baseline was of borderline statistical significance (p=0.060). Mean SV for entire period was -9.3 ± 6%. CO fell to -22.4% from baseline (p=0.037) and overall mean CO was -11.1 ± 6%. During all three drainage periods SV and CO rose, and on instillation of fluid both then fell. The largest changes occurred during the third drain/fill phase, with SV varying between -4.7% and -19.5% and CO varying between -22.4% and -5.3%.

Contrary to the changes in SV and CO, TPR progressively rose throughout the study period to +53.4% above baseline (p=0.016). Mean TPR for the study period was +19.7 ± 18%. During drainage periods, mean TPR fell but then rose during fluid instillation. Again, changes were greatest during drain/fill phase 3 with TPR varying between -1.7% and +50.2%. A similar pattern of response (fall in TPR with an inadequate rise in SV and CO) was also seen to explain the fall in BP at 20min during the 3.86% glucose dwell. Haemodynamic data are summarised in figure 10-b.

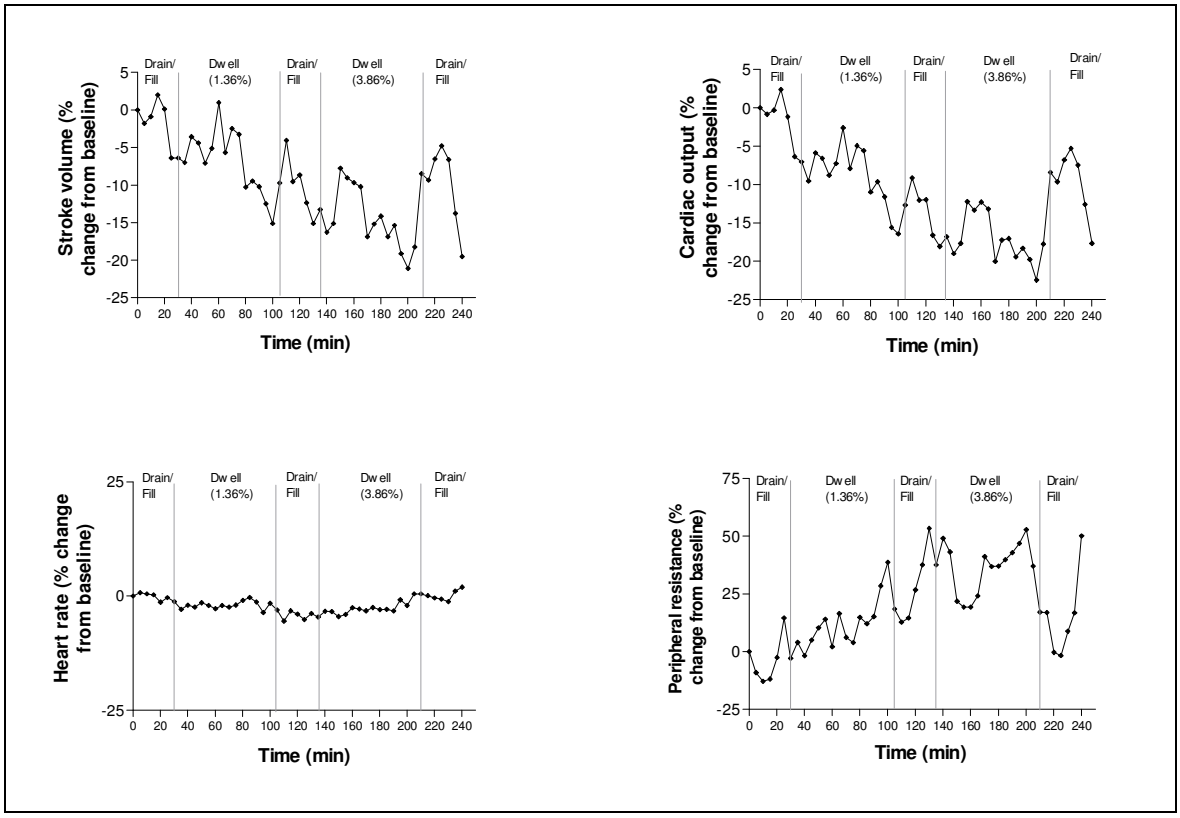


Figure 10-b.
Haemodynamic data. SV and CO progressively decline throughout the study period (p=0.060, p=0.037 respectively). During each drain/fill phase, SV and CO rise as fluid is drained out of the peritoneal space and fall upon instillation of fluid. TPR progressively rises during the study period (p=0.032). During dialysate fluid drainage, TPR falls and then rises again upon instillation of dialysate. HR remains constant, with no significant change comparing start and end mean values (p=0.28).

The haemodynamic and BP patterns described above are population means. Analysing patients individually, 6/8 patients showed similar trends with respect to BP, HR, SV, and TPR. The three diabetic patients were not demonstrably different from the non-diabetics. Of the two that differed from the population mean, one patient did not display a pronounced rise in TPR and fall in CO throughout the overall study period. The other patient did display a similar overall pattern but had a higher SV/CO and therefore a higher BP during the 3.86% glucose dwell. This latter finding

was similar to our previous work showing a similar pattern during hypertonic glucose CAPD dwells (chapters 8 and 9). The two patients who differed from the group did display the same haemodynamic changes during the drain/fill phases with a rise in SV and CO coupled to a fall in TPR during drainage, and a rise in TPR plus a fall in SV and CO during instillation. These two patients were not examined on the same study day, did not have the same peritoneal transporter status and both had UF volumes that were similar to the group mean.

10.4.3 Ultrafiltration volumes and sodium removal

Mean ultrafiltration (UF) volume for the overall dwell period was 542 ± 232ml. Overall, sodium was retained with a mean of +12.8 ± 38mmol. There was no correlation between BP, SV or TPR and either UF volume or sodium loss. Mean serum sodium at the start of the study period was 133.8 ± 4mmol/L and did not change post study, with a mean of 133.8 ± 3mmol/L.

10.4.4 Peritoneal transport characteristics and urea kinetic modelling

All patients had a weekly Kt/V of greater than 2.0 (mean 2.5, range 2.0 to 3.4). Mean weekly creatinine clearance was 78.1l/wk (range 61.8 to 115.5), with mean residual renal function 4.4 ± 3.6ml/min. There were no correlations between BP and residual renal function, weekly creatinine clearance or Kt/V.

10.5 Discussion

This study reports significant systemic haemodynamic effects during APD. Changes were seen in CO and TPR over the entire study period and in BP, SV, CO and TPR during drain/instillation periods. To our knowledge, this is the first report examining the haemodynamic response to APD.

The most significant changes in BP were seen during drain/fill phases. During the first and third drain phases, BP fell when dialysate was drained out of the peritoneal cavity. Mirroring this fall in BP, TPR fell whilst SV and CO both rose. For two of the drain phases the rise in CO was inadequate and did not prevent BP falling. Conversely, BP and TPR rose when dialysate was instilled into the peritoneal cavity, and associated with this there was a fall in SV and CO. This is likely to be a 'volume effect' due to mesenteric capacitance vessel compression by the dialysate fluid. This effect may have been greatest in the third drain/fill phase as this followed the dwell period with 3.86% glucose dialysate during which UF is likely to have been greater. Intravascular volume would therefore be more contracted, and the volume drained would be larger.

TPR rose progressively throughout the APD treatment by more than 50% from baseline. In response to this, a progressive fall in CO was demonstrated (with a trend towards a fall in SV). These changes however did not significantly affect overall BP. The rise in TPR may be due to a

cooling effect, with each drain/fill cycle further cooling the mesenteric vessels and leading to vasoconstriction. A greater cooling effect may occur during APD as compared with CAPD due to the increased number of drain/fill cycles with the former, as the method of warming the APD dialysate in the reservoir bag on top of the Homechoice™ machine may not deliver a fully mixed and accurate fluid temperature. Potentially, the temperature of instilled dialysate could be more accurately measured and manipulated with online sensors and heating, which may provide a way of modulating the haemodynamic response to APD. Haemodialysis patients experiencing progressive UF demonstrate a rise in TPR in association with an increase in peripheral sympathetic nerve activity [81]. Therefore, an alternative explanation for the rise in TPR during our study would be sympathetic activation in response to the UF of APD. This latter explanation seems less likely as there were no correlations between UF volume and BP, SV or TPR.

The data from the experiments described in chapters 8 and 9 demonstrate a differential haemodynamic response to 1.36% and 3.86% glucose-based dialysate fluids, with higher BP, HR and SV (but similar TPR) seen with 3.86% glucose dialysate. This was not seen our current study (except for in one patient). During our previous studies with CAPD patients, the protocol ensured that all conditions (including dialysate fluid temperature) were identical except for the dialysate fluid type. However, the progressive rise in TPR during this current study may reflect that there was a significant cooling effect as APD progressed. This may have

exerted a more powerful effect on systemic haemodynamics than the effects of dialysate glucose. Furthermore, dwell times were much shorter during this current study and a similar UF volume was removed over a shorter period of time. Fluid shifts, plasma refill rate and therefore effective blood volume may therefore have been affected differently.

The link between APD and a faster rate of decline in RRF remains controversial. Some authors have found that APD is associated with a faster decline in RRF [136-138], whereas others have not [200-205]. An adverse haemodynamic response to APD as compared with CAPD has been postulated as a possible factor in speeding decline of RRF but has never before been documented. We have demonstrated significant changes in BP and haemodynamics during drain/fill periods, and it is possible that the increased number of drain/fill cycles with APD (up to 10 per night) as compared to CAPD may impact on RRF. Dehydration episodes in PD patients have also been shown to increase the decline of RRF [203]. Therefore the shorter dwell phases with more rapid ultrafiltration that are seen with APD may explain the fall in BP a third of the way through the 3.86% glucose dwell, and are also a potential factor that may affect RRF. Equally, the haemodynamic changes seen over the entire study period (possibly mediated by systemic cooling) may be important. In health, the kidneys receive 20-25% of CO, and many conditions that cause a fall in CO also lead to reduction in renal blood flow [206]. Systemic cooling in rats has been demonstrated to cause renal vasoconstriction and reduce renal blood flow, although it is not

known whether the same phenomenon occurs in humans [207]. In addition, diseased kidneys have additional impairment of autoregulation, and may therefore be less able to maintain renal blood flow in the face of falling CO. However, it must be noted that our short-term study did not address or measure any changes in renal blood flow, urine output or RRF. Therefore, although we demonstrate significant changes in systemic haemodynamics, the effect that these changes have on RRF is entirely speculative.

In conclusion, we have demonstrated that APD exerts significant haemodynamic effects, with rising TPR (possibly signifying a cooling effect) and falling CO over time. We have also shown that drain/fill periods are associated with significant change in haemodynamics and BP. We speculate that these changes may have the potential to affect renal perfusion and therefore the rate of decline of RRF.

11. Results: regional left ventricular function in response to the haemodynamic changes of peritoneal dialysis

11.1 Introduction

Following our demonstration of regional left ventricular wall motion abnormalities occurring in response to haemodialysis and of significant haemodynamic changes that are evident during CAPD, we performed a study to examine whether CAPD could induce subclinical myocardial ischaemia. In view of the rapid haemodynamic and BP effects occurring during fluid instillation and drainage (chapter 10), regional LV function was assessed by echocardiography before and after a PD exchange.

11.2 Methods

11.2.1 Patients

The same patients that participated in the experiment in chapter 9 were recruited. Baseline patient characteristics are described in chapter 9 and in table 9-a. In contrast to the patients studied in chapter 4, none of these patients had atherosclerosis and only two had LVH.

11.2.2 Study protocol

Patients attended for a single study day. Following a dwell period with 1.36% glucose fluid, an exchange with 2.5L of 3.86% glucose-based dialysate was performed. All fluids were produced by Baxter Healthcare (Norfolk, UK) and were warmed to 37^0C prior to instillation. 2-D echocardiography was performed immediately before draining fluid from the abdomen and then following fluid instillation using commercially available equipment (1.5-3.6 MHz 3S probe, Vivid 3®, GE medical systems, Sonigen, Germany). Images were subsequently analysed for regional wall motion abnormalities using Echo-CMS (chapter 3). BP and haemodynamic data were recorded continuously using the Finometer (chapter 3). We collected blood in lithium-heparin tubes for cTnT analysis at baseline and at three hours after the end of the exchange.

Primary endpoints were the number of new RWMAs developing in response to the haemodynamic changes of a PD exchange.

All patients gave informed consent prior to commencement, and ethical approval for the project was granted by Derbyshire Research Ethics Committee.

11.3 Results

11.3.1 Regional LV function

There was a very low frequency of new RWMAs, occurring in only 3.9% of LV regions. Six patients did not demonstrate any new RWMAs. As a result, mean SF did not change following the exchange in two of the regions assessed; in the remaining eight regions mean SF increased significantly. Overall mean SF for all regions also increased significantly following the exchange from 2.8 ± 1.0% to 3.9 ± 0.94% ($p<0.001$). Data for pre and post SF for each region and overall are shown in table 11-a. EF did not change significantly following the exchange with means of 69.6 ± 12% before the exchange and 76.6 ± 5% after (p=ns).

	Pre SF (%)	**Post SF (%)**	***p*-value**
Basal Septal	2.0 ± 1.3	2.0 ± 1.0	ns
Mid Septal	3.6 ± 1.4	4.4 ± 1.3	<0.05
Apical (4 chamber)	1.7 ± 0.9	2.6 ± 1.1	<0.05
Mid Lateral	2.7 ± 1.1	4.5 ± 1.4	<0.001
Basal Lateral	3.6 ± 1.6	5.5 ± 2.1	<0.001
Basal Inferior	2.8 ± 1.8	4.2 ± 1.8	<0.001
Mid Inferior	3.9 ± 1.4	5.2 ± 1.5	<0.01
Apical (2 chamber)	2.8 ± 1.6	3.6 ± 1.1	ns
Mid Anterior	2.9 ± 1.6	3.6 ± 1.4	ns
Basal Anterior	2.6 ± 1.7	4.1 ± 1.8	<0.001
Overall	2.8 ± 1.0	3.9 ± 0.9	<0.001

Table 11-a.
Regional function in each of the LV regions assessed, and overall mean regional function before and after the PD exchange.

11.3.2 Blood pressure and haemodynamics

BP varied significantly during the exchange, falling on drainage of fluid and rising upon instillation. Mean SBP was 160.1 ± 11mmHg prior to drainage and fell to 156 ± 11mmHg post drainage, and rose from 165 ± 11 before instillation to 174 ± 13mmHg after ($p<0.008$). BP data are summarised in figure 11-a.

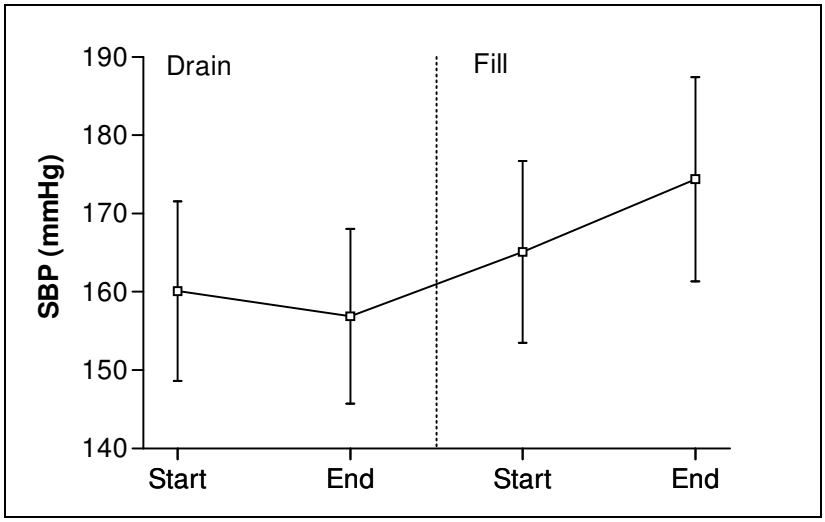


Figure 11-a.
SBP at the start and end of fluid drainage and instillation.

Mirroring the changes in BP, TPR also fell during fluid drainage from 29 ± 8% to 19 ± 8%, whilst during fluid instillation TPR rose from 27 ± 10% to 40 ± 9% ($p<0.001$).

The observed changes in CO were less. Although CO rose during fluid drainage from -13 ± 8% to -11 ± 8%, and fell during fluid instillation from -13 ± 7% to -16 ± 7%, these changes did not reach statistical significance (p=ns). The changes in SV were similar to CO and also did not reach

statistical significance. HR varied very little throughout. Haemodynamic data are summarised in figure 11-b.

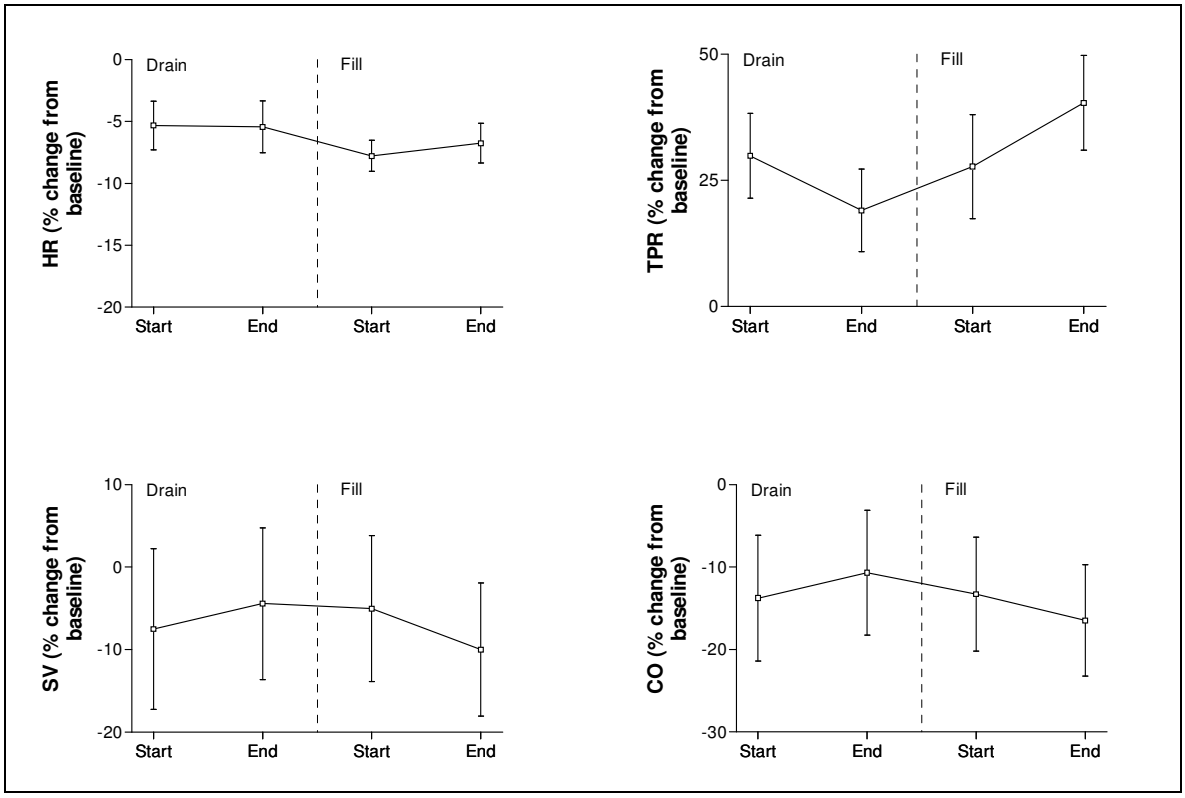


Figure 11-b.
Haemodynamics at the start and end of fluid drainage and instillation.

11.3.3 Troponin levels

cTnT levels did not change at all following the exchange. Mean values were 0.02 ± 0.01µg/l both before and three hours after the study period (p=ns).

11.4 Discussion

This study confirms that significant changes in BP and systemic haemodynamics occur during drainage and instillation of fluid in and out of the abdominal cavity. In a cohort of patients with no atherosclerosis and little structural heart disease, there was no evidence that these changes led to subclinical myocardial ischaemia.

The fall in BP and TPR with an inadequate rise in CO during fluid drainage with the opposite seen during instillation are similar to the results observed during exchanges in chapter 10. The most likely explanation for this is that peripheral resistance is altered significantly by compression of the mesenteric vessels, which occurs during instillation of fluids and is relieved when fluid is drained. However, it remains unclear why HR and CO do not increment adequately to prevent the fall in BP during drainage, even in a cohort of patients with relatively little cardiac disease. Certainly, autonomic neuropathy is widespread in dialysis patients and it may be that a blunting of the baroreflex arc could explain this. This would also fit with absence of any change in heart rate in the same patients during the experiment described in chapter 9. However, autonomic function was not measured as part of this study.

Despite these significant changes in haemodynamics, we did not observe any evidence of myocardial stunning. There were very few regions that demonstrated new RWMAs and overall SF in all LV regions remained

unchanged or increased. In addition, we did not observe any change in cTnT levels. In contrast to the patients described in chapter 4, this cohort was relatively free of cardiac disease and no patients had clinically apparent atherosclerosis. In keeping with this, baseline cTnT levels were also low. It is possible that CAPD patients with a greater degree of vascular disease and LVH would be more susceptible to subclinical myocardial ischaemia in response to these haemodynamic fluctuations, but this remains to be tested.

It was interesting to observe that regional LV function in many regions increased following the exchange. This is similar to unaffected LV areas in the haemodialysis patients that also displayed increased wall motion by the end of dialysis. This suggests that myocardial performance (in terms of contractility) increases in an attempt to respond to the significant changes in haemodynamics. However, as evidenced by the inadequate rise in CO, this is insufficient to prevent changes in BP. This may in part be due to the inadequate chronotropic response.

In conclusion, in a cohort of patients with relatively low risk for myocardial ischaemia, we found no evidence that the haemodynamic consequences of a PD exchange lead to myocardial stunning.

12. Conclusions

The risk of cardiovascular death in dialysis patients is grossly elevated. As the dialysis population continues to expand whilst donor kidneys remain scarce, this excess of mortality is becoming an increasingly important issue. There are many different factors that negatively impact on the cardiovascular system in the dialysis patient. However, our results suggest that the haemodialysis process itself may be implicated by inducing haemodynamic perturbation sufficient to induce subclinical myocardial ischaemia.

In two separate groups of dialysis patients, we have demonstrated the occurrence of dialysis induced LV regional wall motion abnormalities. Although there are other reports of silent ischaemia occurring during dialysis [28, 43-52] our work is the first to suggest that it is possible to reduce this phenomenon by improving intradialytic haemodynamic stability. Our studies show that biofeedback dialysis and the simple and universally available technique of reducing dialysate temperature are both effective in this way. This is important as repeated episodes of ischaemia and myocardial stunning may be cumulative and contribute to the genesis of chronic heart failure in patients with ischaemic heart disease [59]. Therefore, as haemodialysis is repeated thrice weekly, it is possible that repeated dialysis induced myocardial stunning may contribute to chronic cardiac dysfunction. It remains to be seen whether improving the

haemodynamic tolerability of dialysis would be an effective intervention to reduce the development of heart failure in this patient group.

Exploring additional haemodialysis techniques purported to reduce IDH, we found that acetate-free PHF is associated with a lower BP without increased instability and significantly less deterioration in systemic haemodynamics as compared to low-flux bicarbonate-based dialysis. We observed a non-significant trend towards less IDH during PHF; this difference may have been statistically significant if participant numbers were larger or more sessions studied. We suggest that one possible explanation for the improved haemodynamic response to PHF is the absence of acetate that may be important in maintaining myocardial contractility. Although these results imply that PHF has potential benefits, the lack of clear separation in terms of IDH suggests that PHF is not likely to be the most efficient technique to reduce dialysis induced regional LV dysfunction.

Equally, within a setting of empirical reduction of dialysate sodium, Diacontrol® (DC) did not appear to have any short tem clinical advantage in terms of haemodynamic stability over standard dialysis. In addition, DC was not able to reduce plasma conductivity to as great a degree, with the range of lower conductivity settings limited by software programming on the dialysis monitor.

Mirroring the published literature, we found conflicting results regarding the acute effects of dialysis upon cTnT levels. In one study (chapter 5) we

found no rise in cTnT post dialysis, even in the context of dialysis induced ischaemia. However, in chapter 6 we did see a rise in troponin with standard dialysis, whereas with PHF cTnT levels fell. In addition we also observed higher predialysis cTnT levels in IDH prone patients. The acute rise in cTnT would be consistent with subclinical myocardial damage occurring during the dialysis procedure, but it appears that this is only intermittently detected with blood sampling immediately post dialysis. This may be because cTnT levels may take up to 12hours to rise following an ischaemic insult; therefore the studies that found no difference in pre and post dialysis troponin levels do not refute the development of dialysis induced myocardial cell damage. An alternative, practicable approach when looking for dialysis induced changes in cTnT would be to measure post dialysis cTnT levels at 44hours (i.e. measured at immediately before the subsequent dialysis session). An acute rise in cTnT has been demonstrated in this way following dialysis sessions complicated by IDH, but not following sessions in which the patients remained stable [78].

We have also shown that peritoneal dialysis has significant haemodynamic effects. We observed higher blood pressure, SV and CO during 3.86% glucose dwells compared to 1.36% glucose, Icodextrin and 1.1% amino acid solutions. We also demonstrated that there was a significant degree of hyperglycaemia and hyperinsulinaemia in response to CAPD with hypertonic glucose dialysate, and that his was associated with the acute rise in BP. In addition, a differing haemodynamic response

to conventional and newer biocompatible PD solutions was observed, the cause of which is at present unclear.

Cardiovascular mortality rates are similar between haemodialysis and peritoneal dialysis populations [15], which in part is explained by some risk factors such as LVH and vascular calcification that exist in both groups. However, the two techniques of dialysis have many differences; as such it is not surprising that their short term effects on the cardiovascular system also differ. It is conceivable that the effects that we have described in this thesis attributable to each type of dialysis have the potential to negatively impact on long term cardiovascular risk, but in different ways. HD causes repetitive episodes of subclinical ischaemia that may lead to long term LV dysfunction and cardiac failure. In contrast to HD there was no evidence that the short term haemodynamic changes associated with CAPD led to myocardial stunning. However, CAPD with hypertonic glucose solutions leads to acute rises in BP, hyperglycaemia and hyperinsulinaemia, all of which predict poor cardiovascular outcomes in non dialysis patients. Importantly, we have shown that both dialysis modalities are potentially modifiable to lessen these short term effects. We have demonstrated that improving intradialytic BP and lessening IDH reduces the genesis of LV RWMAs during HD. The adverse haemodynamic and metabolic effects of CAPD are modifiable by using dialysis regimes consisting of low-glucose, biocompatible fluids. Whether modifying these short term effects of dialysis has the potential to improve long term cardiovascular outcomes of dialysis patients remains to be explored.

In addition, we demonstrated that APD exerts significant haemodynamic effects, with rising TPR (possibly signifying a cooling effect) and falling CO over time. We also showed that drain/fill periods are associated with significant change in haemodynamics and BP. We speculate that these changes, in particular the greater number of drain/fill phases as compared to CAPD may have the potential to affect renal perfusion and therefore the rate of decline of RRF. This is potentially important as RRF is not only crucial in maintaining adequacy in many peritoneal dialysis patients, but maintenance of RRF has been shown to affect survival.

In conclusion, the results presented in this thesis show that both haemodialysis and peritoneal dialysis are associated with significant, but differing derangements in blood pressure and systemic haemodynamics. Associated with this, haemodialysis has a negative impact on regional left ventricular function whereas CAPD with hypertonic glucose causes an acute rise in blood pressure, hyperglycaemia and hyperinsulinaemia. In this population that has overwhelmingly high cardiac mortality rates, cardiovascular risk reduction is key to ensuring optimal outcomes. The findings of this thesis suggest several novel, modifiable mechanisms related to the short term effects of dialysis that are potentially implicated in the development of uraemic cardiomyopathy.

12.1 Future work

This thesis explores the short term cardiac and haemodynamic effects of dialysis. However, some of the results are themselves hypothesis-generating and there remain several unanswered questions.

For definitive proof of dialysis induced myocardial stunning, further work is now needed to measure myocardial blood flow in conjunction with LV function. We are currently collaborating with the cardiology group at the Medical Research Council Clinical Sciences Centre, Imperial College London led by Professor Camici in order to measure myocardial blood flow during dialysis. This study combines the gold standard of positron emission tomography to measure myocardial blood flow with echocardiography to measure regional left ventricular function. Standard dialysis are compared Hemocontrol® in this way.

We have also commenced a study to assess the long-term development of heart failure in response to repeated dialysis-induced myocardial ischaemia, and to determine whether a reduction in the frequency of dialysis RWMAs improves long term outcomes in terms of LV systolic function. In addition, this study will also allow characterisation of risk factors that predict the development of dialysis induced ischaemia. In particular, we aim to correlate new RWMAs with cTnT levels immediately before the subsequent dialysis session. Furthermore, we are also

planning studies to assess whether increasing tissue oxygen delivery has a beneficial effect on dialysis induced stunning.

Cooling the dialysate improves IDH and our results show for the first time that it has a beneficial effect on intradialytic regional LV function. Furthermore, cooling the dialysate is possible on all dialysis monitors and is extremely simple to perform. However, concerns regarding unpleasant symptoms of cold have led to the relative under use of this intervention. Further work is therefore required to define the optimal dialysate temperature to maximise potential benefits whilst avoiding excessive thermal symptoms.

It is possible to minimise glucose and GDP exposure in CAPD patients by combining Icodextrin and amino acid based fluids with biocompatible glucose based solutions. This approach theoretically should minimise the adverse changes in BP, glucose and insulin that we have demonstrated and should be studied in terms of longer term cardiovascular risk reduction. It also remains to be clarified as to whether the haemodynamic consequences of APD can accelerate the loss of residual renal function as compared to CAPD, and if so how the technique of APD could be altered (possibly by modulating dialysate temperature) to reduce this.

13. List of abbreviations

ABPM	ambulatory blood pressure monitoring
AFB	acetate free biofiltration
ANOVA	analysis of variance
APD	automated peritoneal dialysis
BFD	biofeedback dialysis; in this thesis this refers exclusively to Hemocontrol®
BIA	bioimpedance analysis
BP	blood pressure
CAPD	continuous ambulatory peritoneal dialysis
CFR	coronary flow reserve
CI	95% confidence intervals
CK	creatinine kinase
CKMB	creatinine kinase MB
CO	cardiac output
CRP	C-reactive protein
cTnI	cardiac troponin I
cTnT	cardiac troponin T
DBP	diastolic blood pressure
DC	Diacontrol®
$DC_{13.5}$	Diacontrol, target plasma conductivity 13.5mS/cm
$DC_{13.6}$	Diacontrol, target plasma conductivity 13.6mS/cm
$DC_{13.7}$	Diacontrol, target plasma conductivity 13.7mS/cm
DC_H	Diacontrol, matched with standard dialysis for end dialysis plasma conductivity 13.7mS/cm
DC_L	Diacontrol, matched with standard dialysis for end dialysis plasma conductivity 13.5mS/cm
DC_M	Diacontrol, matched with standard dialysis for end dialysis plasma conductivity 13.6mS/cm
ECW	extracellular water
EF	ejection fraction
ELISA	enzyme linked immunosorbent assay
ERF	established renal failure, CKD stage 5
GDPs	glucose degradation products
Hb	haemoglobin
HD	haemodialysis; standard bicarbonate haemodialysis
HD_{35}	dialysis using dialysate temperature of 35^0C
HD_{37}	dialysis using dialysate temperature of 37^0C
$HD_{13.2}$	standard haemodialysis, dialysate conductivity 13.2mS/cm
$HD_{13.6}$	standard haemodialysis, dialysate conductivity 13.6mS/cm
$HD_{13.4}$	standard haemodialysis, dialysate conductivity 13.4mS/cm
HD_H	standard haemodialysis, matched with Diacontrol for end dialysis plasma conductivity 13.7mS/cm

HD_L	standard haemodialysis, matched with Diacontrol for end dialysis plasma conductivity 13.5mS/cm
HD_M	standard haemodialysis, matched with Diacontrol for end dialysis plasma conductivity 13.6mS/cm
HDF	haemodiafiltration
HF	haemofiltration
HOMA-IR	homeostasis model of assessment index - insulin resistance
HR	heart rate
HRP	horseradish peroxidase
ICW	intracellular water
IDH	intradialytic hypotension
IDWG	interdialytic weight gain
IL6	interleukin 6
IMB	ionic mass balance
IQR	interquartile range
LA	left atrium
LV	left ventricle
LVH	left ventricular hypertrophy
LVMI	left ventricular mass index
MAP	mean arterial blood pressure
NYHA	New York Heart Association
OR	odds ratio
PD	peritoneal dialysis
PDF_{AA}	1.1% Amino acid based peritoneal dialysis fluid, Nutrineal®
PDF_{BIO}	biocompatible glucose-based peritoneal dialysis fluid, Physioneal®
PDF_{CONV}	conventional glucose-based peritoneal dialysis fluid, Dianeal®
PET	Positron emission tomography
PHF	paired haemodiafiltration
RBV	relative blood volume
RRF	residual renal function
RWMA	left ventricular regional wall motion abnormality
SBP	systolic blood pressure
SD	standard deviation
SE/SEM	standard error of the mean
SF	percentage shortening fraction (measure of regional LV wall motion)
SF_{MEAN}	Mean regional LV motion for all regions
SF_{WMA}	Mean regional LV motion for those regions with new RWMAs
SF-36	short form 36 quality of life questionnaire
SPECT	single photon emission computed tomography
SV	stroke volume
TBW	total body water
TMB	tetramethylbenzydine
TNFα	tumour necrosis factor alpha
TPR	total peripheral resistance
UF	ultrafiltration

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15. Appendices

15.1 Temperature questionnaire

1. Have you felt cold during dialysis in the last two weeks?

Every session	(4)
Sometimes	(3)
Once	(2)
Never	(1)

2. In the last 2 weeks, have you had to use blankets or extra clothes during dialysis to keep warm?

Every session	(4)
Sometimes	(3)
Once	(2)
Never	(1)

3. Have you felt uncomfortable due to symptoms of cold during dialysis in the last 2 weeks?

Every session	(8)
Sometimes	(6)
Once	(4)
Never	(2)

4. In the last 2 weeks, have you shivered during dialysis?

Every session	(8)
Sometimes	(6)
Once	(4)
Never	(2)

5. Overall, in the last 2 weeks whilst on dialysis, have you felt:

Much better than usual
Slightly better than usual
No different from usual
Slightly worse than usual
Much worse than usual *(this question not scored)*

15.2 Dialysis thirst score

Thirst is a problem for me

Never (1) occasionally (2) sometimes (3) quite often (4) very often (5)

I am thirsty during the day

Never occasionally sometimes quite often very often

I am thirsty during the night

Never occasionally sometimes quite often very often

My social life is influenced because of my thirst feelings

Never occasionally sometimes quite often very often

I am thirsty before dialysis

Never occasionally sometimes quite often very often

I am thirsty during dialysis

Never occasionally sometimes quite often very often

I am thirsty after dialysis

Never occasionally sometimes quite often very often

Bots CP, Brand HS, Veerman ECI, et al. Interdialytic weight gain in patients on hemodialysis is associated with dry mouth and thirst. *Kidney Int*. 2004; 66: 1662-1668.