Characterising fluid status, distribution and dynamics in haemodialysis patients to improve fluid management strategies

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

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<u>Abstract</u>

Both the removal of too much and the failure to remove enough fluid during haemodialysis are associated with morbidity and mortality. It has been suggested that bioimpedance measurements using the Body Composition Monitor (BCM) and relative blood volume (RBV) monitoring can improve fluid management. However, both lack a robust evidence base characterising measurements in the clinical setting.

BCM measurements were made in healthy controls to characterise the effect of measuring outside standard conditions and were made alongside RBV measurements in haemodialysis cohorts with well-defined clinical characteristics that were perceived to influence BCM results.

The results suggested that BCM protocols can be flexible regarding measurement paths and timing of measurement to ensure as many patients as possible can benefit from the technology. The observed tendency for patients with high body mass index (BMI) to finish dialysis fluid-depleted does not appear to be related to systematic bias in the BCM models, but to a greater tolerance of fluid removal. The tendency for elderly subjects to have a degree of excess fluid, as measured by BCM, does appear to be associated with changes in tissue hydration linked to sarcopenia. Standard measurements in patients with localised lower-limb oedema can lead to attempts to remove unrealistic fluid volumes and alternative paths can help optimise targets. Feasibility for a trial of interventions to promote fluid mobilisation from oedematous tissue was not demonstrated. Simultaneous use of RBV with BCM suggested that assumptions underlying current RBV shape analysis use for fluid management can be violated and that there is a need for further studies.

BCM can be used across the haemodialysis population, but an understanding of its application in individuals with particular characteristics is essential. RBV provides complementary information to BCM but there is a need for re-evaluation of RBV interpretation, standardisation of measurement categorisation and development of intravascular volume measurements.

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<u>List of abbreviations</u>

A.C. – Alternating current

ADH – Antidiuretic hormone

ANP – Atrial natriuretic protein

ATM – Adipose tissue mass

BCM – Body composition monitor

BIA – Bioimpedance analysis

BIS – Bioimpedance spectroscopy

BIVA – Bioimpedance vector analysis

BCM – Body Composition Monitor

BMI – Body mass index

BNP – Brain natriuretic peptide

C – Fraction of non-conducting material

CBV - Central blood volume

C_m – Cell membrane capacitance

C_S – Stray capacitance

CKD – Chronic Kidney Disease

CSA – Cross sectional area

CT – Computed tomography

D.C. –Direct current

DEXA – Dual energy x-ray absorptiometry

ECF – Extracellular fluid

ECS – Extracellular solids

eGFR – Estimated Glomerular Filtration Rate

F_c – Characteristic frequency

FFM – Fat free mass

FM – Fat mass

GFR - Glomerular filtration rate

H – Height

Ht - Haematocrit

HD – Haemodialysis

ICF – Intracellular fluid

IDH – Intradialytic hypotensive episodes

IVC – Inferior vena cava

IVF - Intravascular fluid

IDFG – Intradialytic fluid gain

IDH – Intradialytic hypotension

IPC - Intermittent pneumatic compression

ISF - Interstitial fluid

LTM – Lean tissue mass

MDRD - Modified diet in renal disease

MRI – Magnetic resonance imaging

MUST - Malnutrition universal screening tool

Na - Sodium

NHS - National Health Service

OH – Overhydration

R – Resistance

R_0 – Resistance at zero frequency

R_∞ - Resistance at infinite frequency

R_I – Resistance of the intracellular fluid compartment

R_E – Resistance of the extracellular compartment

RAS – Renin Angiotensin System

RBV – Relative blood volume

RRT – Renal Replacement Therapy

SBIA - Segmental bioimpedance analysis

SBIS - Segmental bioimpedance spectroscopy

SGA - Subjective global assessment

TBW – Total body water

UF – Ultrafiltration

VA - Vascular access

W - Weight

X - Reactance

Z – Impedance

α – Tissue dispersion constant

τ – Time constant of a capacitor

ω – Angular frequency

Φ – Phase angle

ρ - Resistivity

ρ_a – Apparent resistivity

1 Preface

1.1 Background to the project

The number of people suffering from renal failure is growing as the prevalence of hypertension and diabetes, two of the major risk factors for the condition, increases (Stengel et al., 2003). Currently there are around 60,000 people in the UK who regularly receive renal replacement therapy (RRT) (Shaw et al., 2013). RRT can be through a form of dialysis or a donated kidney. The most commonly used form of dialysis in the UK is haemodialysis, typically involving three sessions of about four hours each week during which blood is removed from the body, treated and returned.

One of the principal functions of the kidneys is the regulation of fluid levels in the body by controlling how excess water is excreted in urine. A healthy kidney will respond to changes in fluid intake and output to maintain levels within a narrow range. In haemodialysis, fluid status is maintained by removing fluid from the blood using a process called ultrafiltration that is controlled by the dialysis machine.

Haemodialysis is burdensome for patients, both practically - the treatment typically takes three half days out of their week - and clinically. The process of repeated fluid accumulation and removal can put a large stress on the heart, and both under- and over-hydration have associated cardiovascular complications (Jaeger and Mehta, 1999). There is a startling increase in the risk of cardiovascular mortality at all ages in patients receiving haemodialysis as compared to the general population. Foley et al. showed that a 30 year old dialysis patient in the USA had the life expectancy of a typical 80 year old (Foley et al., 1998, Wabel et al., 2009). Five-year survival has improved from 41% for patients who started dialysis in 1997 to 53% for those who started in 2007 (Pruthi et al., 2013) but it is still lower than for most common cancers. Reducing cardiovascular risk is considered to be the most important challenges in clinical nephrology today and optimising fluid management is an essential element of meeting this challenge.

Managing how much fluid to remove at each treatment is not trivial. Clinical assessment has remained central to fluid management and there has been little advancement on established indicators of fluid status, such as blood pressure, oedema, cramping and dizziness. However, there is increasing acceptance that the use of

technology – and in particular bioimpedance and relative blood volume (RBV) monitoring - can form a vital part of fluid management (Covic and Onofriescu, 2013, Balter et al., 2015).

Bioimpedance analysis is based on measuring the impedance of the body to small electric currents. The test can give information on fluid volumes in the body (Kyle et al., 2004). Bioimpedance measurements are simple, non-invasive, harmless and inexpensive. The technology has long played a role in fluid assessments of dialysis patients in the research setting, but has not gained acceptance as a routine tool in fluid management. One of the principal reasons has been the need to reference the fluid volumes from these measurements to results in healthy controls, leading to problems in a patient population in which abnormal body composition (particularly low muscle mass) is common. However, recent developments in body composition modelling (Chamney et al., 2007) have allowed the assessment of hydration status with bioimpedance analysis independent of comparisons to controls. A device based upon this new model, the Body Composition Monitor (BCM) (Fresenius Medical Care, Bad Homburg, Germany), has been developed and the fluid information it provides has been well validated against gold standard body composition measurements (Wabel et al., 2009). The ability to quantify over- or under-hydration has allowed easily interpretable bioimpedance data to be obtained by a device that can be used at the bedside with minimal training (Wabel et al., 2007a).

1.2 Unmet health need

It is well established that there is a need to improve fluid management strategies for haemodialysis patients. Current approaches have scarcely developed in decades and there is a growing evidence of the complications associated with current management approaches (Huang et al., 2015). At the same time, there is an increasingly strong case that using BCM to guide populations to fluid status targets can improve outcomes (Covic and Onofriescu, 2013) and a growing realisation of the dangers of excessively reducing plasma volumes (McIntyre, 2010), which reinforces the need for RBV monitoring. Despite this, there remains a great degree of scepticism about the benefits of BCM and RBV as routine tools and there is relatively infrequent use of them as part of standard patient care.

This in part is due to the lack of a strong evidence base for measurement in the clinical setting. For BCM, the standard wrist-ankle protocols cannot always be used, as there is a high prevalence of anatomical and physiological abnormalities in the dialysis population (O'Hare et al., 2003, Ferreira-Filho et al., 2012). There is an unmet need to develop a knowledge base to make BCM measurement more flexible and effective. For RBV, there are inadequate descriptions for the many factors that impact upon results and how these can be managed.

This project was based in a team with over six years' experience of using BCM and RBV as part of routine fluid management and that has undertaken a number of studies to try to address some of the concerns about the devices. The BCM was validated for paediatric use (Lindley et al., 2010). The need for alternative measurement paths was identified in response to the challenge of measuring bilateral amputees from our patient population and the use of hand-to-hand measurements was validated for these individuals (Keane and Lindley, 2015). A strong association between BCM-measured overhydration (OH) and BMI (Lindley et al., 2012) was demonstrated. Finally, a project was undertaken to evaluate the introduction of BCM into a unit that had never used the technology (Lindley et al., 2014) in collaboration with the NHS Technology Adoption Centre, and a guide for implementing BCM in hospital units (NTAC, 2013) developed.

This project aims to develop this work by characterising fluid status measurements across different paths, in healthy controls and haemodialysis patients, to improve our understanding of how best to use the diagnostic information generated by BCM across the dialysis population. It is also evident that there are groups of patients with certain clinical characteristics in which BCM measurements need further validation or characterisation. There is uncertainty about the validity of BCM at extremes of body habitus (Carter et al., 2005) and there is a lack of guidance on how to manage patients with abnormal distribution of fluid, such as those with lower-limb oedema. Combined use of RBV with BCM allows the association between fluid status and intradialytic changes in blood volume to be investigated.

It is hoped that the knowledge generated in this study will facilitate the translation of BCM use from being primarily used in research to a technology that can be used and interpreted by staff on dialysis units. A more individualised approach to making and

interpreting BCM measurements can help to benefit patients across the whole haemodialysis population. An increased knowledge base and wider dissemination of this knowledge will ultimately improve fluid management strategies throughout the NHS and the evidence suggests this will be a significant step towards improved cardiovascular outcomes in this patient group (Wizemann et al., 2009).

1.3 Research questions

Hypothesis: Improving the knowledge base around bioimpedance measurements and their relationship with RBV will allow improved and individualised fluid management strategies that can be applied across the haemodialysis population.

Research question 1: Can characterisation of alternative measurement paths and timing for the BCM, and the use of simultaneous RBV monitoring, lead to more individualised and effective fluid management?

Research question 2: Is the tendency for high BMI haemodialysis patients to finish dialysis fluid depleted according to the BCM associated with an artefact in the mathematical model or with systematic differences in the prescription and delivery of treatment?

Research question 3: Is the presence of malnutrition associated with BCM-measured fluid overload?

Research question 4: (a) Can BCM measurements be used to assess and monitor localised oedema and (b) Can easily administered, non-invasive interventions mobilise persistent localised oedema so that it can be recruited into the circulation?

1.4 Structure of the report

In line with the stated aims of this project, the thesis is structured around an overarching narrative which is underpinned by four specific research questions.

This preface, chapter 1, briefly sets out the rationale behind the work and the hypothesis examined.

The background to the research is then examined in detail in chapter 2. The consequences of kidney failure and the role of renal replacement therapies are explored in the context of fluid homeostasis. Approaches to fluid management in haemodialysis are critiqued and the need for objective measures outlined. Finally, the technical aspects of the work are outlined in chapter 3 in sufficient detail to understand method sections in subsequent chapters.

The four specific research questions will be evaluated individually in chapters 4-7. Each chapter includes an introduction, a methods section, results, discussion, limitations and implications for clinical practice.

The conclusion of the thesis, chapter 8, draws together the findings of each of the studies, puts them into the context of the central aim of the work, looks at the limitations of the studies and considers what further work is needed.

2 Introduction

2.1 Fluid balance

2.1.1 Fluid balance in health

Water is the major constituent of the human body and plays a part in numerous functions, including regulation of oxygen and carbon dioxide, transport of nutrients and waste products, heat regulation and chemical distribution (Tortora and Derrickson, 2009). The body regulates fluid levels primarily through the kidney's ability to control how excess water is excreted in urine.

Due to the important functions relating to waste removal and regulation, the kidneys receive almost 25% of the cardiac output, meaning that in adults around 1200 ml/min of blood can pass through the kidneys (Tortora and Derrickson, 2009). The blood splits into many arteries which supply around two million nephrons, and these form the functional units of the kidneys. Here, the glomeruli create a hydrostatic pressure gradient which forces water and solutes from the blood plasma into the renal tubules. In the tubules, two processes occur which are central to the homeostatic functions of the kidney. Selective reabsorption causes about 99% of the filtered fluid and many useful solutes to return to the blood via reabsorption at the tubule cells (Tortora and Derrickson, 2009). Conversely, tubular secretion acts to remove further waste products into the filtered fluid, which is then collected and passed to the ureter as urine.

There are two main control systems that regulate urine production: osmoreceptors monitor the serum osmolality and baroreceptors monitor intravascular volume. The osmoreceptors are cells in the hypothalamus that monitor changes in extracellular tonicity. As sodium accounts for the majority of extracellular fluid (ECF) tonicity, the cells are essentially monitoring sodium concentration (Verbalis, 2007). Changes in sodium concentration indirectly indicate fluid balance disturbances through the effect of dilution or concentration and the osmoreceptors act to address the disequilibrium. Baroreceptors exist in the right atrium and great veins and monitor intravascular volume via transmural pressure. The numerous receptor points reflect the fact that there can be varying volume status across the vasculature (Louden, 2012). Pressure increases or

decreases indicate hyper- or hypo-volemia respectively and stimulate actions to restore normal pressure.

Antidiuretic hormone (ADH) and the renin-angiotensin-aldosterone system (RAS) provide the principal responses to fluid disturbances. ADH increases the permeability of distal tubules and the collecting duct to increase reabsorption of water without sodium, thereby causing excretion of concentrated urine (Abbrecht, 1980). It is produced in response to hypovolemia-induced reductions in pressure at the baroreceptors and osmoreceptor stimulus from high tonicity. The RAS is triggered in response to stimuli from baroreceptors only. It is a cascade reaction, based on renin secretion by the kidneys, renin-facilitated formation of angiotensin I and angiotensin II and finally the production of aldosterone. The chain of events acts to preserve fluid, by increasing sodium reabsorption in the distal tubular cells of the kidney, increasing plasma osmolality and stimulating ADH release and the thirst mechanism (Abbrecht, 1980).

The different receptors and response mechanisms have quite different characteristics. Plasma osmolality is one of the most tightly controlled parameters in human physiology and the osmoreceptors are very sensitive to small changes in tonicity (Verbalis, 2007), whereas it is not until there is an approximate 10% drop in blood volume that baroreceptors are triggered (Guyton and Hall, 2006). However, the ADH response triggered by the baroreceptors is much stronger and long lasting than that from osmolality changes (Louden, 2012).

2.1.2 Fluid balance in disease

2.1.2.1 Fluid balance in chronic kidney disease

Chronic kidney disease (CKD) is an umbrella term encompassing many different disease processes and is defined by the presence of abnormal kidney structure and/or function. CKD is classified into five stages based on the glomerular filtration rate (GFR), with decreasing GFR indicating worsening CKD.

As kidney function declines, individuals will need to consider a form of RRT. Currently there are nearly 60,000 people in the UK who regularly receive RRT (Rao et al., 2015). RRT can be through a form of dialysis or a donated kidney, with transplanted kidneys

accounting for more than half of the people receiving RRT and haemodialysis being the most commonly used form of dialysis in the UK.

When somebody is receiving RRT, fluid balance is generally managed by the particular RRT technique in addition to residual renal function - where present - which can be augmented by the use of diuretics. In CKD patients who have yet to reach the need for RRT, fluid balance is largely maintained by the remaining function of the kidney with the use of diuretics and sometimes dietary advice to limit fluid intake.

2.1.2.2 Fluid balance in haemodialysis

The kidneys fail in different ways; some haemodialysis patients have sufficient renal function to maintain their hydration close to normal, others pass no urine at all and are dependent on the treatment and their ability to manage their salt and fluid intake to control their fluid status.

Excess fluid tends to accumulate in the ECF, across both the intravascular fluid (IVF) and interstitial fluid (ISF) compartments. Up to a certain point, the fluid will proportionately increase the volume of each compartment, but eventually the blood volume will plateau and the greater compliance of the interstitium means that fluid will almost exclusively accumulate there, manifested as oedema (Guyton and Hall, 2006).

The accumulated fluid is removed using a process called ultrafiltration during haemodialysis. Ultrafiltration for thrice weekly haemodialysis can be equivalent to the whole plasma volume being removed during a four hour session (Thijssen et al., 2013). This puts a huge strain on the cardiovascular system and the body reacts with compensatory mechanisms to try and maintain haemodynamic stability. These mechanisms include vasoconstriction in arterioles and venules to increase systemic vascular resistance and ensure that central blood volume is maintained (Prakash et al., 2002).

Ultrafiltration removes fluid from the blood volume alone, so the excess fluid in the interstitium must be able to pass into the blood before it can be removed, a process called vascular refilling. The capillary walls act as the main barrier between the IVF and

ISF where fluid exchange takes place (Korthuis, 2011). The forces driving the fluid exchange were first described by Starling (Starling, 1896). The net filtration pressure across the capillary wall is the sum of the difference in hydrostatic pressure in the capillary and the interstitium and the difference in oncotic pressure between the capillary and interstitium (Korthuis, 2011). As fluid is removed from the blood volume, there is a reduction in the hydrostatic pressure gradient from the capillaries to the interstitium and, given that plasma proteins are too large to pass through dialyser membranes, an increase in the oncotic pressure gradient across the capillaries (Chamney et al., 1999). Both these changes have the effect of refilling the vascular space with fluid to replace that lost in ultrafiltration.

There are many factors that affect the refilling rate. There are treatment related factors - ultrafiltration rate, plasma sodium concentration, dialysate temperature (Palmer and Henrich, 2008) and patient related factors, including the response of vasoactive substances and vascular tone adjustment, body size and plasma volume, fluid overload, regional blood flow distribution, plasma protein concentration and transcapillary pressure gradient (Schneditz et al., 1992, Santoro et al., 1998). Many of these factors are patient specific and can vary from session to session. This makes it difficult to tailor ultrafiltration rates according to refilling rate and a standard approach to ultrafiltration is often taken across whole patient groups.

Unless refilling from the interstitial volume can compensate for ultrafiltration, blood volume will drop and this can cause intradialytic hypotension (IDH). This is defined as an acute drop in blood pressure; it is a common and serious complication of haemodialysis (van der Sande et al., 2000). The body's response to a drop in blood volume is stimulated by the baroreceptors, since ultrafiltration removes isotonic fluid. However, the pathophysiology of IDH is still not well understood. The body's autonomic response to a drop in blood volume can be compromised in haemodialysis patients, who may have congestive heart failure and diastolic dysfunction, autonomic neuropathy or disturbed acid-base and electrolyte balances (Thijssen et al., 2013). IDH is also related to decreased venous compliance, decreased systemic vascular resistance, decreased stroke volume and cardiac index (Kooman et al., 1992, Converse et al., 1992, Nakamura et al., 1991, Boon et al., 2004), all of which inhibit the body's response to haemodynamic stress. The problems associated with ultrafiltration and IDH can also be

compounded by excessive interdialytic fluid gains (IDFG) - related to salt intake, social drinking, sodium loading during haemodialysis or short haemodialysis sessions – and haemodialysis treatment itself can cause heat accumulation and induce vasodilation in cutaneous blood vessels (Schneditz and Levin, 2001).

Interventions for prevention of IDH include a number of different approaches. These include optimisation of the ultrafiltration rate - such as treatment duration and frequency, ultrafiltration profiles or RBV biofeedback systems - or interventions to improve the cardiovascular response to ultrafiltration and plasma osmolality changes – such as use of midodrine, dialysate temperature changes or dialysate sodium profiling.

As well as the cardiovascular complications linked to fluid removal, more recent work has demonstrated the damaging effects of ultrafiltration related circulatory stress and reductions in perfusion of a number of other end organs, leading to structural and functional abnormalities of the brain (Eldehni and McIntyre, 2012), ischemic damage to the gut resulting in endotoxin translocation into the circulation (McIntyre et al., 2011) and a reduction of residual renal function in the kidney (Hur et al., 2013).

2.1.2.3 Fluid management in haemodialysis

In haemodialysis, the removal of fluid is managed by giving a patient a `target weight'. This is the weight after dialysis that minimises the cardiovascular risks due to fluid overload without causing discomfort or compromising residual renal function. The accumulation of fluid in the body is assessed before each dialysis session using changes in body-weight and the dialysis machine is programmed to restore the patient to their target weight. Routine fluid management of dialysis patients includes dietary advice and the use of antihypertensive medications, but is fundamentally related to the patient's target weight. If this is too high, patients will be systemically overhydrated throughout the interdialytic period (Kuhlmann et al., 2005). If the target weight is too low, patients will be excessively dehydrated and at risk of IDH complications, loss of any residual renal function and increased post-dialysis fatigue (Lindley and Keane, 2014).

Historically, a patient's target weight was defined on the basis of clinical assessments and changed in response to symptoms during dialysis (Jaeger and Mehta, 1999).

Hypertension, puffy tissue or breathlessness can indicate overhydration, whereas hypotension, cramping, dizziness or nausea are frequently used as signs of dehydration. Unfortunately these indicators can result from conditions that are unrelated to hydration and patients can be fluid overloaded with no obvious symptoms. The result is that the management of fluid status can be reduced to a somewhat `trial and error' approach.

The clinical examination has been the cornerstone of fluid management in patients with kidney failure. Yet it is widely accepted as being far from adequate (Ishibe and Peixoto, 2004). Evidence shows that clinical examination has poor sensitivity and specificity in identifying fluid imbalance (McGee et al., 1999, Chung et al., 1987). Part of the problem with the clinical examination is the reliance upon parameters that are indirectly linked to fluid status and affected by other physiological effects. Blood pressure, for example, is a fundamental part of clinical assessments, but has been shown to have very poor correlation with fluid status in this population (Lindley and Keane, 2014). The drive for normalisation of blood pressure as a central aspect of fluid management invariably leads to a focus on reducing target weights to try to reduce fluid overload and blood pressure. This idea is the basis of the 'probing' approach to fluid management, which involves systematic reduction of target weight until the point when the patient experiences symptoms relating to IDH (Canaud and Lertdumrongluk, 2012). This approach would invariably use the term 'dry weight' in place of target weight, stressing the approach of drying patients out as much as possible. While shown to be successful at normalising blood pressure and reducing cardiovascular risk (Charra et al., 1992), this approach clearly puts patients more at risk of the complications relating to excessive fluid-depletion.

A number of novel techniques have been developed to be used in addition to clinical examinations:

- *Lung comets:* When excess fluid accumulates in the lungs, the air-fluid interface at the alveoli produce a pronounced hyperechoic artefact known as a 'comet' on ultrasound assessments. It has been shown that the number of comets observed is directly related to the degree of excess lung water and also to survival, both in patients with lung congestion (Zoccali et al., 2013) and in haemodialysis patients (Siriopol et al., 2013). There is currently a large multinational trial assessing the

effect of using lung comets as a method of fluid management in haemodialysis patients (Zoccali et al., 2011).

- *Biomarkers:* Atrial natriuretic peptide (ANP) is secreted in response to fluid overload as stimulated by increased atrial transmural pressure (Ishibe and Peixoto, 2004) and as such ANP levels can be used as an indicator of fluid status. However, there is significant variability in results (Kouw et al., 1993) and it also has the drawback of not being able to differentiate fluid depletion from normal hydration. There is no evidence that it can be used in clinical practice. Brain natriuretic peptide (BNP) has also been given much attention, particularly in heart failure patients, but suffers from similar limitations as ANP. Both markers have good prognostic power for left ventricular hypertrophy and dysfunction and mortality (Zoccali et al., 2001), but the ability to be used in routine clinical management and improve results is lacking (Nishikimi et al., 2001, Ishizaka et al., 1994).

- *Inferior vena cava diameter:* The intravascular volume can be indirectly assessed by ultrasound measurements of the diameter of the inferior vena cava (IVC), which have been shown to correlate well with central venous pressure. This assessment can also be supplemented by measuring changes in the IVC diameter during deep expiration, known as the collapsibility index. The use of these indices for target weight assessment has been shown to be able to differentiate patients under-hydrated at the end of haemodialysis with those who are normo- or hyper-hydrated (Cheriex et al., 1989). Furthermore, it has been shown that IVC diameter can detect clinically unapparent volume depletion and that using the results to inform target weight changes improved blood pressure and heart rate (Leunissen et al., 1993). However, measurements post-dialysis do not account for fluid redistribution from the extravascular space, so it is important to keep in mind that the technique is based on intravascular fluid, not whole body excess fluid. There are also problems in interpreting IVC diameter in patients with heart failure (Ishibe and Peixoto, 2004).

- *Bioimpedance and blood volume monitoring:* The most established techniques for aiding traditional, clinical examination-based fluid management are bioimpedance and blood volume monitoring. These techniques are considered in detail in sections 2.3 and 2.4.

2.1.2.4 Fluid balance and oedema formation

In some tissues the hydrostatic pressure gradient exceeds the osmotic pressure gradient along the length of the capillary bed, causing a large net fluid loss due to filtration and the need for filtered fluid to be returned to the blood via lymphatics (Taal et al., 2012). Oedema forms when the capillary filtration rate exceeds the lymphatic drainage rate (Topham and Mortimer, 2002). The lower limbs are subject to higher hydrostatic pressures due to gravity, increasing transcapillary filtration into the interstitium (Stick et al., 1993b) and this is one of the reasons that oedema is often found in the lower limbs.

At normal hydration the interstitium is relatively non-compliant such that small increases in ISF will produce large increases in interstitial hydrostatic pressure, opposing further fluid leakage from the IVF to the ISF. However, as the tissue becomes oedematous, the compliance of the ISF drastically increases, allowing it to accommodate significant amounts of excess fluid (Korthuis, 2011). The increased compliance reduces the compensatory hydrostatic pressure increases in the ISF and so reduces the rate of refilling, making oedematous fluid more difficult to remove during a short dialysis session.

Venous and lymphatic function is dependent on the muscle pump for return of fluid from the extremities. The movement of the limbs drives the venous pump and the pressure changes in the tissue provides a rhythmic opening of the lymphatics, increasing lymph transport.

2.2 Body composition assessment
2.2.1 Body composition models

To allow quantitative analysis of body composition it is necessary to generate models that describe relationships between specific body components in a numerical way. The basis of such modelling is that there are properties of the human body that are constant across all individuals. For many years, most research was based on the two compartment model of fat-mass (FM) and fat-free mass (FFM), however these were based on observations on a small, ethnically uniform, male group of individuals (Wang et al., 2008). When it became clear that the assumptions that underpinned this approach were insufficient, more components were added to the models to help to improve the

prediction ability, leading to 6-compartment models based on fat, water, bone mineral, protein, soft-tissue minerals and glycogen (Wang et al., 1998).
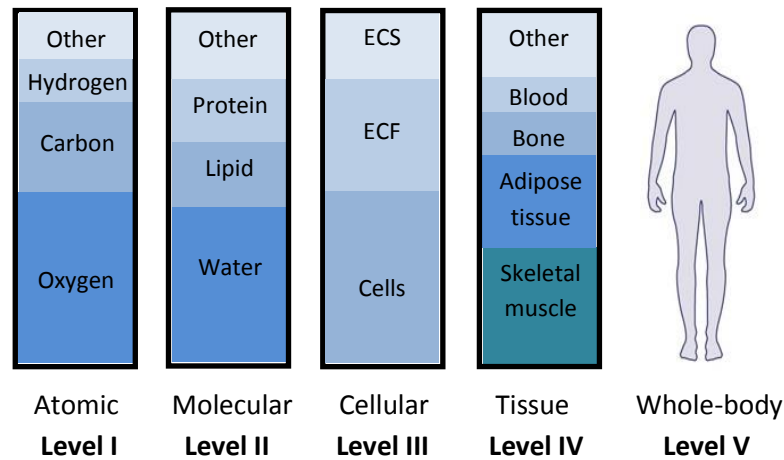
Figure 2-1: Five levels of body composition. ECF is extracellular fluid and ECS is extracellular solids. Adapted from (Wang et al., 1992)

In an attempt to provide a framework for consistent description of multi-compartment body composition models and to facilitate research in the area, Wang described a comprehensive five level model of body composition (Wang et al., 1992). The five levels run from the descriptions at the atomic level through to descriptions of the body as a whole (fig. 2-1) and provide definitions and equations to quantitatively relate different constituents with the unit weight, body weight.

Level I, the atomic level, describes the body in terms of the fundamental building blocks which make it up. Atoms combine together to form thousands of different types of molecules in the body, and the description of these molecules make up level II, the molecular level. Due to the number and complexity of the molecules in the body, it is necessary to categorise them into related molecular species: water, lipid, protein and other. Level III represents the cellular level, which organises molecules into the functional components of the living body, cells. The model describes the body at this level with cells, ECF and extracellular solids. The components of level III are grouped into tissues which make up the tissue-system level, IV. Finally, level V indicates the whole-body level which looks at body size and shape.

The practical application of this framework lies in the ability to quantify the relationship between known compartments that are tightly controlled, measure specific

compartments directly or indirectly and deduce the size of further compartments by simple arithmetic equations for body weight. An example of this is the measurement of FM from a measurement of ECF, using the two compartment model for FM and FFM based on the level II molecular level:

- It is well described that total body water (TBW) is tightly controlled at 73% of FFM

- There are a number of common methods for measuring TBW (e.g. tracer dilution), allowing the estimation of FFM

- The two compartment model defines body weight as the sum of FM and FFM, meaning that from a measure of TBW and body weight, estimates of FM and FFM can be made.

Deciding which body composition level to use in practice is very much dependent on the purpose of the body composition measurement. Level V, whole body, includes some of the simplest and most used assessments such as height, weight and body mass index (BMI). These are often used for population based research and categorisation, but it is well known that BMI, for example, is flawed as a measure of fat due to the fact that individuals of the same sex, height and weight can have markedly different fat and muscle masses. At the other end of the spectrum, level I, atomic level models tend to be applied to sophisticated tests, such as neutron activation, and used primarily in research and as reference measurements (Heymsfield et al., 1997).

2.2.2 Body composition in kidney disease

Because fluid status is frequently perturbed in patients on haemodialysis, many of the body composition models and measurements that are related to fluid status are invalid. If we consider the example described above, excess fluid in haemodialysis patients accumulates in the FFM so the assumption of 73% hydration of FFM is invalid and will cause overestimation of FFM and underestimation of percentage FM.

To overcome this, body composition in haemodialysis was often described using a Level III, cellular approach by measuring ECF and TBW. Target weights could then be reviewed based on reference ranges for ECF normalised by intracellular fluid (ICF) or TBW. However, this approach is limited due to the fact that there are inter-individual

differences in lean and adipose tissue compartments which alter the normalised ECF independent of fluid status.

One approach to account for differences in lean and fat proportions was to consider the volume of excess fluid in a patient as a separate compartment. Kraemer proposed a four compartment model, consisting of the excess fluid, fat, muscle and remaining 'basic' components (Kraemer, 2006). This introduced the concept of normo-hydrated weight - being the weight of the body when all the tissues have normal fractions of ICF and ECF - and overhydration (OH) - which is the fluid volume above or below that of a norm-hydrated subject, for positive and negative values respectively. Chamney et al. refined this idea and proposed a 3-compartment model (Chamney et al., 2007). This model is centred on the concept that, in health (when kidney disease is not present), lean tissue and adipose tissue compartments are normally hydrated and, crucially, they have fixed proportions of ICF and ECF across all individuals. On this basis, it is relatively straightforward to show that simply by measuring ECF and ICF and knowing body weight and height, simultaneous equations can allow the quantification of normally-hydrated lean tissue mass, normally-hydrated adipose tissue mass and OH (for details see (Chamney et al., 2007)).
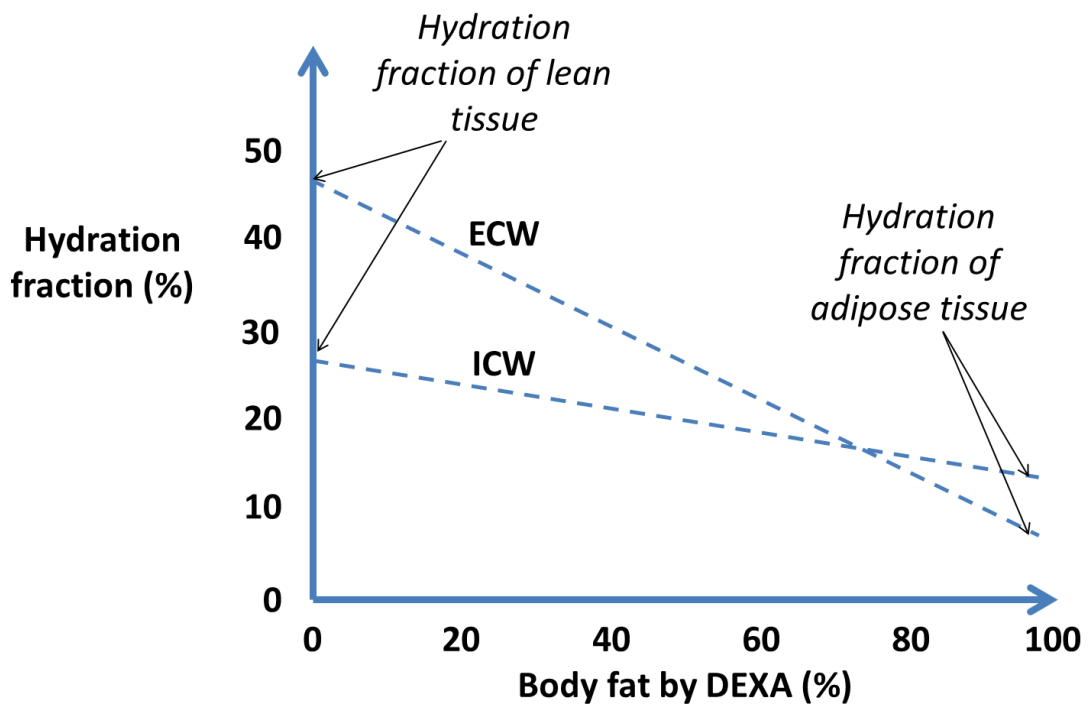
Figure 2-2: Estimation of the fractional hydration of ECF and ICF in lean and adipose tissue, by extrapolation of regression lines based on dilution based fluid volumes and DEXA body fat

This model theoretically allows the measurement of OH over a wide range of body compositions, as long as the assumption about constant fractions of ECF and ICF in the lean and adipose tissue compartments is valid. The values for these fractions were determined experimentally. Simultaneous measurements of DEXA body fat, ECF with bromide dilution and TBW with deuterium dilution (allowing an estimate of ICF by subtraction of ECF) were made. Percentage body fat was plotted against ECF and ICF and a regression line allowed an estimation of the fractional ECF and ICF at the hypothetical states of 0 and 100% body fat - i.e. the percentage ECF and ICF for lean and adipose tissue (see fig.2-2).

The model is commonly used with bioimpedance assessments of ECF and ICF for measuring normo-hydration weight and OH in particular, but also for lean and adipose tissue. There are a number of studies that have attempted to validate the measurements, (Wabel et al., 2009, Wabel et al., 2007b) but, for normo-hydration weight and OH, this is very difficult as no gold-standard exists. There is a growing body of evidence supporting a link between measurements of OH using this model and outcomes (Wizemann et al., 2009, Moissl et al., 2013, Onofriescu et al., 2014), which further supports the clinical validity of the values from the model. However, it must be acknowledged that all measurement techniques have their limitations and even the criteria methods used in the development of this model have a certain degree of uncertainty which will feed into that of the model. This should be considered for each application of the model.

2.2.3 Body composition measurement

Table 2-1 summarises some of the most common body composition measurements (Siri, 1993, Duren et al., 2008, Heymsfield et al., 2005). We can broadly describe them as being direct or indirect. Direct measurements include dilution methods and neutron activation, whereas indirect measurements rely on the results of a direct measurement. By definition, indirect methods will have greater uncertainty due to the inter-individual variance in biological assumptions which underpin the direct or criterion methods that they rely on (Duren et al., 2008). However, it must be noted that no body composition measurements are free from comparison to a reference and, as such, a set of assumptions about the biological conditions of an individual. This biological variability

must be considered alongside the experimental error when evaluating any technique (Siri, 1993).

Technique	Examples	Type	Use	Biological uncertainties	Measurement uncertainties
Anthropometry	Weight; BMI; skinfold thickness	Direct; Indirect	Nutritional status	Population specific; direct measures cannot distinguish fat and lean tissue	Equipment calibration; inter-user variability
Bioimpedance	BIA BIS	Direct; Indirect	Fluid volumes	BIA is population specific. Both BIA/BIS are dependent on assumptions about shape, and resistivity	fluid distribution; electrode positioning
DEXA	Pencil beam; fan-beam	Direct	Fat, lean and bone	Attenuation properties of different tissues in reconstruction algorithms	Beam hardening
Densitometry	Under-water weighing; Air displacement plethysmography	Indirect	Fat mass	Assumes reference body composition for all components except variable adipose tissue	Assumptions on the amount of air in the body; requires compliant subject
Dilution	Deuterium; bromide	Direct	Fluid volumes	Assumptions on tracer kinetics	Assay uncertainty
Whole body counting	^{40}K counting; Neutron activation	Direct	Level I, atomic compartments	Assumptions on ^{40}K abundance	Reliance on highly technical equipment.
Imaging	CT; MRI	Direct	Level IV tissue compartments	Relationships between tissue type and pixel values	Finite resolution; attenuation correction;

Table 2-1: Comparison of different body composition measurements. BIA is bioimpedance analysis, BIS is bioimpedance spectroscopy, DEXA is dual energy x ray absorptiometry, ^{40}K is the isotope potassium-40, CT is computed tomography, MRI is magnetic resonance imaging.

2.3 Bioimpedance

2.3.1 Introduction

Bioimpedance is defined as the ability of biological tissue to impede electric current (Grimnes and Martinsen, 2011) and refers to the measurement of the electrical properties of biological tissue. It is used in an extremely wide range of applications,

from measuring the characteristics of individual cells through to optimising performance in sports science and monitoring body composition and physiology.
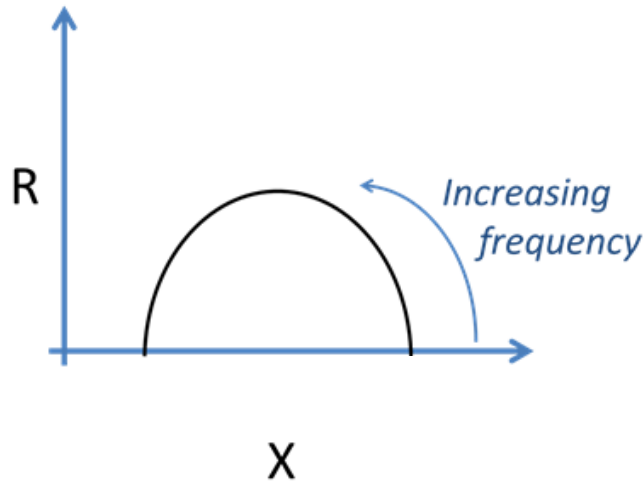
Figure 2-3: Cole-Cole plot

The principle that underpins clinical applications is the relationship between impedance and the volume of fluid - the conducting material - in the tissue under investigation. This relationship can be characterised empirically, allowing estimation of body fluid volumes from a measurement of bioimpedance, and this simple approach has been and remains the basis of most devices. However, as far back as the early twentieth century, pioneering work around the physical and chemical properties of tissue that underpin bioimpedance was underway. Kenneth Cole and his brother Robert investigated the frequency-dependent impedance of capacitively coated spheres in suspension (Cole, 1928) and described the circular arc, now commonly known as a Cole plot (see fig. 2-3), that is found if impedance at different frequencies is plotted on an Argand diagram. As technology improved in the latter part of twentieth century, measurement processes improved and bioimpedance could be applied to body composition analysis. Thomasset developed the concept of differentiating ECF and TBW with needle electrodes by measuring at low and high frequency respectively (Thomasset, 1963), which was then replaced by the less-invasive surface electrode measurement technique (Hoffer et al., 1969).

There is now a bewildering array of bioimpedance analysers available for health-related applications, from cheap devices designed for home monitoring of body composition up to expensive and complex systems for specialist and niche fields.

2.3.2 Electrical properties of tissue

2.3.2.1 Defining bioimpedance

Impedance (Z) is a complex quantity composed of a resistive component (R) and a reactive component (X), as defined by equation 2-1 (where *i* denotes the square root of -1). Following normal mathematical handling of complex numbers, this allows impedance to be displayed graphically on an Argand diagram, where resistance and reactance describe the real and imaginary parts of impedance respectively (fig. 2-4), and allows the calculation of the real terms impedance magnitude (|Z|) and phase difference (φ) based on equations 2-2 and 2-3.
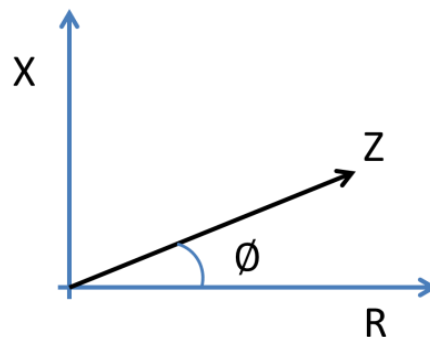
Figure 2-4: Argand diagram showing the relationship between impedance (Z), resistance (R), reactance (X) and phase angle (∅)

Equation 2-1
$$Z = R + iX$$

Equation 2-2
$$|Z| = \sqrt{R^2 + X^2}$$

Equation 2-3
$$\emptyset = tan^{-1}\left(\frac{X}{R}\right)$$

Bioimpedance specifically refers to measuring the impedance of biological tissue. If an electric current is introduced into tissue and the potential difference is measured, the impedance magnitude |Z| can be calculated based on Ohm's law as the ratio of the potential difference and the current. If the phase difference between the current and potential difference is measured, the resistance and reactance can be calculated.

Impedance can equally be described by its inverse term, admittance, which is composed of the real term conductance (how easy it is for a current to flow) and susceptance (how

susceptible a material is to polarisation). These terms can be used to help describe bioimpedance theory, but measurement processes tend to use the term impedance and its constituent terms.

2.3.2.2 Conductive properties of tissue

When a current is introduced to tissue, it is conducted through the body by the movement of ions. At the cellular level, the body can be described as a bath of fluid containing cells. Cell membranes are formed by a phospholipid bilayer which acts as an insulator separating a volume of conducting fluid outside the cell, the ECF, and a volume of conducting fluid inside the cell, the cytoplasm. The arrangement of an insulating layer separating two conductive regions defines a capacitor in an electric circuit. As such, the impedance of the body can be considered as composing of a viscous resistance to ion movement, which is described electrically as a resistance, and from the interaction of ions with cell membranes acting as capacitors, forming the reactive part of impedance.

Tissue	Conductivity (S/m) 1 Hz - 10kHz	1MHz	Φ_{max} at <10 MHz	Anisotropy
Human skin, dry	10^{-7}	10^{-4}	80°	?
Human skin, wet	10^{-5}	10^{-4}	30°	?
Bone	0.01	-	20°	?
Fat	0.02-0.05	0.02-0.05	3°	Small
Lung	0.05-0.4	0.1-0.6	15°	Local
Brain	0.1	0.15	15°	?
Liver	0.2	0.3	5°	?
Muscle	0.05	0.6	30°	Strong
Whole Blood	0.7	0.7	20°	Flow dependent
Urine	0.5	0.5	0°	0
Saline, 9% at 20°C	1.3	1.3	0°	0
Saline, 9% at 37°C	2	2	0°	0

Table 2-2: Typical electrical properties of tissue, data adapted from (Grimnes and Martinsen, 2011). Φmax defines the maximum phase difference

At the tissue level, impedance of the body is extremely non-uniform and anisotropic. Table 2-2 shows typical conductivity values (inversely proportional to resistivity) for different tissues, with corresponding phase angles and the degree of anisotropy displayed. The disparity highlights the significant effect body composition has on any bioimpedance measurement; for example, the conductivity of muscle is around ten

times that of fat. The effects of anisotropy are particularly notable for muscle tissue, which can have values of conductivity up to ten times greater parallel to muscle fibres as compared to perpendicular, dependent on measurement frequency (Epstein and Foster, 1983).

2.3.2.3 Frequency dependence of bioimpedance
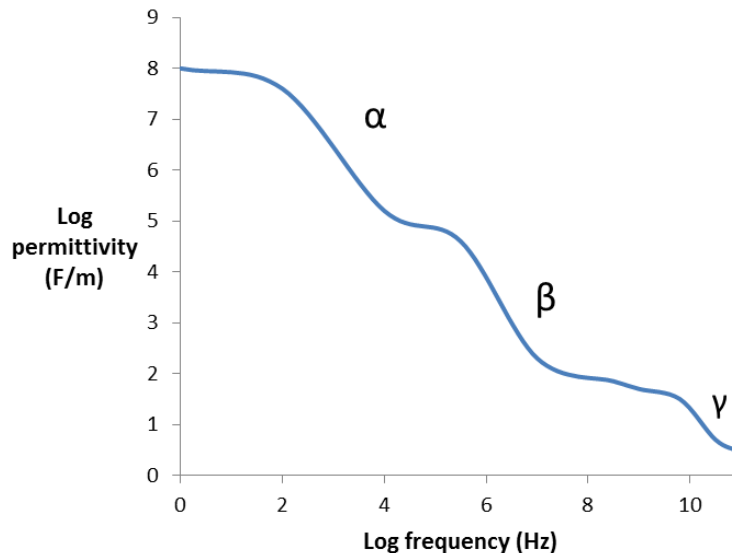
Figure 2-5: Idealised version of the typical frequency dependent permittivity of biological tissue. Adapted from (De Lorenzo et al., 1997)

Bioimpedance is heavily dependent on the frequency of the applied current. This is primarily related to the fact that cell membranes are poor conductors but good capacitors. Zero-frequency current, known as direct current (D.C.) is not able to pass through a dielectric, such as cell membranes, and so the impedance is based solely on the resistive properties of the ECF. Alternating current (A.C.) signals stimulate a changing potential over cell membranes, causing them to store and discharge electric charge, allowing the passage of electric current into the ICF and generating the reactive component of impedance.

Permittivity is a measure of how easily bound charges are moved or polarised in the presence of an electric field and is related to reactance (Dean et al., 2008). Because tissue contains many different charged ions and molecules that have their own response to an alternating electric field, biological tissue displays a frequency dependent distribution of permittivity values known as dispersion (Schwan, 1957). The

characteristics and mechanisms responsible for each region were described by Schwan as alpha, beta, delta and gamma regions (fig. 2-5) (Schwan, 1993). Most bioimpedance measurements are based on the region of beta-dispersion (tens of kHz to 100MHz) where dispersion is due to the cellular structure of tissue and the dielectric properties of cell membranes discussed above. At lower frequencies, in the alpha region, electrode polarisation is very high and the dispersion mechanisms are not well understood. The gamma and delta regions are related to the polarisation of water molecules and do not allow the investigation of cellular level body composition (Dean et al., 2008).
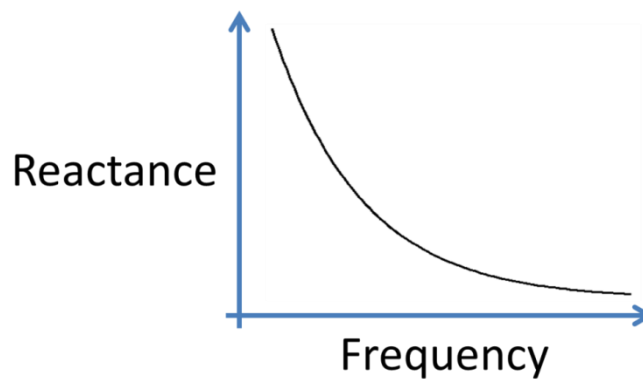
Figure 2-6: The change in reactance with A.C. signal frequency

Within the region of interest to bioimpedance measurements, the relationship between reactance and frequency can be seen in figure 2-6: increasing the frequency reduces the time between discharge of the membranes and the build-up of charge on the membranes which opposes current flow.

2.3.2.4 Cole-Cole model

Kenneth Cole performed pioneering work around the electrical properties of cell membranes. His work looking at the bulk properties of a cell suspension showed empirically that when resistance is plotted against negative reactance over a range of frequencies it produces an arc of a circle with centre below the axis. The depressed centre is due to the fact that cell membranes are imperfect capacitors and also the variation in cell type gives different time constants (Cornish et al., 1993). The equation describing the empirically determined impedance locus, known as the Cole equation, is defined as:

Equation 2-4
$$Z = R_\infty + \frac{R_0 - R_\infty}{1 + (i\omega\tau)^\alpha}$$

Where: R_0 is the resistance at zero frequency; R_∞ is the resistance at infinite frequency; ω is the angular frequency (where $\omega = 2\pi$ x frequency); τ is the time constant - the constant of a capacitive circuit describing the rate of charge accumulation and discharge; and α is a parameter relating to the dispersion characteristics of the tissue.

2.3.2.5 Equivalent circuit of a cell
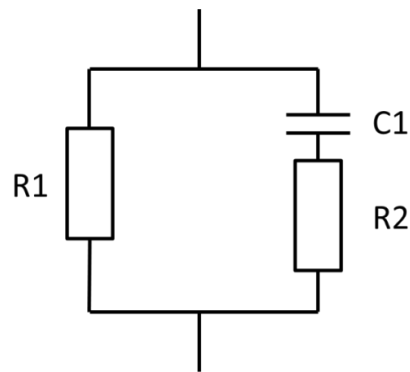
Figure 2-7 Equivalent circuit model of biological tissue. R1 represents the resistance of the ECF, R2 the resistance of the ICF and C1 the capacitance of the cell membranes

Various models have been proposed to simulate the electrical properties of tissue and the empirical observations outlined by Kenneth Cole. The most commonly employed model consists of a parallel circuit with a resistor in one arm and a resistor and capacitor in the other arm, corresponding to the ECF and ICF respectively (fig. 2-7). The response of this modelled circuit with changing frequency describes an arc as predicted by Cole's model

As frequency increases, the reactance of the cell membranes decreases (see fig. 2-5) and the effective conducting volume increases as current penetrates into the cells, reducing the resistance. Reactance is zero for D.C. currents, begins to increase with frequency - as current begins to interact with the cell membranes - before reaching a plateau when this effect is balanced by the reducing absolute reactance. Continued increases in frequency reduce the reactance further until, at infinite frequency, the rate of charge and discharge at the cell membranes becomes so high that the effect of the cell membrane becomes insignificant and the reactance will once more be zero. At this point the current

will pass through the ECF and ICF in proportion to the relative volumes and conductivities of each compartment (De Lorenzo et al., 1997). The intersection of the low frequency impedance loci and the resistance axis is R_0 and represents the resistance of the ECF while the intersection of the high frequency loci and the resistance axis, R_∞, represents the resistance of the TBW (figure 2-8). The frequency at which reactance is a maximum, the peak of the Cole-Cole plot, is known as the characteristic frequency, f_c.

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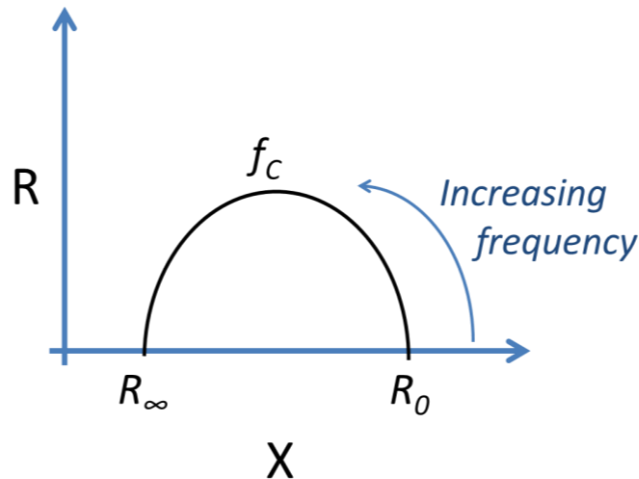
Figure 2-8: **Cole-Cole plot showing the changes in reactance**

This equivalent circuit model of cell properties allows the Cole equation (equation 2-4) to be given in more physiological terms. The measured frequencies, R_0 and R_∞ can be related to compartmental resistances. The resistances of the intracellular and extracellular spaces, R_E and R_I are summed in parallel to get the resistance of the total body water, R_∞. Using the formula for adding parallel resistances, R_∞ can be given in terms of R_E and R_I (equation 2-5).

Equation 2-5: $$R_\infty = \frac{R_E R_I}{R_E + R_I}$$

Equation 2-6 $$\tau = (R_E + R_I)C_m$$

The time constant of the circuit, τ, is given by the cell membrane capacitance, C_m, multiplied by the sum of R_E and R_I (equation 2-6) (Cornish et al., 1993). If equations 2-5 and 2-6 are substituted into equation 2-4, the Cole equation can be re-written in terms of fluid compartment resistances and cell membrane capacitance (equation 2-7).

Because the calculation of R_I incorporates the uncertainty of measuring R_0 and in measuring R_∞, whereas the calculation of R_E is based only on measurement of R_0, there is greater uncertainty associated with R_I and the physiological parameters based upon it.

Equation 2-7 $$Z = \left(\frac{R_e}{R_e + R_i}\right) \times \left(R_i + \frac{R_e}{1 + \left(i\omega C_m(R_e + R_i)\right)^\alpha}\right)$$

2.3.3 Measuring bioimpedance

2.3.3.1 Measurement approach

Clinical applications of bioimpedance can be categorised based on the approach to recording the impedance data. There are temporal measurements which monitor and analyse impedance changes; the primary example of this approach is the use of impedance derivatives measured across the thorax to calculate haemodynamic parameters such as cardiac output (Moshkovitz et al., 2004). Alternatively, electrical impedance tomography uses bioimpedance as an imaging tool, for example in the assessment of lung volume and ventilation (Kotre, 1997). By attaching electrodes around the circumference of a body segment and applying currents between different poles, tomographic images based on differences in conductivity can be formed.

For assessment of body composition, instantaneous bioimpedance measurements are made across a single path. Within this field of measurement, there are many different approaches, based on the different anatomical regions of investigation, different properties of the applied signal and different methods of analysis of the data obtained.

2.3.3.2 Measurement site

For all non-implantable bioimpedance devices, surface electrodes on the skin are used to provide a conducting path to the tissues. 'Bi-polar' arrangements refer to measurement between two single electrodes which are used to inject current and measure the potential drop. 'Tetrapolar' arrangements refer to measurements in which two pairs of electrodes are used, one pair to inject current and one pair to measure the potential drop (fig. 2-9). Tetrapolar arrangements have an advantage in that the separation allows a more uniform current density in the region of the measurement electrode (Khan et al., 2005).
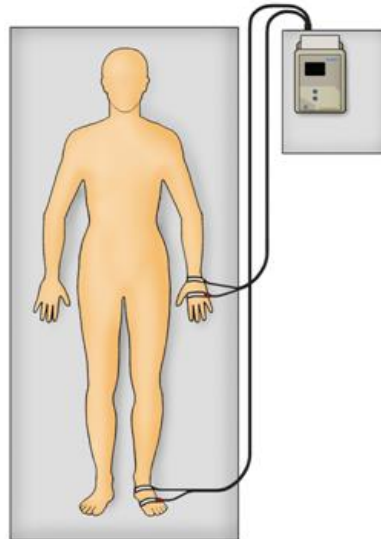
Figure 2-9: Standard tetrapolar electrode arrangement for a whole body bioimpedance measurement between wrist and ankle. (Used with permission from LTHT Medical Illustration).

The positioning of the electrodes defines the region of the body to be measured. 'Whole-body' techniques indicate the extrapolation of information based on certain segments of the body to generate information on whole-body composition. This is most commonly based on measurement between wrist and ankle (fig. 2-9), but can also be measured between each wrist or between each ankle. The use of foot to foot measurements are common in commercial devices designed for home use; pressure contact electrodes on foot pads can be incorporated into bathroom scales to allow a body composition measurement by simply standing on the device (Khan et al., 2005). Similarly, the practical ease of measuring from hand to hand has led to the development of cheap devices that have plate electrodes built into handles on a device which can be held with outstretched arms for a simple measurement.

Whole-body measurements do have limitations. Modelling the body as a series of cylinders (see chapter 2.4.4.1) is based on the knowledge of the length of these cylinders, but for practical ease height is universally used as a surrogate for the circuit length. This is sometimes corrected for by a factor which uses standard anthropometric ratios (De Lorenzo et al., 1997), but will add some uncertainty into results. Also, the trunk only accounts for 10% of impedance of the body despite accounting for 50% of body mass (Kyle et al., 2004), whereas the forearm only accounts for about 1% of body mass yet contributes 25% to whole body impedance (Fuller and Elia, 1989). This means

that changes in body composition in the trunk will not be well reflected in whole-body measurements, whereas changes in the arm and leg will be overly reflected in whole-body parameters.

These limitations can be avoided by use of segmental bioimpedance, which allows the isolation of one or more segments for measurement. This can give information about one segment alone or several segments can be measured individually and summed together to give an equivalent estimate to whole body approaches (Zhu et al., 1999). Avoiding some of the limitations of whole-body measurements, segmental approaches generally give a more precise estimation of the properties of each segment and can allow the assessment of regional fluid distributional changes during haemodialysis. Electrodes can be positioned at the segment boundaries, with measurements of the distance between them defining path length, or alternatively using standard whole-body electrode placements on both sides of the body (8-electrode technique) and making variations on which electrodes are used for current and voltage (Gibson et al., 2008). This, in effect, isolates individual regions of tissue. However, standardisation of electrode positioning remains a concern in all segmental approaches (Lorenzo and Andreoli, 2003).

2.3.3.3 Measurement signal

The measurement of impedance is dependent on the interaction between a current passing through the tissue under investigation and the voltage measured across this tissue. Measurements can be based on applying a voltage and measuring current, or applying a current and measuring voltage change. Bioimpedance devices tend to use a current with amplitudes in the range of 20 µA to 10mA which is below minimum levels of harm (Khan et al., 2005). A high input impedance is necessary to avoid effects due to the skin impedance and the variable impedance at the electrode-skin interface due to different contact pressures (Khan et al., 2005).

The frequency of the applied signal has a significant effect on what information can be inferred from a test. Low frequency signals are restricted to the ECF while high frequency signals pass through both ICF and ECF. By increasing the number of measurement frequencies, it becomes possible to get more information on differences in

ECF and ICF. Bioimpedance devices can be based on measurements at a single frequency, dual-frequency or multi-frequency.

Single frequency: Given that measurements at zero frequency relate to the ECF only while at infinite frequency they relate to the TBW, most single frequency devices use a frequency somewhere in between in an attempt to make a measurement including ECF and ICF. Hoffer showed that the resistance index (height squared over resistance; H^2/R) using 100 kHz measurements is proportional to TBW (Hoffer et al., 1969) and this proportional relationship is the basis of most regression based prediction equations used for single frequency devices.

Often 50 kHz is used as the measurement frequency, as it is usually close to the characteristic frequency where the reactive component is at a maximum. However, in reality, f_c can have quite a range of values – usually between 30 and 60 kHz but including up to 130 kHz in healthy adults (Stahn et al., 2012). At 50kHz, the signal does not fully penetrate the cell and so the device cannot truly express the resistance of the ICV (Kyle et al., 2004). However, the high correlations between ECF, ICF and TBW mean that relationships between impedance and any of these volumes can be defined and used for analysis (Matthie et al., 1998). This means that in theory, any frequency in the beta dispersion range could be used to predict ECF, ICF and TBW.

Dual-frequency: The dual frequency approach acknowledges the fact that the resistivity of the ICF and the ECF are different and the two volumes can be differentiated by measuring at high and low frequency based on the parallel circuit model. Typically, the relationship between resistance index and fluid volume is used separately for the calculation of ICF and ECF.

Multi-frequency: To fully characterise the impedance spectrum it is necessary to measure at many frequencies and this approach is known as bioimpedance spectroscopy (BIS). Typically, a sufficient number of measurements are made across the beta dispersion range to allow a Cole-Cole plot to be fitted to the data and allow the extrapolation of R_∞ and R_0 from an impedance locus. For the BCM, measurements are made at 50 frequencies.

2.3.4 Analysing bioimpedance data

2.3.4.1 Fundamental concepts of analysis

The resistance of an object of uniform resistivity is related to its shape and ability to carry charge. It can be described using a length (L), cross sectional area (A) and resistivity (ρ) by equation 2-8, and knowing that the volume of a cylinder is area multiplied by length we can generate an equation to relate the volume of conducting material to resistance (equation 2-9) (Kyle et al., 2004). This relationship underpins the relationships between bio-electrical and physiological parameters.

Equation 2-8
$$R = \frac{\rho \times L}{A}$$

Equation 2-9
$$V = \frac{\rho \times L^2}{R}$$

This relationship, along with the fact that FM and fat free mass (FFM) have very different electrolyte content and resistivities (Kushner and Schoeller, 1986), makes it relatively simple to generate some equations to estimate body composition compartments from bioimpedance measurements. However, these concepts are underpinned by a number of assumptions which are important for a good understanding of the limitations of bioimpedance.
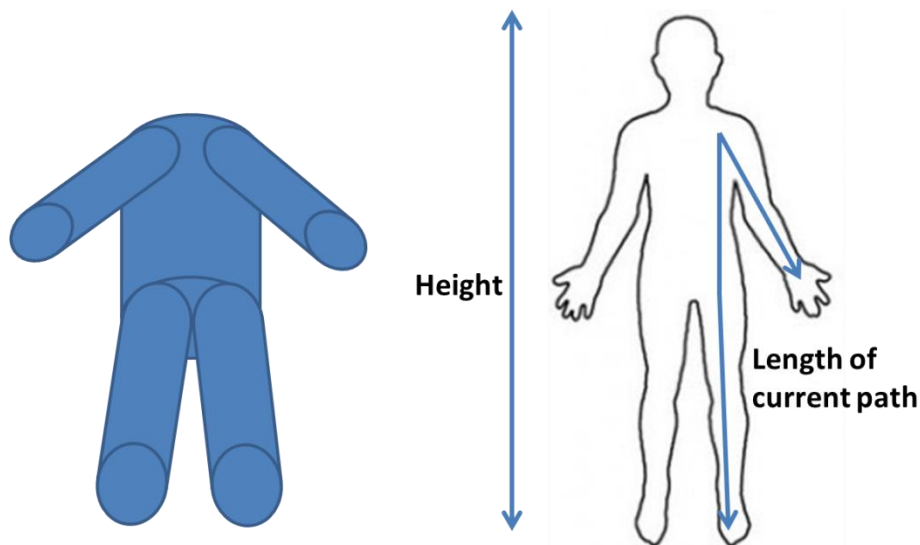
Figure 2-10: For many approaches to analysing bioimpedance, the body is assumed to consist of a series of cylinders (left) and in whole-body measurements, height is generally used as a surrogate for the length of conducting material

Geometry: Equation 2-8 is based upon measurement of a cylinder of uniform conducting material. For most approaches to analysing bioimpedance, the human body is assumed to comprise five cylinders: two arms, two legs and the trunk (fig. 2-10). The length of the tissue under investigation tends to be estimated by height for whole body measurements and can be measured directly for segmental measurements. Also, in reality, the limbs are not cylinders but more like a series of truncated cones and the disparity between limb and trunk CSA means that, for whole body measurements, the majority of the impedance comes from the limbs and little for the trunk (section 2.1.3.2).

Models for whole body bioimpedance expressly, or as part of the predictive equations, will account for these geometrical factors. This is based on estimating the effects on a "standard person" or as part of a reference population. Van Loan et al. calculated a factor, k_b, for BIS measurements to account for differences in shape of human body segments from cylinders (Van Loan et al., 1993). This was done by anatomical measurements of length and circumference of limbs and corrected the relationship in equation 2-8 to give rise to a modified equation:

Equation 2-10 $$V = \frac{k_b \rho h^2}{R}$$

Here, K_b is a constant at 4.3 and h is the height of the subject. However, for any individual, this approach is only valid for proportional changes in geometry as compared to the reference. If there is the case of highly localised changes, inaccuracies will be introduced into the results. Examples of localised changes include abnormal limb lengths with reference to height, or preferential build-up of fat in the abdomen (increasing the CSA of trunk but not limbs).

Resistivity: The value of ρ used for analysis tends to come from empirical estimates, but will have some variability and, as such, will impact upon the accuracy of body composition parameters. Different values have been put forward for sex-specific ECF and ICF resistivities (Van Loan et al., 1993, De Lorenzo et al., 1997), which could be explained by differences in limb shape and composition between sexes. The resistivity

of ECF is close to that of saline, but ρ for the ICF is dependent on the type of cell and is estimated with less certainty (Jaffrin and Morel, 2008).

It is also assumed that the tissue under investigation is a uniform body of conducting material, which is clearly not the case for the human body. Hanai looked at how resistivity of a fluid changed when it contains non-conducting spheres, due to the fact that current must flow around these spheres rather than travelling directly through the fluid (Hanai, 1968). This work, known as "mixture effects", showed that if spheres take up a fractional volume of C then the overall resistivity of the fluid is described by equation 2-11.

Equation 2-11 $$\boldsymbol{\rho_a = \frac{\rho}{(1-C)^{3/2}}}$$

Where ρ_a is the apparent resistivity, accounting for mixture effects, and c is the fraction of non-conducting material. Applying this theory to bioimpedance, cells and other bodies are modelled as non-conducting spheres in a bath of fluid. However, section 2.1.2.5 shows that the volume of conducting material is dependent on the frequency: at low frequencies the conducting volume is the ECF and the non-conductors are the cells containing ICF and other bodies; whereas at high frequencies the conducting volume is the TBW - that is ECF plus ICF - and the non-conductors are limited to the other bodies in this volume (De Lorenzo et al., 1997).

Body composition also has a significant effect on resistivity due to the highly insulating properties of body fat. Gender (Kyle et al., 2001), age (Tengvall et al., 2009) and ethnicity (Deurenberg et al., 2002) are also associated with differences in FFM/FM ratios which will affect the resistivity. Zhu et al showed that resistivity is different between segments, which is likely related to differences in fat and fat-free mass content of the segments (Zhu et al., 2006).

2.3.4.2 Bioimpedance analysis

Bioimpedance analysis (BIA) generally refers to analysing single or dual-frequency measurements using regression equations that relate impedance to a physiological

parameter based on data obtained from a control population. This technique makes use of the principle described in equation 2-9 which links the volume of a conducting material, to its length and, resistance and resistivity. A regression equation is generated from gold standard measures of fluid volumes in a population alongside bioimpedance measures of the resistance of the body at a specific frequency (e.g. equation 2-12 (Cox-Reijven and Soeters, 2000)).

Equation 2-12
$$TBW = 0.08 + \frac{0.458 \times h^2}{R} + 0.06 \times w$$

Here, *h* is height and *w* is weight.

Because of the high intercorrelation between ECF, ICF and TBW it is possible to generate equations to predict any of these volumes from an impedance measurement (De Lorenzo et al., 1997). However, simple regression is very much affected by population factors, so equations are population specific. Many propose different equations for males and females but it is not common to have different equations for other important characteristics that vary between populations. Caution is warranted in the use of these equations in altered body composition or fluid status.

2.3.4.3 Bioimpedance spectroscopy

By making use of some of the known electrical properties of tissue, analysis techniques have been developed that have a stronger theoretical basis than BIA techniques. Bioimpedance spectroscopy (BIS) refers to making impedance measurements at many frequencies, allowing an approximation of the Cole plot and estimation of the Cole parameters, in particular R_E and R_I. The most common approach to extrapolating the measured impedances to obtain the Cole parameters is to use non-linear least squares iterative fitting to generate a Cole plot based on the measured data.

The measured data will often have artefacts due to stray capacitances that are particularly noticeable at high frequencies and interfere with the analysis of the data. This effect had been attributed to the time delay introduced from the measurement cables and a function was introduced to the Cole equation on this basis (De Lorenzo et al., 1997). More recently, it has been proposed that the time delay compensation does

not adequately explain or correct this effect and a function based on correcting for stray capacitances has been proposed (Buendia et al., 2010). This is the basis of the term used in the BCM (equation 2-13), which is multiplied by the Cole equation (described in section 3.2). ω is the angular frequency and C_s is the stray capacitance.

Equation 2-13: $$e^{-i\omega C_s}$$

Knowing R_E and R_I it is possible to calculate each volume directly if we know the resistivity of the two compartments, corrected for Hanai's mixture theory. Calculation of ICF is less accurate than ECF, due to the accumulation of errors in estimating R_0 and R_∞ for estimation of ICF and the greater uncertainty in ICF resistivity compared to ECF.

More simply, R_E and R_I can be used as indicators of the relative amount of ECF and ICF without the need for any volume equations and all the assumptions that they involve (Chanchairujira and Mehta, 2005). This does however mean that the problems in differentiating body composition and fluid status associated with BIA must be considered here also.

2.3.4.4 Bioimpedance vector analysis

The limitations of using empirically based regression equations (as in BIA), or complex models (as in BIS) are avoided in bioimpedance vector analysis (BIVA). This is a single frequency technique, typically measured at 50 kHz using a whole-body approach. Rather than attempting to convert the impedance data into body composition parameters, the raw measured resistance and reactance values, normalised to height, are plotted as bivariate vectors on a graph (figure 2-11). The graphs contain reference ellipses at 50%, 75% and 95% based on gender and sex specific reference populations (Piccoli et al., 1994, Piccoli et al., 2002). The vector of an individual can be compared to the normalised plot and a judgement made relating to fluid and nutritional status.

The advantage of this method is that it relies on few assumptions about the underlying basis of impedance and the bio-electrical properties of tissue, but adopts a simple, empirically-based, graphical method that can be used to indicate abnormal hydration status and monitor changes in hydration status as the vector moves. However, the

method relies on reference data that are population specific and, similar to BIA, it is not able to differentiate fluid status and body composition. For example, a vector below the bottom left of the ellipse could equally be a normally hydrated obese individual or a muscular person with fluid overload.
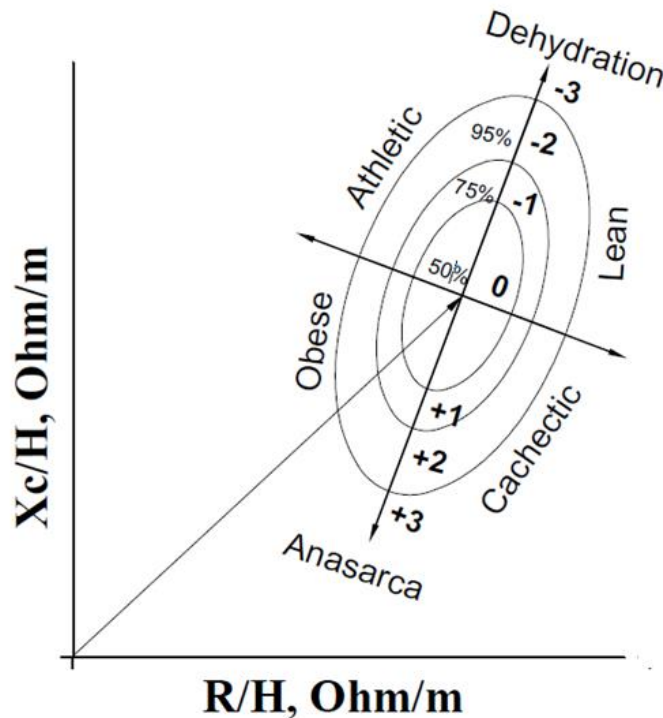
Figure 2-11: 'Piccoli' nomogram showing the bivariate distribution of the impedance vector, displayed as height normalised R and X values, in a reference population. Used with permission: Piccoli A, Pastori G: Department of Medical and Surgical Sciences, University of Padova, Padova, Italy.

2.3.4.5 Phase angle

Phase angle is one of the raw electrical parameters generated in a bioimpedance measurement and so, as with BIVA, it is free from assumptions and models inherent in the more complex analyses. By definition, phase angle is a measure of the lag between current and voltage, introduced by the storage of electric charge in cell membranes due to their dielectric properties. This makes the parameter inextricably linked to the reactance and also to the body cell mass - the intracellular mass of the body - and has been proposed as a useful marker of nutrition (Małecka-Massalska et al., 2015).
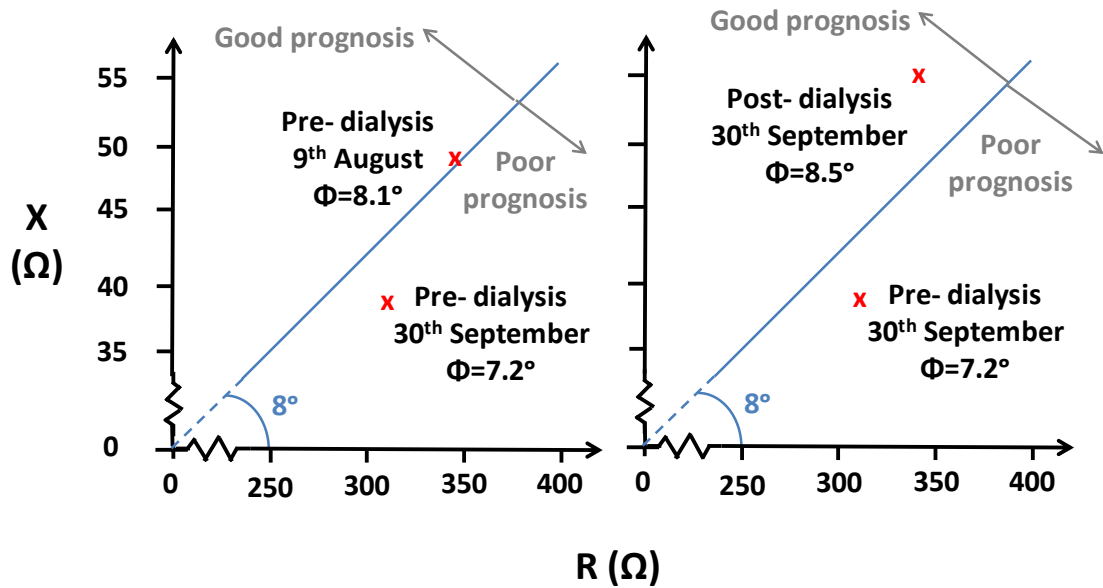
Figure 2-12: The effect of fluid status and fluid management on the ability of phase angle as a marker of cellular health or nutrition. The graph on the left shows a single patient with pre-dialysis BCM measurements less than two months apart. The graph on the right shows the same patient with BCM measurements pre- and post-dialysis on the same day. The line of identity defines the phase angle of 8°, proposed as an indicator of poor survival (Abad et al., 2011)

However, because the reactance is not only dependent on the body cell mass, but also a function of the ECF (the greater the ECF compartment, the less likely current will interact with membranes), direct interpretation of the parameter can be confounded by variability in the ECF compartment (Baumgartner et al., 1988). Figure 2-12 displays data from measurements on one patient plotted on the same R-X graph that is used to display a Cole-Cole plot. The plot on the left shows a measurement on the 30th September where OH was -0.9 litres pre-dialysis, suggesting an excessively low target weight. This was increased and a repeat measurement made less than two months later, when OH was 3.8 litres. There was no suggestion that nutritional state had changed and the move from a region of good prognosis to poor prognosis is likely to be entirely related to the way target weight was set. The plot on the right shows the same patient measured pre- and post-dialysis, clearly showing the change in phase angle over a four hour dialysis treatment that is due solely to fluid removal, taking the individual from the poor prognosis region to the good prognosis region.

2.3.5 The use of bioimpedance in haemodialysis

2.3.5.1 Introduction

The sensitivity of bioimpedance to changes in fluid status and the need for objective measurements to guide fluid management in haemodialysis have inevitably led to a large body of work assessing the use of bioimpedance in haemodialysis. This has predominantly been confined to the research setting and had gained little acceptance as a routine tool in fluid management. Historically, one of the principal reasons for this has been the need to reference the fluid volumes from these measurements to results in healthy controls - leading to problems in a patient population in which abnormal body composition (particularly low muscle mass) is common.

2.3.5.2 Historical overview

Early attempts at utilising bioimpedance for fluid management were based on measuring ECF using BIA volumes and comparing them to matched controls with normal kidney function. Increased ECF indicated a degree of fluid overload (Kouw et al., 1993). This approach was refined by Chamney et al. who used BIS to measure ECF in a group of normally hydrated controls and used the results to define normal ECF volumes as a proportion of body weight (Chamney et al., 2002). This relationship was used to define the dry weight as the point at which a subject's ECF intersected the slope defined by normality (fig. 2-13). However both of these approaches neglect to account for differences in body composition, which can mean two individuals of the same ECF/weight ratio can have wide range of fluid status, due to different lean tissue and fat tissue combinations.

Lopot took a similar approach by considering hydration state to be described by an ECF/TBW ratio (Lopot et al., 2002). He described the association between ECF/TBW and age due to increased body fat percentage with ageing and suggested the use of an age normalised ratio for patient management. Although this did account for some population based confounding factors, the confounding due to subject specific body composition was still not addressed.
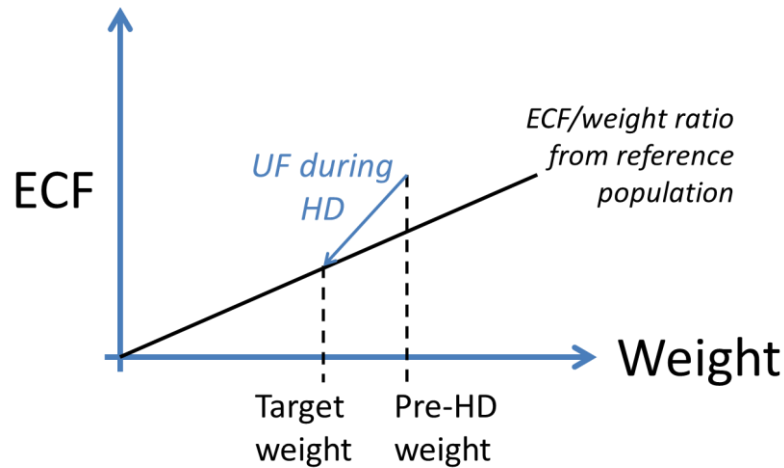
Figure 2-13: Objective definition of target weight based on BIS measured ECF and the idealised ECF/weight relationship based on a reference population. Adapted from (Chamney et al., 2002).

This problem was resolved by re-thinking body composition models for patients who have altered fluid status (see section 2.2.2). The model developed by Chamney et al. has allowed the assessment of hydration status with bioimpedance analysis independent of comparisons to controls and population normalised body composition (Chamney et al., 2007). A bioimpedance device based upon this new model, the Body Composition Monitor (BCM) (Fresenius Medical Care, Bad Homburg, Germany), has been developed and the fluid information it provides has been well validated against gold standard body composition measurements (Wabel et al., 2009).

Segmental bioimpedance techniques are also being developed to avoid many of the problems relating to geometry. Zhu and colleagues have outlined in a series of papers that the use of bioimpedance measurements of the calf can offer a more accurate method of measuring fluid volumes (Zhu et al., 2006, Zhu et al., 1999), but it is not clear yet how this can be applied in routine fluid management.

2.3.5.3 Current applications and consensus opinions

The development of more appropriate models for analysis and devices with better specifications have led, for the first time, to a more widespread acceptability by clinicians of the technology and better engagement with bioimpedance as a routine part of fluid management strategies (Covic and Onofriescu, 2013). A number of studies have produced data to support this approach. Moissl et al performed a cohort study that

attempted to optimise fluid status with the BCM over a three month period and showed improved fluid status, quality of life, blood pressure and symptoms (Moissl et al., 2013). In addition, three randomised controlled trials have been performed using BCM to guide fluid management which have shown improvements in hard end points such as mortality, hospitalisation and blood pressure (Onofriescu et al., 2014, Hur et al., 2013, Onofriescu et al., 2012). Despite this, the BCM device is not universally accepted and is still used much more in research than in patient management. The BCM has been available in the UK since 2008 but very few centres are using it. Anecdotally, this is often because units given a device for evaluation have considered the results to be unrealistic, probably due to lack of training in the identification of poor readings or the surprisingly degree of dehydration that is often observed in large patients.

2.4 Blood volume monitoring

2.4.1 Introduction

Being the accessible compartment used for haemodialysis, the blood volume is acutely reduced during a haemodialysis session and the ability of the body to respond to this and maintain the blood volume is a key determinant of achieving good fluid balance. It is therefore no surprise that measurement and monitoring of the blood volume during haemodialysis is used widely to aid fluid management strategies.

2.4.2 Measurement of blood volume

Measurement of absolute blood volume is not simple. Tracer dilution is established as the gold standard but is not suitable for routine assessment (Puri et al., 2014). More recently, a method has been developed for absolute blood volume measurement in haemodialysis patients based on measuring changes in haematocrit (Hct) after administration of a bolus of saline (Kron et al., 2014, Schneditz et al., 2014). The simplicity of the measurement and the fact that the technology is now readily available on dialysis machines suggests this could allow widespread assessment of absolute blood volume, but further work is needed to look at the clinical significance and utility of these measurements.

However, the assessment of RBV during haemodialysis is an established technique. RBV is defined as the instantaneous blood volume at a specified time during a dialysis

session, expressed as a percentage of the blood volume at the commencement of treatment.

If we consider any element of the blood volume that is constant throughout haemodialysis, the concentration at the start of treatment (C_0) and at time t (C_t) can be used to calculate the RBV at time t as a percentage:

Equation 2-14
$$RBV_t = \frac{C_0}{C_t} - 1$$

A number of different parameters have been proposed as suitable for monitoring, including haemoglobin (Hb), Hct, plasma proteins and plasma conductivity (Santoro et al., 1996). The apparatus for measuring Hb and Hct tend to use optical absorbance or transmission based on the Lambert-Beer law (Santoro et al., 1996), whereas for total plasma proteins, measurement is based on the speed of ultrasound in blood (Schneditz et al., 1990, Johner et al., 1998).

2.4.3 Principles of measurement and common artefacts

The measurement of RBV is based on the concept that the degree of haemoconcentration reflects the change in blood volume. There are two fundamental assumptions that underpin this technique: that the amount of substance being measured is constant throughout the measurement session; and that there is uniform mixing throughout the blood volume, or that the distribution of the substance is constant throughout the session (Dasselaar et al., 2005). These assumptions need some consideration.

The main factors that can affect the amount of the substance being measured are haemolysis, blood leaks or blood transfusion during haemodialysis, although these are not common. However, the distribution of red blood cells and plasma proteins during haemodialysis is a more complicated and variable issue. The concentration of Hb in the capillaries is lower than in larger blood vessels – referred to as the Fahraeus effect (Goldsmith et al., 1989) – which means that the venous or arterial Hct measured by RBV is higher than whole-body Hct (Chapdelaine et al., 2011). The difference in these two Hct values is known as the F-cell ratio. During ultrafiltration it has been shown that

the F-cell ratio rises due to fluid shifts from the microcirculation to the macrocirculation, which is thought to be a compensatory mechanism to maintain central blood volume (Mitra et al., 2004). This causes a reduction in measured Hct and increase in RBV, such that most commonly used devices overestimate change in RBV at modest changes and underestimate it at large RBV changes (Chapdelaine et al., 2011).

There are other redistribution effects that are important to consider. Postural changes from supine to standing can cause fluid to shift from the circulation to the interstitium due to gravitational forces with an associated rise in whole-body Hct. However the haemoconcentrated blood in the limbs is relatively slow in mixing such that measured increase in Hct is underestimated (Lundvall and Bjerkhoel, 1995). Furthermore, if there is a rapid return to supine position the haemoconcentrated blood is rapidly mixed, increasing systemic Hct despite the increase in blood volume, before reducing as refilling from the interstitium occurs (Lundvall and Bjerkhoel, 1995). Eating causes blood to be moved from the central circulation to the splanchnic bed, where the Hct is lower, causing a drop in measured RBV (Dasselaar et al., 2005). Despite that this effect is redistribution rather than a true drop in blood volume, the shift away from the central circulation can lead to IDH and this would be independent of vascular refilling. A large proportion of the blood volume resides in the splanchnic and splenic vascular beds and this forms a reserve which is used to buffer changes in blood volume in the central circulation (Ribitsch et al., 2015). There has been a demonstrated link between norepinephrine and reduced RBV, but it was unclear whether this was related to increased plasma protein content or reduced plasma water (Nette et al., 2006).

2.4.4 Use of RBV in haemodialysis

The appeal of RBV monitoring in HD is the potential to identify IDH before it occurs, thereby reducing the number of events and associated adverse effects. In theory this should also aid setting more appropriate target weights (Lopot et al., 1996).

Interpretation of RBV data can largely be split into analysis of the magnitude of RBV changes during haemodialysis and the shape of the RBV curve. Typically, the magnitude of RBV changes is defined in a number of ways, be it the absolute change in RBV over a single session, or the change over a period of the treatment. Alternatively,

Lopot et al identified very early in the use of RBV that the shape of a curve had diagnostic significance (Lopot et al., 2007) (figure 2-14). When plotting RBV against time over a single treatment, curves that were relatively flat were assumed to indicate plasma refill rates exceeding ultrafiltration rates, suggesting the presence of fluid overload. Curves with a linear decrease in RBV, or a period with a flat-line followed by a linear decrease were interpreted as being close to dry weight. A fourth characteristic curve shows an early linear decrease followed by a period with a flat-line. This is not well explained but must be due to redistribution as the decline can be much higher than the ultrafiltration rate.
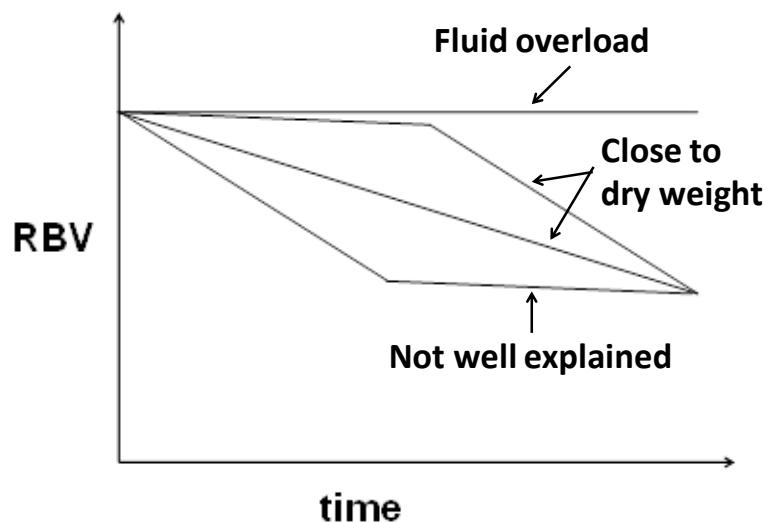
Figure 2-14: Characteristic shapes of RBV curve

Unfortunately, it appears that the potential of this technology has not been realised in clinical practice. Part of the difficulty in the use of RBV is that inter- and intra-patient variability makes interpretation non-trivial and there are no single, typical criteria that can reliably predict IDH in an individual. It has been shown that the plasma refilling coefficient varies markedly between patients undergoing haemodialysis (Iimura et al., 1996, Santoro et al., 1998), with a removal of 2 litres over 1 hour giving anywhere between 0.7% and 21.9% reduction in RBV (Koomans et al., 1984). It then follows that critical RBV limits will also vary between subjects, and this has been reported (Andrulli et al., 2002). Removing the inter-subject variability by defining individual critical RBV limits improves the reliability and this has, in one study, been shown to predict IDH events with a variation of less than 5% RBV (Barth et al., 2003), but there remains significant intra-individual variation in the RBV response to ultrafiltration (Krepel et al.,

2000). This variation will in part be related to the fact that RBV does not account for initial fluid status or absolute blood volume. Maduell et al showed a clear association between OH measured by BCM and RBV change (Maduell et al., 2013) and novel techniques for measuring absolute blood volume are highlighting the significance of this parameter (Kron et al., 2015). To try and avoid these issues, a critical Hct has been suggested as better predictor of IDH than the change in blood volume measured by RBV (Steuer et al., 1994).

This variability also seems to be present in analysis of RBV curve shape. For example, Lopot et al. suggested that less than 1% of cases typically show curves with a linear decrease followed by a flat-line (Lopot et al., 2000), whereas a different centre reported that after analysing 2240 curves, 91% had this shape (Dheu et al., 2009). Furthermore, clear definitions of the different shapes are lacking. The flat-line shaped curves are defined as being a change in RBV of less than 5% (Steuer et al., 1994), 3% (Reddan et al., 2005) or 1.5% (Agarwal et al., 2009) per hour.

Alternatively, the rate of change in blood volume has been proposed as a useful tool in managing patient's target weights. It has been shown that as dry weight is approached, the rate of decay moves from an exponential pattern to a linear decay, due to a switch from two-pool kinetics based on interstitial and intravascular volumes, to single-pool kinetics when the interstitial volume is depleted (Ishibe and Peixoto, 2004, Mitra et al., 2002).

The use of RBV as a diagnostic monitoring tool usually results in changes to the dialysis prescription for future sessions, but a potentially more powerful application of the technology is the continuous response of the dialysis machine to the instantaneous RBV data – so called biofeedback. This involves directly linking RBV results into an automated management programme which responds to changes in RBV by adapting the UF rate or dialysate sodium, to either increase or reduce fluid removal as necessary (Chapdelaine et al., 2011). This works on the assumption that the physiological variations occurring during haemodialysis necessitate different Na and ultrafiltration rates at different times. The user pre-defines limits for changes in blood volume and the device will alter ultrafiltration rate and dialysate sodium concentration to maintain the RBV slope along the defined trajectory while attempting to achieve the target

ultrafiltration volume within the treatment time. This typically involves starting a session with the maximum ultrafiltration rate that the user allows, to compensate for potential reductions in ultrafiltration rates later in the session.

2.4.5 Current applications and consensus opinion

Attempts to use a Hct threshold for guiding fluid management have shown mixed results (Hecking et al., 2013), possibly because of the high intra- and inter-patient variability. The Crit-Line Intradialytic Monitoring Benefit (CLIMB) study showed a negative result (Reddan et al., 2005) but there has been considerable discussion about the design of this study (Chapdelaine et al., 2011). Further trials are underway to evaluate the use of biofeedback controlled ultrafiltration on IDH (Leung et al., 2014, Hecking et al., 2012).

3 Methods

3.1 Introduction

The research presented in this thesis centres on two measurement techniques used as part of fluid management strategies − BCM and RBV. This chapter will detail the common methods of use for each technology and also detail statistical software used and research governance arrangements.

3.2 Bioimpedance measurements

3.2.1 Standard BCM measurement

The Body Composition Monitor (BCM; Fresenius Medical Care, Bad Homburg, Germany) is a whole-body, bioimpedance spectroscopy device. Measurements of resistance, reactance and phase angle are made between the wrist and ankle after injection of a current of around 50-800μA at 50 frequencies, logarithmically spaced between 5 kHz and 1 MHz (FMC, 2009). The device then uses a curve fitting routine based on the Cole-Cole model (see section 2.3.4) to fit a curve to the measured data points, allowing estimation of the Cole parameters. The extracellular resistance (R_E) and intracellular resistance (R_I) are used in the fluid model described by Moissl et al (Moissl et al., 2006) to calculate ECF and ICF and these parameters are used in Chamney's body composition model (Chamney et al., 2007).

Healthy controls had height measured using a stadiometer and weight measured using calibrated scales. They were positioned in a supine position for a minimum of 3 minutes before a measurement was taken. Haemodialysis patients were positioned supine, or as close to supine as was comfortable. Pre-dialysis measurements were made on the non-fistula side after the patient had been supine for at least 3 minutes; post-dialysis measurements were taken immediately after the needle sites had stopped bleeding and been dressed.

For all measurements, voltage measuring electrodes were positioned on the dorsal surface of the hand and foot at the wrist bone and ankle and the current injecting electrodes positioned about 10 cm distal to the voltage electrodes, across the knuckles, to ensure uniform current density at the measurement electrodes (FMC, 2009).

Measurements were checked visually for artefacts, and repeated until the difference in the measured OH was no greater than 0.2 litres between readings. In almost all cases only two readings were required and the discrepancy between the first and second was usually no more than 0.1 litre.

3.2.2 8-lead BCM measurement

For the purposes of research, the manufacturers of the BCM, Fresenius Medical Care, developed a modified version of the device, known as the 8-lead BCM, which is not commercially available. This device has two additional cables which allow 8 leads to be connected to both hands and both feet, with a voltage and a current lead at each site (fig. 3.1). This allows the possibility of making BCM measurements across a number of paths and also allows the isolation of individual segments for measurement as described in section 2.3.3.2.

#	Voltage	Current	Segments measured
1	RH-RF	RH-RF	Right arm, right trunk, right leg
2	LH-LF	LH-LF	Left arm, left trunk, left leg
3	RH-LF	RH-LF	Right arm, R-L trunk, left leg
4	LH-RF	LH-RF	Left arm, L-R trunk, right leg
5	RH-LH	RH-LH	Right arm, top trunk, left arm
6	RF-LF	RF-LF	Right leg, low trunk, left leg
7	RH-LF	RH-RF	Right arm, right trunk
8	LH-RF	LH-LF	Left arm, left trunk
9	LH-RF	RH-RF	Right leg, right trunk
10	RH-LF	LH-LF	Left leg, left trunk
11	RH-RF	RH-LH	Right arm
12	LH-LF	RH-LH	Left arm
13	RH-RF	RF-LF	Right leg
14	LH-LF	RF-LF	Left leg
15	LH-LF	RH-RF	Right trunk
16	RH-RF	LH-LF	Left trunk
17	RH-RF LH-LF	RH-RF LH-LF	Full whole body
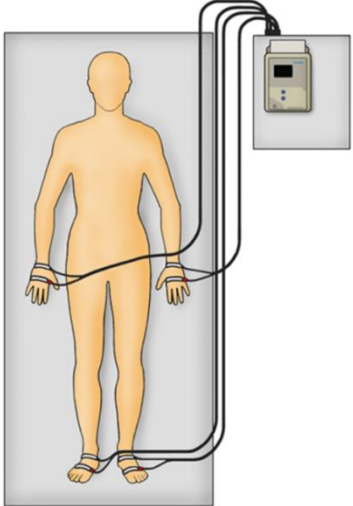
Figure 3-1: Specifications of an 8-lead BCM measurement. Illustration used with permission from Medical Illustration, Leeds Teaching Hospitals Trust.

For 8-lead measurements, electrodes were positioned as in the standard BCM measurements only on both hands and both feet. Measurements of resistance, reactance and phase angle were made at the same 50 frequencies as in the standard BCM, for

seventeen combinations of voltage and current (for full details, see appendix 9.1) which allows body composition measurements of the segments detailed in fig 3-1. The 8-electrode device does not display Cole-plots or body composition data to allow real-time assessment of artefacts or consistency, so measurements were taken in singular and the raw impedance values were extracted.
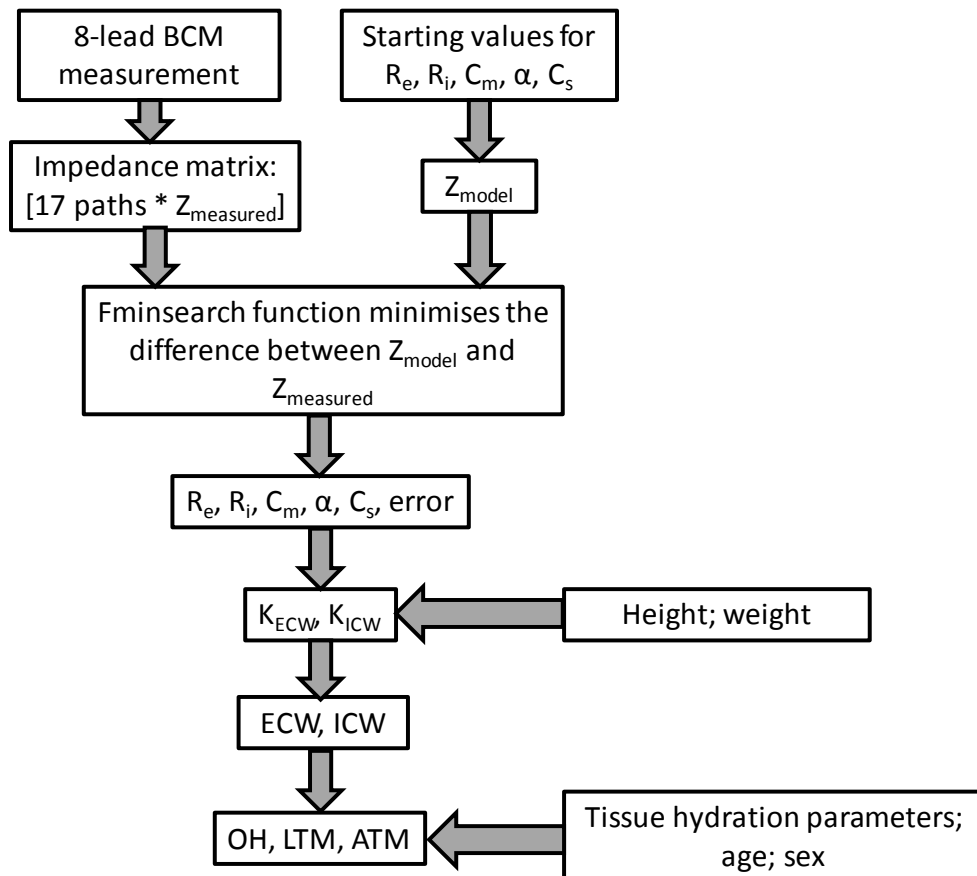
Figure 3-2: Flowchart showing how the data from 8-lead measurements was analysed. R_e is extracellular resistance, R_i is intracellular resistance, C_m is the cell membrane capacitance, α relates to the dispersion characteristics of the tissue and C_s is the stray capacitance

Programmes were written in Matlab (v. 2014a; Mathworks In, MA, USA) to process 8-lead BCM data. The analysis is described in fig. 3-2. Measured data were stored as a matrix of complex impedance data at 50 frequencies for 17 measurement paths. The data from each measurement, $Z_{measured}$, were analysed by fitting a Cole plot (equation 3-1) to the measured data. The equation used to fit the data is a combination of the Cole equation (equation 2-7) and a stray capacitance exponential term (equation 2-13) as discussed in section 2.3.4.3. The programme used some initial values for the Cole

parameters which were altered through a series of iterations to minimise the difference between the measured data and modelled data (Z_{model}). The error was calculated as the sum of the squared error of ($Z_{measured} - Z_{model}$) at each point, normalised to the mean error across all frequencies, for both real and imaginary components of Z. The Matlab algorithm "fminsearch" was used to minimise the error. R_E and R_I were then used with the volume and body composition models (Moissl et al., 2006, Chamney et al., 2007) using optimised tissue hydration parameters (courtesy of Fresenius R&D) to calculate OH, lean and adipose tissue compartments.

$$Z_{Model} = \left(\frac{R_e}{R_e + R_i}\right) \times \left(R_i + \frac{R_e}{1 + \left(i\omega C_m(R_e + R_i)\right)^{\alpha}}\right) \times \left(e^{-i\omega C_s}\right)$$

Equation 3-1: Modelled Cole equation for parameter optimisation

The analysis programme was validated by re-analysing data that had been used in standard BCM measurements. The raw data from twenty measurements on subjects with a wide variety of body composition and fluid status (mean (range) of BMI: 29 (16 to 50) kg/m^2; and of OH 0.5 (-3.8 to 5.9) litres) were analysed with the programme and the agreement between the body composition parameters can be seen in figure 3-3. For OH, the bias was 0.1 litres with 95% limits of agreement of around -0.1 to 0.3, showing very good agreement and verifying that the programmes are working similarly to commercial device analysis. For lean tissue and adipose tissue, biases of -0.4 and 0.3 litres were observed with larger confidence intervals. This is likely to be related to known issues in modelling R_I which has a larger influence on lean tissue and adipose tissue than OH. However, for the purposes of the study lean tissue and adipose tissue are secondary outcome interests and the agreement was deemed sufficient.
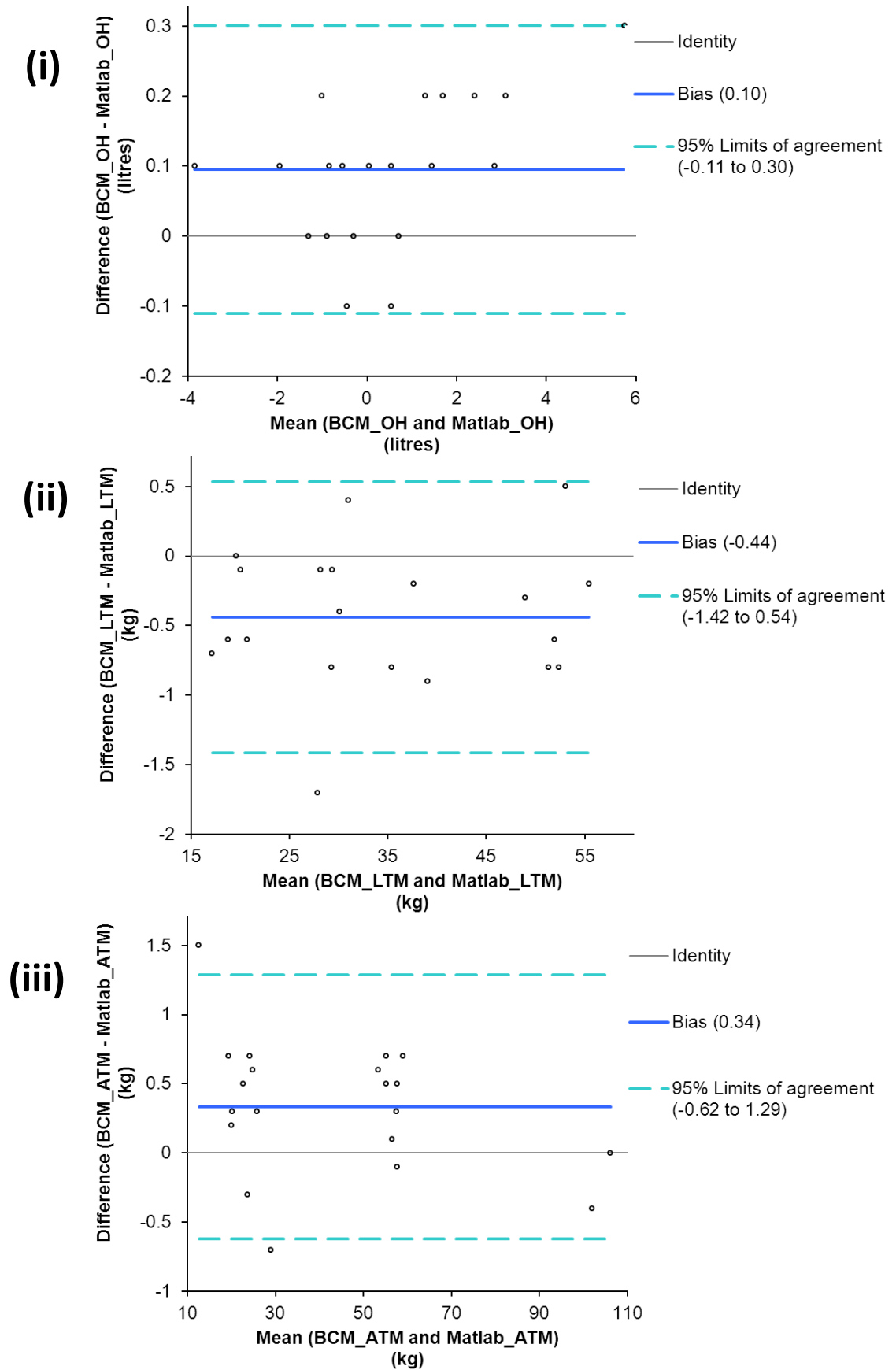
Figure 3-3: Validating the data analysis programme against commercial analysis for OH (ĭ), lean tissue (ĭĭ), adipose tissue (ĭĭĭ) using Bland-Altman analysis

3.3 Blood volume measurements

3.3.1 Measurement technique

Relative blood volume measurements were made using the Crit-Line III Monitor (Hema-metrics, Kaysville, UT, USA). A sterile, disposable cuvette was positioned between the arterial blood line and the dialyser blood inlet and the sensor clip placed onto the cuvette. The sensor emits optical light at specific wavelengths and monitors the absorption and reflection of the light as it passes the whole blood in the cuvette. This allows it to make a measure of haematocrit content and percentage change in blood volume, relative to the start of the measurement period. The results of each haemodialysis session were downloaded and imported into Microsoft Excel (Microsoft Corporation) to allow analysis. Device calibration was checked using a verification filter monthly, as recommended by the manufacturer.

3.3.2 RBV classification

RBV measurements were classified based on the shape of the curve as advocated for informing fluid management and also were used as the basis of a novel approach to classifying the refilling characteristics of patients.

The basis of using shape classification of RBV slopes is outlined in section 2.4.4. The shape of each blood volume curve was defined as one of four characteristic shapes described in figure 3-4.
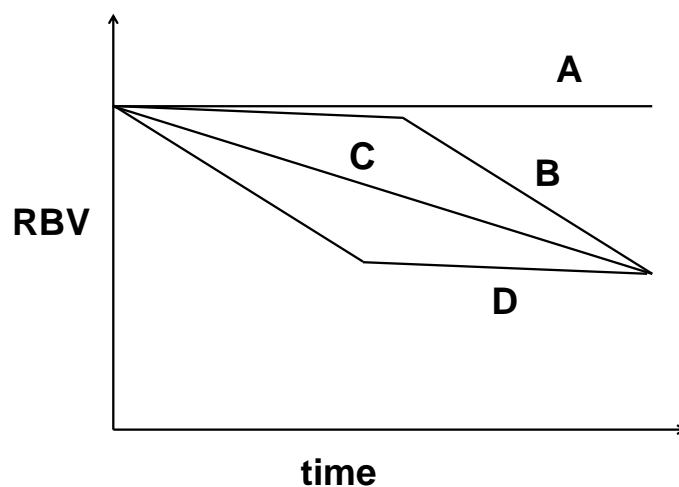
Figure 3-4: Characteristic shapes of RBV curves

A) Flat-line: 'A' slopes are characterised by a flat line throughout a haemodialysis session, with a maximum slope of 3% per hour.

B) Late reduction: 'B' slopes are characterised by a flat slope over an initial period of the haemodialysis session (<3% per hour for at least one hour) followed by a more rapid reduction in blood volume for the remainder of the session (average slope >3% per hour).

C) Linear reduction: 'C' slopes are characterised by a linear reduction in blood volume throughout the haemodialysis session (average slope >3% per hour)

D) Early reduction: 'D' slopes are the inverse of 'B' slopes and are characterised by an initial rapid blood volume slope (average slope >3% per hour for at least one hour) followed by a flat slope for the rest of the session (average slope <3% per hour)

The concurrent use of RBV and BCM measurements allowed a novel method of classifying patients based on their refilling ability. The drop in RBV over the full dialysis session reflects the difference between the volume of fluid removed from the blood by ultrafiltration and the refilling volume. However, the refilling volume is dependent on the pre-dialysis fluid overload, which can be measured by BCM. As such, patients can be classified as exhibiting good or poor refilling capacity based on the RBV drop over a dialysis session, for a given ultrafiltration volume and pre-dialysis OH. The cut-off values chosen here were based on experiences of using the two technologies locally and can be seen in Table 3-1. Sessions which indicated good refilling are denoted as 1 and those with poor refilling denoted as 0.

	UF < OH	UF < OH + 1 litre	UF > OH + 1 litre
UF < 2 litres	5%	10%	10%
UF > 2 litres	10%	15%	20%

Table 3-1 Critical drop in blood volume over a 4-hour dialysis session that indicates inadequate refilling, based on a dialysis session of 4 hours, pre-dialysis OH (OH) and ultrafiltration volume (UF).

Consequently, each individual haemodialysis session is given a classification based on a combination of a 0 or a 1 with a letter A, B, C, D.

3.4 Haemodialysis treatments

All haemodialysis subjects included in these studies had prescriptions for standard regimes of three sessions of four hours per week. Dialysate temperature was 36°C and sodium was 137mmol/l as standard. All haemodialysis patients come from units where BCM use is standard as part of fluid management and informs target weight decisions alongside clinical examination. RBV measurements are available on some, but not all of the dialysis machines used in the dialysis units and is rarely used as part of routine care.

3.5 Statistical analysis

Bland Altman analysis was done using Analyse-it for Microsoft Excel (version 2.26). All other analysis was done using the statistical software package 'R' version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

3.6 Research governance

This work was undertaken in accordance with the principles of the Declaration of Helsinki and ethical approval was granted by a local ethics committee (References: 13/YH/0148 for the work in chapter in 4; 12/YH/0497 for the work in chapter 5; and 14/YH/1091 for the work in chapter 6).

4 Characterising BCM and RBV measurements in haemodialysis

4.1 Objective

Bioimpedance and RBV monitoring are considered as promising tools for improving fluid management in haemodialysis patients (Rosner and Ronco, 2014) and have started to be implemented in routine practice in some centres. However, despite some 25 years of research into both these technologies in haemodialysis there lacks a sufficiently robust evidence base in the clinical setting to allow users to make management decisions with confidence. This study aims to characterise some important features of BCM and RBV use in normal clinical practice to allow users to make measurements and interpret the results with confidence in a range of clinical scenarios.

Research question 1: Can characterisation of alternative measurement paths and timing for the BCM, and the use of simultaneous RBV monitoring, lead to more individualised and effective fluid management?

4.2 Introduction
4.2.1 Bioimpedance

Many of the limitations around bioimpedance relate to assumptions made during data analysis, including assumptions about temperature, resistivity, limb shape and fluid distributions (Hoenich and Levin, 2003). Assessing these limitations as applied to the technology as a whole is not easy. Bioimpedance devices use many different forms of analysis and the impact of these assumptions upon a clinical measurement is very dependent on the form of measurement and analysis. Despite the fact that many groups have reported on these factors (Huang et al., 2000, Zhu et al., 1999, Zhu et al., 1998, Moissl et al., 2006), the sheer number of measurement and analysis techniques and lack of understanding of the differences between them make it difficult to interpret the literature in this area.

The important factors affecting bioimpedance measurements can be split into two types: those on the microscopic scale, such as temperature, ionic concentrations, tissue resistivity; and those on the macroscopic scale, such as body composition, body geometry, fluid distribution and measurement path. This investigation aimed to evaluate the effect of some of the macroscopic assumptions on BCM measurements.

BCM manufacturer's guidance states that measurements should not be made on an arm with peripheral vascular access (FMC, 2009). However, in practice this requirement would exclude a large number of patients from having BCM measurements. Patients can, over time, end up with multiple vascular access sites over both sides of the body, or in some cases it may not be possible to avoid a vascular access site, e.g. patients with amputations, bandaged limbs, localised fluid. It is also advised that measurements should be made pre-dialysis and that the patient should be recumbent for at least 2 minutes before measurement (FMC, 2009). This is due to the effect that ultrafiltration (Abbas et al., 2014) and posture (Zhu et al., 1998) have on fluid distributions in the body. Due to the different shapes and sizes of the limbs, shifts in fluid from one compartment to another can have significant effects on the whole body impedance.

In order to allow greater understanding of the effect of making measurements outside the manufacturer's protocol, the effect of changing measurement path and time of measurement on body composition parameters was characterised.

4.2.2 Relative blood volume measurements

The principle obstacle to RBV monitoring gaining acceptance as a simple, user-friendly diagnostic tool is the inter- and intra-subject variation in the blood volume response to ultrafiltration (Krepel et al., 2000). In any one measurement session, the RBV slope will be a function of a large number of variables as outlined in table 4-1.

Real changes in blood volume can be simplistically described as the difference between ultrafiltration removing fluid from the blood and refilling of the blood. The ultrafiltration rate is dependent on the amount of fluid to be removed and the time taken to remove it, along with any profiling used to introduce variable ultrafiltration rates. The refilling rate is more complicated and is described in more detail in section 2.1.2.2.

However, it is fundamentally affected by differences in hydrostatic and oncotic pressures in the blood volume and the interstitium and by the integrity of lymphatic return.

Response	Variable	Factors
Real changes in blood volume	Ultrafiltration rate	Treatment time Ultrafiltration volume Ultrafiltration rate profiling
	Refilling rate	Fluid status Absolute blood volume Venous and interstitial compliance Dialysate sodium concentration Body size Plasma protein Antihypertensive medications Integrity of lymphatics
Apparent changes in relative blood volume	F-cell ratio	Postural changes Ingested food and drink Dialysate and core temperature Exercise
	Haemolysis	Haemodialysis equipment

Table 4-1: Factors affecting RBV measurement

In addition to these real blood volume changes, there are a number of situations that can lead to apparent RBV changes as recorded by monitors. These are described in section 2.4.3, but generally relate to changes in the local and global haematocrit concentrations during the measurement period.

Some of the variables can be controlled to some degree – ultrafiltration rates can be manipulated, patients can be advised not to eat etc. – but others, such as interstitial compliance, are very difficult to investigate, never mind control. This has led to a prevailing lack of consensus on how to use the technology, and of the hard evidence to justify its use (Hecking et al., 2012). There are studies advocating the use of RBV that have controlled some of these factors for the research (e.g. with fluid restrictions, enforced treatment times, standardised prescriptions etc.) and in this situation, RBV technology has been shown to be associated with a reduction in IDH and symptoms (Steuer et al., 1996, Balter et al., 2015), but this is not likely to be generalizable to routine management of a whole group of patients. Cohort based, controlled studies looking at clinically important outcomes are lacking.

One of the parameters associated with both inter-subject and intra-subject variation in RBV changes is initial fluid status (Maduell et al., 2013). For a long time there has been no simple measure of fluid status, so it has not been incorporated into most algorithms for interpreting RBV data. OH measured using BCM can provide a simple assessment of initial fluid status that could inform RBV interpretation in routine practice.

4.3 Methods

4.3.1 Subjects

Two cohorts of subjects were involved in the study. The inclusion criteria for each group are detailed here.

Healthy control subjects:

- 48 subjects stratified by age-decade between 20 and 70+ and by sex
- No history of kidney disease or heart failure
- No visible localised fluid accumulation such as oedema
- Ability to be weighed
- No limb amputations

Haemodialysis patients:

- 48 subjects over 18 years old
- No more than one recorded episode of IDH within the preceding two months
- No visible localised fluid accumulations
- Ability to achieve target weight, defined by a clinician with access to BCM results

Pilot work comparing BCM measurements from hand to hand and from hand to foot showed standard deviations of the mean difference in hydration values of around 1.0 litres (Keane and Lindley, 2015). Recruiting 48 subjects into each cohort would allow differences of 0.3 litres to be measured between the primary two paths at the level of 5% type I error with 80% power using a two sided t-test. This is deemed an acceptable sample size; differences below 0.3 litres will fall below the limits of reproducibility of

the device (Wieskotten et al., 2013). Further research questions investigated will be exploratory and will not be powered for a particular outcome.

4.3.2 Procedure

For healthy volunteers, standard and 8-lead BCM measurements were made as described in chapter 3. Height and weight were recorded immediately before BCM measurements.

For haemodialysis patients, height was taken from their clinical notes and pre- and post-dialysis weights were obtained as part of normal care. A standard BCM and an 8-lead BCM measurement were made before and immediately after dialysis and RBV was monitored (see Chapter 3). Planned and achieved ultrafiltration volumes were recorded. Repeat measurements were made between 2 weeks and 4 weeks after the initial measurement.

4.3.3 Analysis

4.3.3.1 Model generation

Data were analysed using linear mixed-effects models. For healthy controls, subject was taken as the random effect and path, sex and age were taken as fixed effects. For haemodialysis patients, the same model set up was used, only with the addition of pre-/post-dialysis as an interaction term if it significantly altered the model or as a normal predictor variable if not. For healthy controls, measurement from hand-to-foot on the dominant side of the body was taken as the reference path, whereas for haemodialysis patients, measurements from hand-to-foot on the contralateral side of the body to the most recently used vascular access (VA) was taken as the reference path.

To present the data, results for a 60 year old female acted as a reference and the adjustments for age and sex are detailed. The mean value for the dependent variable from the reference path was presented alongside the bias, p-values and 95% confidence intervals associated with making measurements on each of the whole body paths. Significance levels were set at 0.05.

To examine the model, plots of standardised residuals against fitted values were used to check the assumption of homoscedasticity and a Q-Q plot of the residuals was used to assess normality.

4.3.3.2 Characterising BCM measurements of body composition

The raw data from the 8-lead BCM were processed as outlined in chapter 3 to give estimates of OH, lean tissue and adipose tissue for each path. The difference in estimated OH, lean tissue and adipose tissue respectively between the 6 primary paths (see figure 4-1) was characterised. Extracellular resistance (R_E) was taken from the modelled impedance data for each of the five body segments to investigate segment specific changes in R_E.
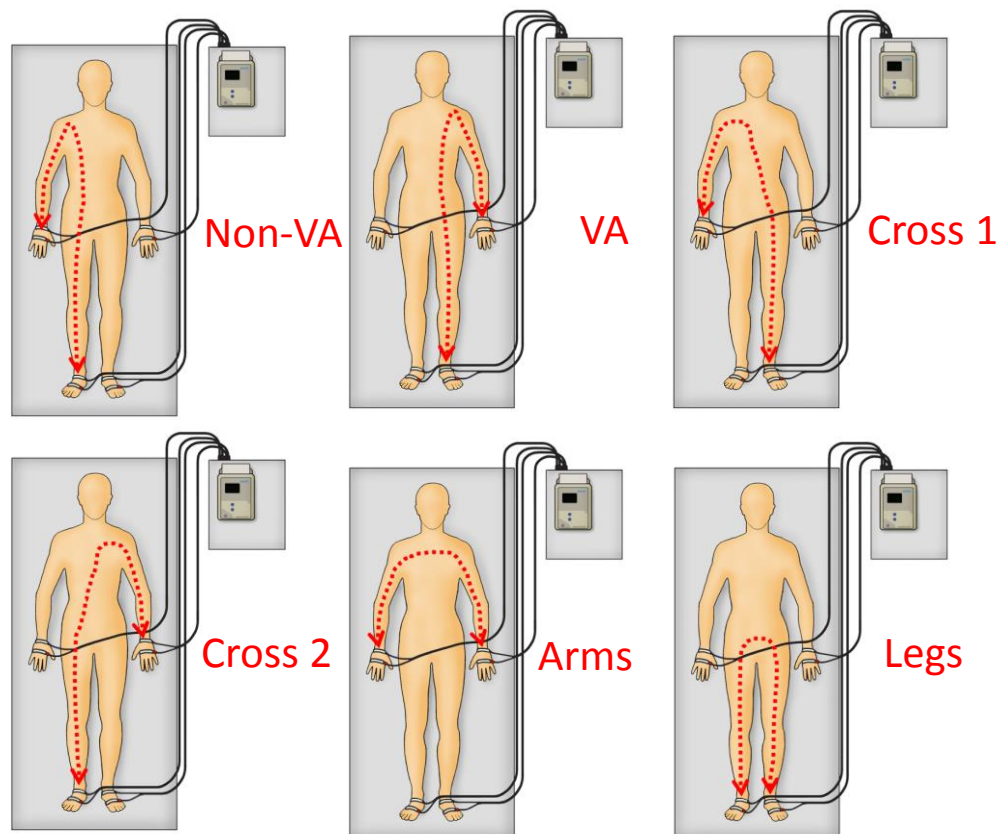
Figure 4-1: Primary 6 measurement pathways. VA is vascular access and the configurations here are shown for a person with left sided VA

To consider the repeatability of the model for haemodialysis patients, the repeat measurements were re-analysed in the same way and a qualitative description of the differences in the two analyses was made.

4.3.3.3 Characterising changes in body composition parameters during HD

To investigate the validity of post-dialysis measurements, the agreement between change in BCM-measured OH from the reference path and change in weight was assessed using Bland-Altman analysis. Furthermore, the consistency of lean tissue and adipose tissue from the start to end of dialysis was assessed using paired t-tests.

4.3.3.4 Characterise RBV measurements

The RBV chart from each session was defined based on its shape, as outlined in previous literature, and based on the novel method of categorising refilling characteristics (see chapter 3). The intra-subject repeatability for curve shape was assessed by comparing the proportions in each category at the first and second measurement session using Cohen's kappa. The relationship between RBV curve shape and fluid status was explored by plotting the pre- and post-dialysis BCM-measured OH for all subjects with each RBV shape category. Furthermore, quantitative comparisons were made between mean pre- and post-dialysis OH in the RBV slope groups using one-way analysis of variance. Because there are numerous criteria for categorising curve shape, sensitivity analysis was undertaken to re-classify all the curve shapes based on a conservative definition of a 'flat line' of 1.5% per hour (Sinha et al., 2010).

4.4 Results

4.4.1 Mixed effects regression model

	Healthy controls	HD patients
Age (years)	49 (17)	60 (16)
Height (m)	1.71 (0.11)	1.70 (0.12)
Weight (kg)	73 (14)	81 (23)
BMI (kg/m^2)	25 (4)	29 (7)
Sex (males)	24	28
HD vintage (months)	-	30 (6)
Most recent VA (left sided)	-	38
Albumin (g/L)	-	38 (2.8)
Diabetes	-	14
Number of comorbidities: - 1	-	12
- 2	-	4
- 3	-	3

Table 4-2: Subject demographics. Data are mean (standard deviation) for normal data and number of subjects for categorical data. Comorbidities present included acute coronary syndrome, heart failure, cerebrovascular disease, liver disease, peripheral vascular disease and smoking.

The results from the mixed effects regression models are presented based on the results from a 60 year old female as reference, with age and sex adjustments detailed in the table legend. In each case, the mean result from the reference path for a 60 year old female is given and the bias associated with measurements on the other paths.

For the models investigating OH and adipose tissue, age and sex were non-significant predictors in either healthy controls or haemodialysis patients. For lean tissue, age and sex were significant predictors in both healthy controls and haemodialysis patients. For dialysis patients, including pre-/post-dialysis as an interaction term made a significant difference to the model (p=0.02), so data are presented as such.
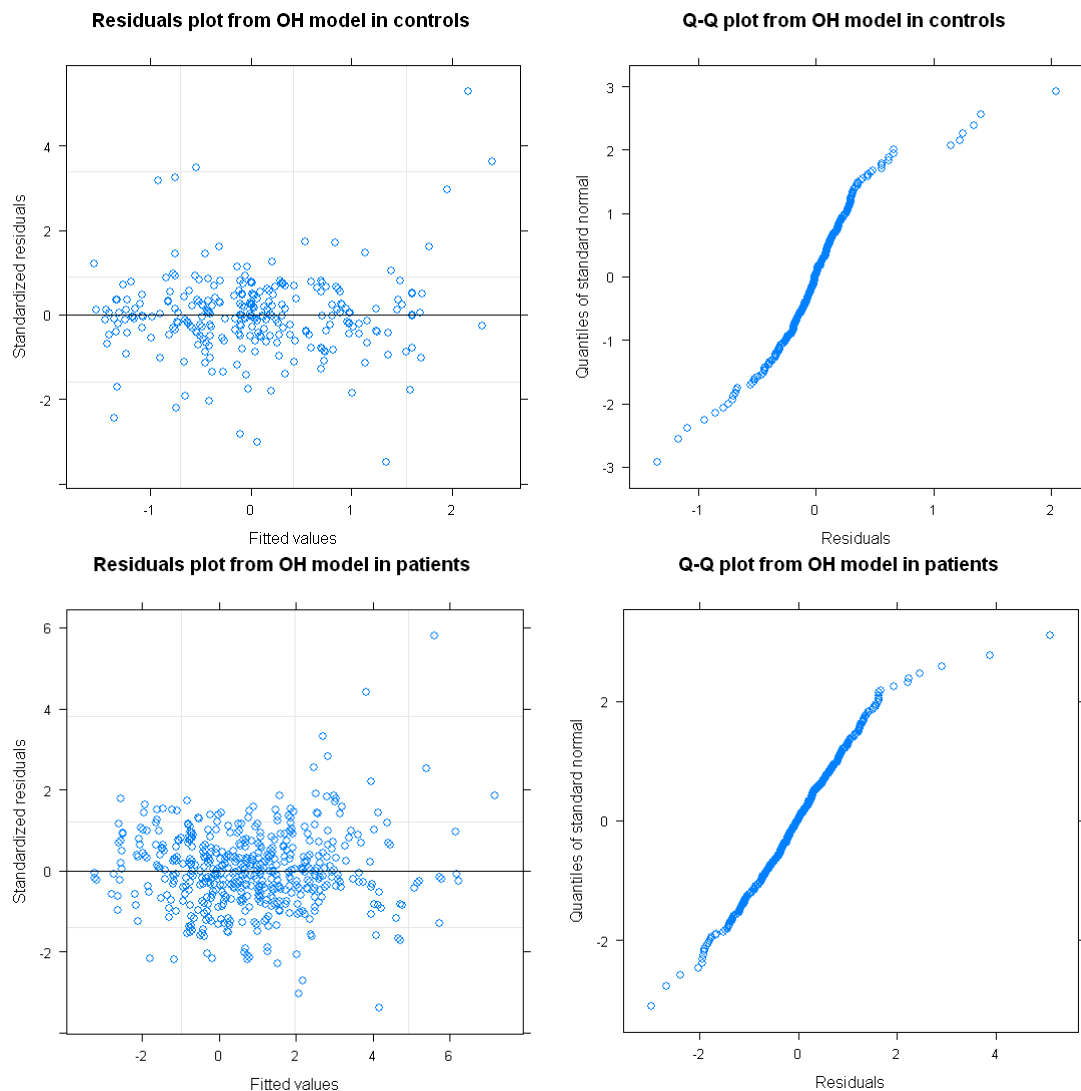
Figure 4-2: Plot of residuals (left) and a 'Q-Q' plot of the residuals (right) for mixed-effects model of OH in healthy controls (above) and haemodialysis patients (below)

For the model investigating OH, plots of residuals against fitted values and Q-Q plots can be seen in figure 4-2, while those for lean tissue and adipose tissue are in the appendix (see section 9.2). Visual inspection of the residual plots suggested random scatter. Although Q-Q plots showed some dispersion from normality; sensitivity analyses were done by checking the outliers for errors, but no clear problems with the data were found. These outliers were predominantly examples of increased discrepancy between hand-to-foot and foot-to-foot measurements in some individuals.

4.4.2 Characterising BCM measurements of body composition

4.4.2.1 Healthy controls

The data from healthy controls show that there is no difference in BCM-measured OH measured between all the whole-body paths other than the foot to foot measurement, which had a bias of 0.8 litres (table 4-3). Considering lean tissue and adipose tissue, there was a significant bias on most of the paths other than some of the cross measurements, including higher lean tissue and lower adipose tissue in the dominant arm and in the hand-to-hand path as compared to the reference path.

	Measurement Path	OH (litres)	Bias (litres)	p-value	Approx. 95% CI
Healthy controls	Dominant	-0.12	-	0.74	-0.86 to 0.61
	Non-dominant	-	0.09	0.24	-0.06 to 0.25
	Cross1	-	0.002	0.98	-0.15 to 0.16
	Cross2	-	0.10	0.20	-0.05 to 0.26
	Arms	-	-0.02	0.81	-0.17 to 0.14
	Legs	-	0.80	<0.01	0.64 to 0.95

Table 4-3: Model for OH in healthy controls. Data are presented for a 60 year old female, where the adjustment for age was -0.003 per year (p=0.65; 95% CI: -0.02 to 0.01) and for sex was 0.28 for male (p=0.22; 95% CI: -0.17 to 0.74)

	Measurement Path	LTM (kg)	Bias (kg)	p-value	Approx. 95% CI
Healthy controls	Dominant	45	-	<0.01	39 to 50
	Non-dominant	-	-1.5	<0.01	-2.5 to -0.4
	Cross1	-	-0.43	0.41	-1.4 to 0.59
	Cross2	-	-1.1	0.05	-2.1 to -0.03
	Arms	-	-2.7	<0.01	-3.7 to -1.7
	Legs	-	4.4	<0.01	3.4 to 5.4

Table 4-4: Model for lean tissue mass (LTM) in healthy controls. Data are presented for a 60 year old female, where the adjustment for age was -0.17 per year (p<0.01; 95% CI: -0.28 to -0.07) and for sex was 19 for male (p<0.01; 95% CI: 15 to 22)

	Measurement Path	ATM (kg)	Bias (kg)	p-value	Approx. 95% CI
Healthy controls	Dominant	23	-	<0.01	12 to 35
	Non-dominant	-	1.4	<0.01	0.36 to 2.4
	Cross1	-	0.44	0.40	-0.57 to 1.5
	Cross2	-	0.95	0.07	-0.06 to 2.0
	Arms	-	2.7	<0.01	1.7 to 3.7
	Legs	-	-5.2	<0.01	-6.3 to -4.2

Table 4-5: Model for adipose tissue mass (ATM) in healthy controls. Data are presented for a 60 year old female, where the adjustment for age was 0.11 per year (p=0.33; 95% CI: -0.11 to -0.32) and for sex was -2.7 for male (p=0.44; 95% CI: -9.7 to 4.2)

4.4.2.2 Haemodialysis patients: alternative paths

Haemodialysis patients showed different pre-dialysis patterns than subjects with normal renal function. In particular, there was a significant difference in BCM-measured OH between measurements made on the side of the body where vascular access is situated as compared to the contralateral side (table 4-6). Unlike controls, there was no difference in lean tissue or adipose tissue between the sides, despite the fact that vascular access is usually on the non-dominant side.

	Measurement Path	OH (litres)	Bias (litres)	p-value	Approx. 95% CI
Pre-dialysis	Non-VA side	1.7	-	<0.01	0.66 to 2.7
	VA-side	-	0.42	0.02	0.08 to 0.76
	Cross1	-	0.02	0.91	-0.32 to 0.36
	Cross2	-	0.41	0.02	0.07 to 0.75
	Arms	-	-0.21	0.23	-0.55 to 0.13
	Legs	-	1.7	<0.01	1.4 to 2.1
Post-dialysis	Non-VA side	-0.12	-	0.82	-1.2 to 0.90
	VA-side	-	0.13	0.61	-0.36 to 0.61
	Cross1	-	0.02	0.93	-0.46 to 0.50
	Cross2	-	0.10	0.68	-0.38 to 0.59
	Arms	-	0.35	0.16	-0.13 to 0.84
	Legs	-	-0.56	0.03	-1.0 to -0.07

Table 4-6: Model for OH in dialysis patients. Data are presented for a 60 year old female, where the adjustment for age was 0.012 per year (p=0.38; 95% CI: -0.02 to 0.04) and for sex was 0.001 for male (p=0.99; 95% CI: -0.88 to 0.89)

	Measurement Path	LTM (kg)	Bias (kg)	p-value	Approx. 95% CI
Pre-dialysis	Non-VA side	25	-	<0.01	19 to 30
	VA-side	-	0.58	0.45	-0.90 to 2.0
	Cross1	-	-0.38	0.61	-1.9 to 0.97
	Cross2	-	1.2	0.12	-0.30 to 2.8
	Arms	-	1.3	0.08	-0.14 to 3.1
	Legs	-	1.5	0.04	0.07 to 3.0
Post-dialysis	Non-VA side	24	-	<0.01	19 to 30
	VA-side	-	-0.43	0.69	-2.5 to 1.7
	Cross1	-	0.14	0.90	-1.9 to 2.2
	Cross2	-	-0.89	0.41	-3.0 to 1.2
	Arms	-	-0.25	0.82	-2.3 to 1.8
	Legs	-	0.15	0.89	-1.9 to 2.2

Table 4-7: Model for LTM for dialysis patients. Data are presented for a 60 year old female, where the adjustment for age was -0.40 per year (p<0.01; 95% CI: -0.56 to -0.25) and for sex was 17 for male (p<0.01; 95% CI: 12 to 22)

	Measurement Path	ATM (kg)	Bias (kg)	p-value	Approx. 95% CI
Pre-dialysis	Non-VA side	32	-	<0.01	19 to 45
	VA-side	-	0.58	0.45	-0.90 to 2.0
	Cross1	-	-0.38	0.61	-1.9 to 0.97
	Cross2	-	1.2	0.12	-0.30 to 2.8
	Arms	-	1.3	0.08	-0.14 to 3.1
	Legs	-	1.5	0.04	0.07 to 3.0
Post-dialysis	Non-VA side	32	-	<0.01	19 to 45
	VA-side	-	-1.0	0.19	-2.6 to 0.48
	Cross1	-	0.36	0.64	-1.0 to 1.8
	Cross2	-	-1.6	0.04	-3.1 to -0.13
	Arms	-	-1.1	0.15	-2.6 to 0.38
	Legs	-	-3.4	<0.01	-4.8 to -1.9

Table 4-8: Model for ATM in dialysis patients. Data are presented for a 60 year old female, where the adjustment for age was 0.22 per year (p=0.22; 95% CI: -0.14 to 0.58) and for sex was 2.4 for male (p=0.68; 95% CI: -9.0 to 14)

4.4.2.3 Haemodialysis patients: changes over a dialysis session
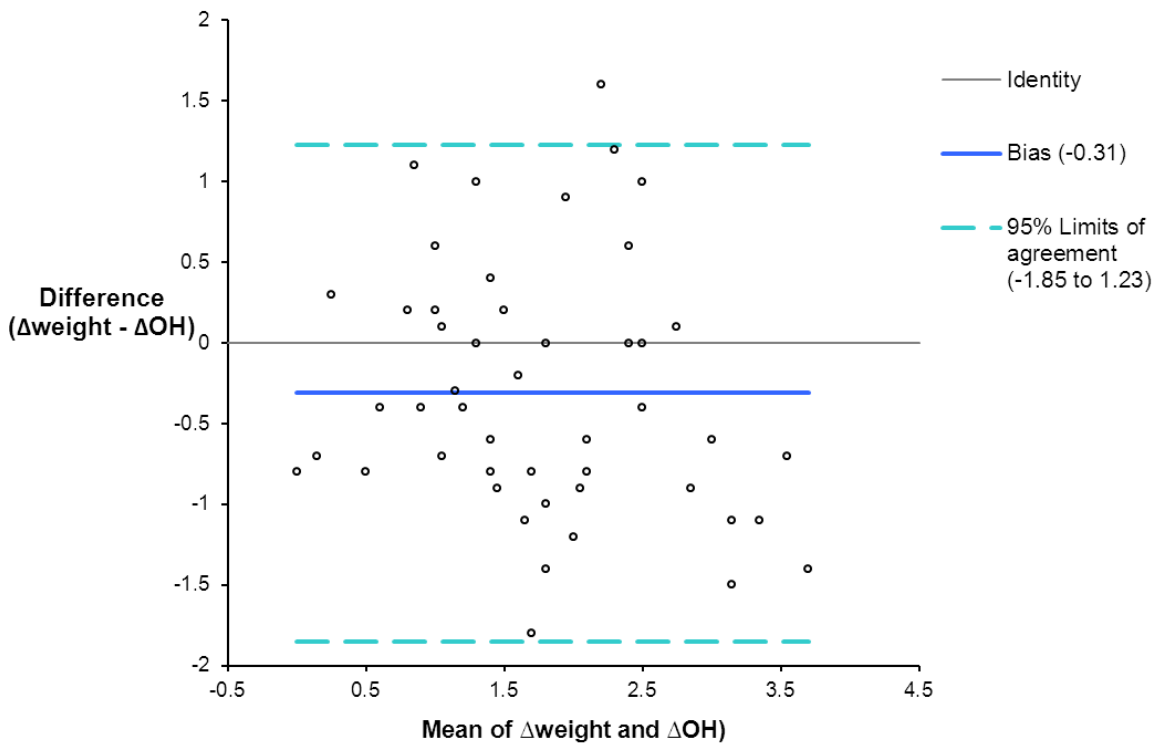
Figure 4-3: Bland Altman analysis of the agreement of change in weight (Δweight) and change in OH (ΔOH)

There was good agreement between change in BCM-measured OH on the reference measurement path ([pre-dialysis OH] − [post-dialysis OH]) with change in weight (fig. 4-3; bias 0.3 kg, 95% CI -1.9 to 1.3 kg). In theory, lean tissue and adipose tissue should not change over dialysis and the mean difference in the measured changes was not different to the null of zero (lean tissue: mean difference: -0.28 kg; p=0.7; 95% CI:-1.8 to 1.2; adipose tissue: mean difference: 0.56 kg; p=0.5; 95% CI: -0.92 to 2.1).

Considering the distribution of changes in BCM-measured OH, the only measurement path that has a significantly different change in OH compared to the reference path is that of the legs. This path shows around 0.6 litre greater change in OH than the reference path over dialysis, indicating that there is a more pronounced change in fluid status in the legs than in the other segments. This was supported by analysis of the changes in raw impedance values from each segment individually, showing greatest reduction in extracellular resistance in the legs and smallest in the arms (table 4-6).

Segment	Mean pre R_E (ohms)	Mean post R_E (Ohms)	Mean % change in R_E (Ohms)
Arm_ref	288	326	13
Arm_opp	283	316	12
Trunk	23	25	6
Leg_ref	248	295	19
Leg_opp	247	294	19

Table 4-9: Segmental changes in ECF resistance (R_E) over dialysis. 'ref' indicates a segment from the reference path and 'opp' from the opposite side.

4.4.2.4 Haemodialysis patients: repeatability

Of the 48 HD patients recruited, 36 had follow up measurements. The model for follow up measurements showed no pronounced differences considering the different sample sizes used, suggesting that the effects from the model are reproducible (see appendix, section 9-3).
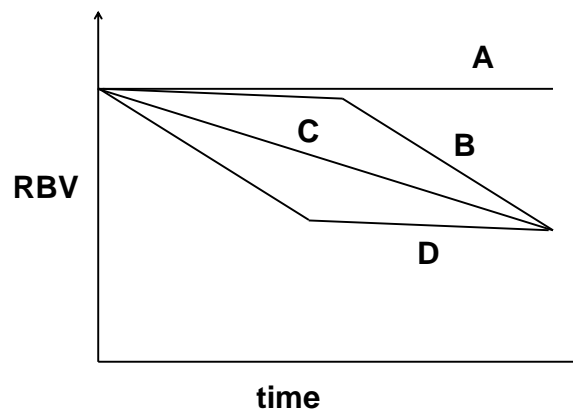
Figure 4-4: Characteristic shapes of RBV curves

4.4.3 Characterising RBV measurements

Of the 48 subjects recruited, RBV measurements were recorded in at least one of the sessions for 47 of them. Based on the criteria outlined in chapter 3, of these 47 the most common characteristics from the first measurement session were of shape 'A' or 'D' (figure 4-4) and the vast majority displayed good refilling characteristics (table 4-10). For those that had follow up measurements, almost a third had a different RBV slope category at the repeat measure and 10% had a different refilling score (full RBV results table in appendix table 9-4). Cohen's kappa was calculated to assess the repeatability of RBV shape between first and second measurement sessions, with the single 'B'

categorised measurements being left out of the analysis as it would invalidate the analysis due to low counts (table 4-11). A kappa value of 0.3 suggested there is no evidence of good repeatability between the 1st and second measurements.

		Shape			
		A	B	C	D
Refilling	0	0	0	4	7
	1	16	1	10	9

Table 4-10 Shape and refilling characteristics of the first measurement session with RBV recorded for each patient.

		2nd session			
		A	B	C	D
	A	4	0	3	4
1st	B	0	0	0	0
session	C	1	1	4	1
	D	4	0	1	9

Table 4-11: Comparison of RBV shape in first and second measurement sessions

The plots of pre- and post-dialysis BCM-measured OH by RBV shape category do not highlight a clear difference in OH patterns between groups, by visual inspection and that across all categories there are subjects who finish dialysis fluid depleted. (fig. 4-5). There was no difference in pre-dialysis OH between the different slope groups, other than the sensitivity analysis of the second session where the small sample of patients with an 'A' curve had the unusual characteristic of being fluid depleted pre-dialysis. Patients from all shape categories finished dialysis with similar fluid status, including in the sensitivity analysis which used a conservative definition of a flat line. Mean ultrafiltration volumes and ultrafiltration volume normalised to ECF were highest in those patients classified as B&C, although the shape definition is dependent on the ultrafiltration volume to some extent. The subjects were divided into tertiles by BMI and each tertile displayed in a different colour in figure 4-5. D shaped curves were less likely to come from the highest BMI tertile.

		A	B & C	D	p-value
Primary analysis	n	16	15	16	1.0
	Good refilling (%)	100	69	56	0.01
	Pre-dialysis mean OH) (l)	1.0	2.2	1.6	0.1
	Post- dialysis mean OH (l)	-0.9	-0.1	-0.1	0.3
	Mean UF volume (l)	1.8	2.5	1.6	0.02
	UF/ECF (%)	11	13	9	0.05
	BMI (kg/m^2)	29.9	27.4	28.3	0.6
Sensitivity analysis	N	8	22	17	0.04
	Good refilling (%)	100	82	59	0.06
	Pre-dialysis OH (l)	1.0	1.9	1.5	0.3
	Post- dialysis OH (l)	-0.9	-0.2	-0.3	0.6
	Mean UF volume (l)	1.5	2.3	1.6	0.02
	UF/ECF (%)	9	13	9	0.01
	BMI (kg/m^2)	28.4	28.6	28.6	0.6

Table 4-12: Patient characteristics and treatment features by shape category groups with a p-value from one-way analysis of variance between the three groups. UF refers to ultrafiltration volume.
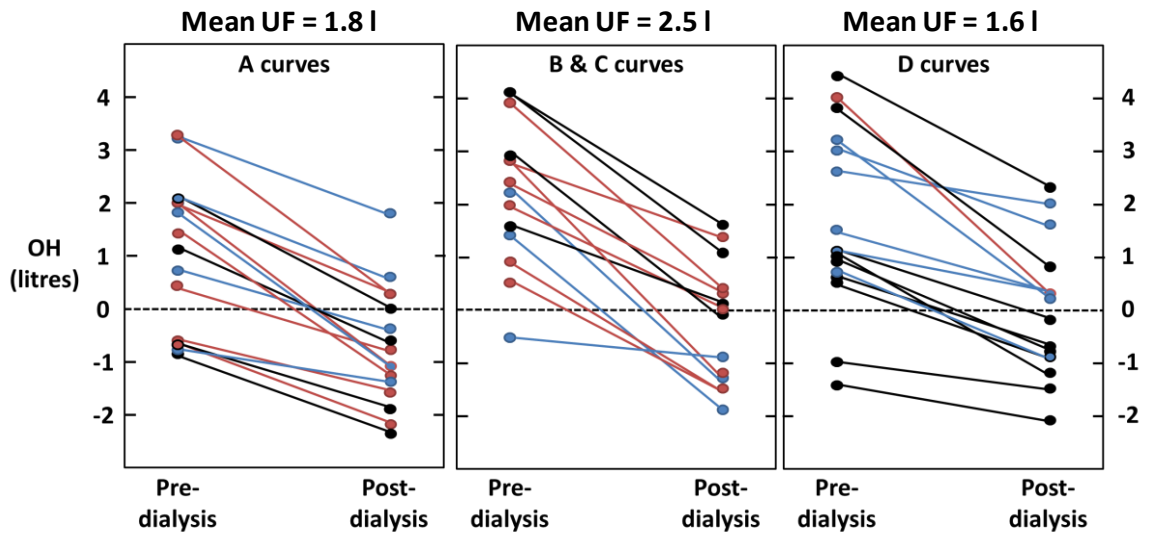
Figure 4-5: Pre and post OH measurements for patients with RBV plots of different curve shape. Blue plots are from the lowest tertile of BMI from this cohort (16.5-24.9 kg/m^2), black plots are the middle tertile (25 to 29.9 kg/m^2) and red plots are the highest tertile (30 to 46 kg/m^2).

Because there is discrepancy in how a flat line slope is defined, sensitivity analysis, using the most conservative definition of a flat line from the literature (1.5% per hour,

(Agarwal et al., 2009)) examined whether the conclusions were still justified when different definitions of a flat-line were used. This showed that of the 16 subjects initially classified as having a flat-line ('A' slope), 8 of these were still classified as being flat-lines and half of these still finished dialysis fluid depleted (mean post-dialysis BCM-measured OH: -0.6 litres, range: 1.8 to -1.9 litres) (full dataset in appendix table 9-4).
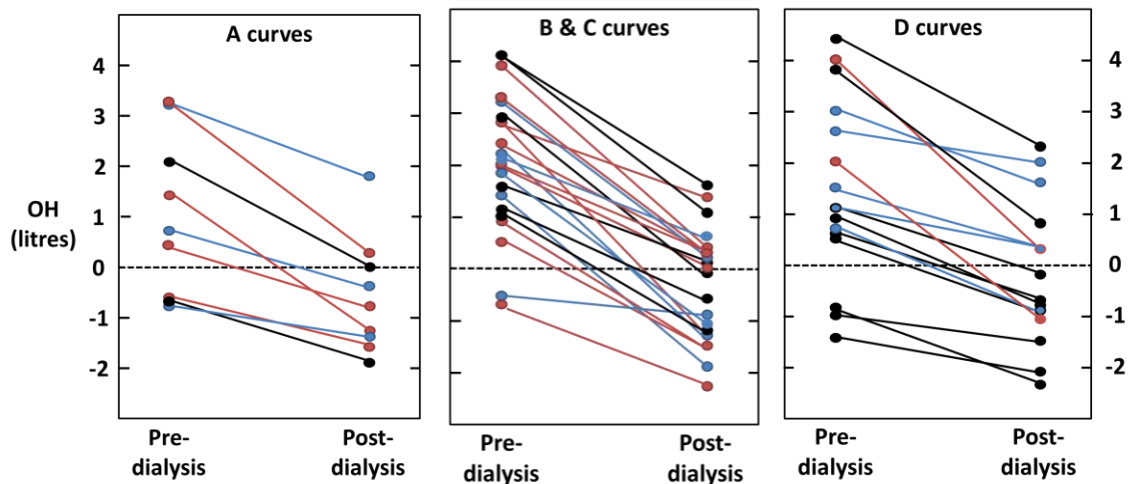
Figure 4-6: Pre and post OH measurements for patients with RBV plots of different curve shape as part of sensitivity analysis with a stricter definition of what constitutes a flat-line. Blue plots are from the lowest tertile of BMI from this cohort (16.5-24.9 kg/m^2), black are the middle tertile (25 to 29.9 kg/m^2) and red plots are the highest tertile (30 to 46 kg/m^2).

4.5 Discussion

4.5.1 Bioimpedance

At a population level, it is becoming well accepted that using BCM as an aide in guiding haemodialysis patient's fluid management improves outcomes (Onofriescu et al., 2014, Moissl et al., 2013). In an uncomplicated individual with relatively common characteristics, generic measurement protocols and decision making algorithms are likely to be beneficial. Yet there is a lack of data to support use of BCM outside this idealised case and there remains a great deal of uncertainty in utilisation of the technology in certain individuals. By making 8-lead BCM measurements before and after dialysis the impact of making alterations to the standard measurement configuration is defined which will allow these measurements to be made with greater confidence.

4.5.1.1 Alternative measurement paths

There is a need for alternatives to the standard measurement path. The haemodialysis population is highly comorbid and is disproportionately prone to amputations and tissue viability problems. There are many clinical conditions that are routinely seen in all haemodialysis units − e.g. heavily bandaged limbs, damaged skin, amputations − that can exclude patients from the standard BCM measurement approach. There are also complications that may not prevent a standard BCM measurement, but will significantly affect the quality of the reading − e.g. use of moisturisers, localised fluid accumulations or contact between body segments such as at the armpit. In either case, alternative pathways may allow measurements to be made on patients who would have otherwise have been managed without BCM. The potential for equivalent measured impedances across different paths was demonstrated two decades ago, when BIA measurements on the each side of the body was compared with cross measurements and relatively small differences in resistance and reactance were observed (Lukaski et al., 1985). However, the only investigation to apply this to BIS measurements using BCM was in preliminary work for this study, where it was shown that BCM-measured OH from the hand-to-hand path is an acceptable alternative to the standard path (Keane and Lindley, 2015).

Segment	Control		Pre-dialysis		Post-dialysis	
	R_E	R_I	R_E	R_I	R_E	R_I
Arm_ref (%) [a]	52	56	50	55	48	54
Arm_opp (%) [b]	52	59	52	51	50	53
Leg_ref (%)	44	47	44	51	45	50
Leg_opp (%)	44	48	43	55	45	52
Trunk_ref (%)	4.0	1.4	4.3	1.6	4.1	1.3
Trunk_opp (%)	4.0	1.3	4.0	1.5	3.8	1.6
Trunk upper (%)	0.2	-	0.3	-	0.1	-
Trunk lower (%)	0.01	-	0.03	-	0.03	-
Trunk_ref-opp (%)	4.0	-	4.1	-	3.8	-
Trunk_opp-ref (%)	4.0	-	4.1	-	3.8	-

Table 4-13: Relative segmental resistances as a proportion of standard whole body path resistances. Differences between the three groups for R_E and R_I were assessed using one way analysis of variance (ANOVA), with [a] indicating p<0.05 for R_E and [b] indicating p<0.05 for R_I

The results from healthy controls suggest there was no significant difference in OH across any whole-body path other than across the legs. Changing from a whole-body measurement to a hand-to-hand or cross measurement will involve substitution of one

limb for another and a change in the pathway through the trunk. Using the 8-lead BCM, the impedances of each limb individually can be isolated (see section 3.2.2) and the relative magnitudes of R_E and R_I in each limb expressed in relation to the resistance of the reference path (whole body measurement on dominant/non-VA side) (table 4-12).

For measurements of R_E, it can be seen that summing the segment resistances for the 6 whole body paths individually supports the results from the model: substituting limbs and trunk paths does not significantly alter the overall path R_E, except the leg to leg path which is noticeably lower (fig. 4-6) – suggesting increased OH as seen in the model results. The higher resistance of the arms seems to be compensated by a lower measured resistance for a current crossing the trunk from arm to arm than from arm to leg.

Estimation of R_I has much greater uncertainty (see section 2.3.2.5) and for segmental measurements, especially, in the trunk, the data were largely uninformative. It was not possible to infer much from segmental R_I data.
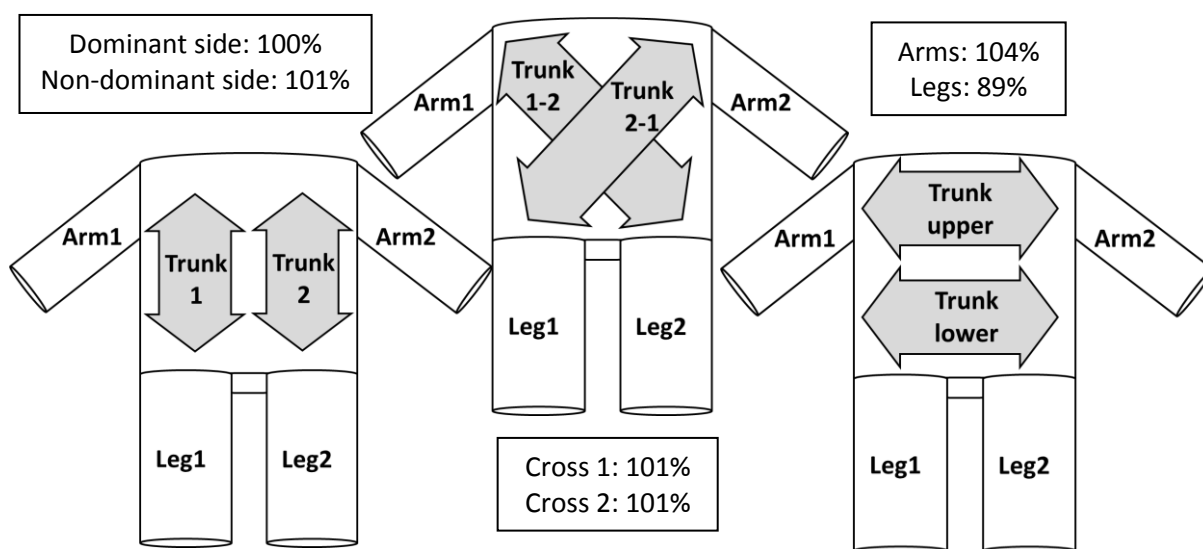
Figure 4-7: Re-calculating whole-body assessments of R_E expressed as a % of the standard measurement path (hand-to-foot on the dominant side) in healthy controls based on the data in table 4-8

The differences in ECF distribution between healthy controls and haemodialysis patients before and after treatment were investigated by assessing differences in R_E between the three groups in each limb (table 4-12). Differences in R_E between controls and dialysis patients will be related to differences in body composition as well as fluid

status, but differences between pre- and post-dialysis measurements will be purely related to differences in fluid. ANOVA suggested that the only significant results were for R_E in the dominant / non-VA arm and for R_I in the non-dominant / VA arm. The higher R_E in the dominant arm is consistent with lower OH in this segment compared to the vascular access arm and the legs, where fluid accumulates. Higher R_I in the non-dominant arm could be explained by a greater difference in muscle mass between the dominant and non-dominant sides in healthy controls compared to dialysis patients, which again is supported by the model results.

Considering the model results for lean and adipose tissue, the dominant side has significantly increased lean tissue and reduced adipose tissue as compared to the non-dominant side and the legs have increased lean tissue and reduced adipose tissue compared to the arms. This is consistent with previous work using BIA that demonstrated a decreased resistance in the dominant arm compared to the non-dominant arm (Bedogni et al., 2002) and a decreased resistance of the legs compared to the arms (Lorenzo and Andreoli, 2003). For the comparison between arms, the decreased resistance is consistent with greater muscle mass, due to the higher proportion of water in muscle than fat, while the decrease in resistance in the arms compared to the legs, the difference could be similarly related to differences in body composition or could be due to the differences in shape. Using limb cross sectional areas and lengths from a study by Zhu et al. and assuming the limbs to be cylinders, equation 2-8 shows that an arm has a resistance 1.2 times higher than a leg, assuming identical resistivities (mean arm circumference 26 cm and length 58 cm; mean leg circumference 39 cm and length 72 cm) (Zhu et al., 2000).

For haemodialysis patients, it has been suggested that the presence of vascular access in a patients' arm can bias measurements of OH and so guidance suggests avoiding these paths. This evidence comes from studies using different bioimpedance analysis techniques. Woodrow et al. used single frequency measurements to show decreased resistance in the fistula arm that was accompanied by increased arm circumference, suggesting increased excess fluid (Woodrow et al., 1997), although this was not replicated in paediatrics (Avila et al., 2015). More recently, two studies from a single centre using segmental BIA (SBIA) have reported that water and lean tissue content is different in the fistula and non-fistula arms (Panorchan et al., 2015, Booth et al., 2011).

However, none of the techniques used in these studies can adequately distinguish excess fluid from lean and adipose tissue, which leaves the possibility that the differences observed relate to differences in lean tissue alone rather than excess fluid, particularly given that fistulae tend to be placed in the non-dominant arm of patients. The results here confirm that the presence of a vascular access tends to increase the measured excess fluid, both in whole-body BCM and by looking at segmental R_E on fistula side compared to the non-fistula (table 4-9). However, the effect (0.4 litres) is similar to the reproducibility of the BCM (0.3 litres) so the clinical significance of this result is debatable.

4.5.1.2 Changes in measurement over a dialysis session

Pre- and post-dialysis BCM measurements allowed assessment of the changes in body composition parameters during haemodialysis, which can provide validation of the BCM models and provide evidence of the effect of making measurements post-dialysis.

In theory, the change in OH over dialysis should equal the change in body weight, while there should be no change in lean tissue or adipose tissue. However, dialysis has been shown to perturb fluid distributions (Shulman et al., 2001) which can influence whole-body bioimpedance measurements (Zhu et al., 1999). Fluid shifts from the limbs into the trunk manifest as an apparent decrease in ECF when measured by whole-body techniques and shifts from the trunk to the limbs as an increase in ECF (Lundvall et al., 1996). Haemodialysis has also been reported to change sodium concentration (Rees et al., 1999) and temperature (Huang et al., 2000), two variables that can affect bioimpedance at the microscopic level.

These results suggest that post-dialysis BCM-measured OH has a small bias of around 0.3 litres with limits of agreement of -1.9 to 1.3 litres, which supports similar measurements done as part of the validation of the BCM (Wabel et al., 2007b). However, El-Kateb et al. reported a similar dataset to that presented here (El-Kateb and Davenport, 2016) with contrasting results, including a significant bias and limits of agreement three times larger than those observed in this study. This discrepancy seems likely to be due to a lack of experience making BCM measurements and highlights the need for some expertise when making measurements (Lindley et al., 2015).

Post dialysis measurements of lean tissue and adipose tissue have non-significant changes over dialysis unlike the validation literature which suggests there is a significant difference (Wabel et al., 2007b).

Despite the good agreement between change in BCM-measured OH and change in weight, the model for OH did suggest there was a degree of ultrafiltration induced changes in fluid distributions, with a larger change in the lower limbs than the upper. This would suggest that more fluid is recruited from the legs than the upper body which is largely in agreement with previous work. Measurements over the first 75 minutes of dialysis using BIS (Shulman et al., 2001) and over the whole haemodialysis session using SBIA (Zhu et al., 2008) and SBIS (Chanchairujira and Mehta, 2001) have demonstrated a greater fractional change in fluid in the legs as compared to arms and trunk. Abbas et al (Abbas et al., 2014) showed that there is a greater % removal of ECV from the legs than other compartments but that as UF rate is increased, there is a preferential recruitment of fluid from the trunk, which correlated with RBV reduction. When RBV was included in the model as an interaction term, there was no significant difference so we were not able to replicate this finding, but the model was not powered to assess this effect.

One of the implications of the greater removal of fluid from the legs than the arms could be that the legs are the last segment that fluid is recruited from. One of the alternative bioimpedance techniques, calf bioimpedance, is based on the normalisation of calf ECF as an indicator of target weight (Seibert et al., 2013, Basile et al., 2015). However, if the legs are the final reserve of excess fluid, there is a chance that achieving normalised ECF fluid status may mean excessive dehydration in other segments, leading to poor perfusion of organs such as the heart, gut and brain and the associated consequences.

4.5.1.3 Summary

In summary, these data can allow BCM measurement and interpretation of the results with greater confidence. This will allow measurement protocols to be much more flexible and individualised than the manufacturer's guidance suggests and can guide future research. This is based on a number of key observations:

- Any of the whole body paths other than foot-to-foot can be used as an alternative to the standard path, with an acknowledgement of the additional uncertainty when interpreting the results.

- The difference in OH when a fistula is present is statistically significant, but arguably is not clinically significant when the uncertainty in other methods of target weight assessment are considered.

- Making BCM measurements post-dialysis introduces a negligible bias to OH measurements but does increase measurement uncertainty, which should be accounted for when interpreting such data. This uncertainty will be reduced with time after dialysis, such as asking patients to move off the dialysis station to be weighed, before a measurement is made.

4.5.2 Characterising RBV slope and refilling

The application of RBV to fluid management in haemodialysis has been inconsistent and developed on a limited evidence base. Yet, despite mixed results from studies looking at the use for improving fluid management, there remains significant interest in the technology and a number of new trials are currently being undertaken (Hecking et al., 2012).

Interpretation of RBV has largely been based upon qualitative categorisation of curves by the user or automated quantitative analysis as part of biofeedback tools. In general, the qualitative approaches are used for assessment of target weight and the automated quantitative analysis is used to prevent IDH. However, both approaches could be thought of as related to the 'probing' concept for fluid management, whereby patients' target weights are progressively lowered until the patient becomes symptomatic, at which point the target weight is increased or ultrafiltration rate reduced (Chazot et al., 2012).

The most common approach to qualitative analysis of RBV is that the patient is overloaded as long as they have a flat-line, or type 'A' curve. This prompts a reduction in target weight until there is evidence that vascular refill cannot keep up with ultrafiltration - manifested as a downward slope in the RBV curve (Lopot et al., 1996). Steuer et al showed that dry weight can be reduced in patients with a flat-line (Steuer et

al., 1998), but with the growing realisation of the harm associate with excessive ultrafiltration, achieving a lower weight is not an appropriate outcome measure in itself. There is less understanding around the other curve shapes. Santoro suggested that increasing ultrafiltration volume can move curves towards a D shape (Santoro et al., 1998) and a small study showed an association between a D type curve and fluid overload (Bonello et al., 2007). Hothi used receiver-operator-curve (ROC) analysis to show that the RBV change in the first hour is the most discriminatory for predicting IDH and that the average shape of the curve for complicated treatments was D-type as compared to a C-type for uncomplicated treatments (Hothi et al., 2008).

These data have for the first time demonstrated that a flat-line does not necessarily indicate fluid overload (fig. 4-4), which could potentially cloud one of the primary assumptions of RBV analysis. Robust physiological explanations of the characteristic shapes are lacking, however it is most likely that the 'D' shaped curves are related to redistribution effects in the early stages of a treatment session. It has been shown that the biggest reduction in central venous pressure occurs during the first hour of dialysis which is accompanied by an increase in myocardial perfusion, after which changes are less pronounced during dialysis and normalise afterwards (Thalhammer et al., 2015). A substantial proportion of the blood is contained in the splanchnic and splenic beds. Yu et al. were able to show that translocation of red blood cells from these beds peaks in the first hour of dialysis and by investigating patients with diffusion only dialysis as well as with ultrafiltration, they demonstrated that this change only occurred during ultrafiltration (Yu et al., 1997). Further work is necessary to investigate these factors as well as others to be able to make better use of RBV analysis.

The problem with using RBV curve classification is compounded by a lack of a consensus on definitions for how to categorise RBV curves. For example, an 'A' curve may be classed as a "flat-line" without any quantitative definition, while elsewhere a flat-line could be anything from 1.5% decrease per hour (Sinha et al., 2010) or even 5% per hour over dialysis (Steuer et al., 1994), This issue has led to the reporting of results with significant disparity. Lopot et al reported that less than 1% of recordings from their centre had the characteristics of a shape D slope (Lopot et al., 2000), yet Dheu et al report that 91% of readings in their paediatric unit had this shape (Dheu et al., 2009). In light of this, sensitivity analysis was undertaken to see how robust the association

between flat-line slopes and fluid depleted states were. This confirmed that even with the most conservative definition of a flat-line, up to 50% of subjects showing a flat-line can finish haemodialysis fluid depleted as measured by BCM (fig. 4-6). It is important that future work with RBV defines objective criteria for better assimilation of reported studies and better understanding of the technology.

There was no evidence of agreement in this study between RBV slopes from serial measurement sessions, up to two months apart. The use of BCM concurrently with RBV allows the opportunity to account for some of the variability between different measurement sessions, with a fluid status assessment, and absolute blood volume measurements could further explain intra-subject variation.

In summary, these data raises some questions about the perceived understanding of RBV dynamics during haemodialysis and highlights the need for further observational studies that use objective and reproducible classifications.

4.6 Limitations

The study was not powered to address the multiple comparisons made by the models - the sample size was based on comparisons between the two primary whole-body paths only. A larger sample would allow better estimates of these different effect sizes. It would also have been interesting to extend the analysis to a group of haemodialysis patients who are defined as being prone to IDH, to investigate the relationship between fluid distributions and dynamics and IDH. However, within the scope of this PhD, it was not feasible to extend the number of patients included in the study any further.

The sample of HD patients came from single renal unit and the generalisability of the results should be considered. The unit is somewhat distinct in the routine use of bioimpedance to guide fluid management over a number of years and sample characteristics show relatively normal hydration post-dialysis (table 4-6).

4.7 Conclusion and implications for practice

These observational data provide an increased evidence base for improving confidence in using BCM in a wider patient group and a wider range of practical situations.

Measurements should preferably be made pre-dialysis and avoid vascular access sites, but both measurements made post-dialysis and/or across vascular access sites can still provide reliable information on fluid status when interpretation acknowledges the additional uncertainty associated with these approaches. Furthermore, all whole-body BCM measurements other than foot-to-foot may be considered when measuring OH in situations where multiple complications are present.

The assumption that a flat-line RBV slope indicates fluid overload, appears to be incorrect, meaning that the widely accepted practice of reducing target weights in response to a flat-line could lead to unnecessary or harmful fluid-depletion. The use of BCM measurements with RBV allows changes in blood volume associated with intolerance to ultrafiltration to be related to a known initial fluid status. The complimentary nature of BCM and RBV supports further studies into how the information from both tests can be combined.

5 Characterising body composition monitor measurements of fluid status in subjects with high BMI

5.1 Objective

There are technical reasons why bioimpedance measurements in patients at high BMI may have increased uncertainty and a number of clinical studies have demonstrated associations between haemodialysis patients' fluid status and BMI. However, the influence of treatment-related factors on these clinical observations has not been well explored and further characterisation of BCM in this group is needed. To be able to differentiate treatment related associations between body habitus and fluid status from associations relating to measurement, this study made BCM tests in subjects with normal renal function as well as haemodialysis patients.

Research question 2: *Is the tendency for high BMI haemodialysis patients to finish dialysis fluid depleted according to the BCM associated with an artefact in the mathematical model or with systematic differences in the prescription and delivery of treatment?*

5.2 Introduction

There remains uncertainty about the use of bioimpedance in individuals with particular characteristics, including those at high BMI. However, much of the literature highlighting these issues are based on BIA devices using equations generated from subjects with relatively normal body habitus and these will not be accurate in extremes of body habitus (Das et al., 2003, Baumgartner et al., 1998).

BCM measurements, based on BIS, use a different approach to analysis which is based on a fluid model (Moissl et al., 2006) and a body composition model (Chamney et al., 2007) (see section 2.3.5). This analysis does avoid the need for population specific regression equations, but BMI may still confound BIS results through its relationship with shape and/or distribution of adipose tissue. In the fluid model used by the BCM,

Moissl et al. introduced a BMI correction for the calculation of fluid volumes to address the effect of changes in body shape (Moissl et al., 2006). However, this correction was based on a sample with a relatively narrow range of BMI and, furthermore, validation of the BCM device is limited at very high BMI (Ribitsch et al., 2012).

There are also a number of observational studies that describe associations between BMI and patients' fluid status in haemodialysis patients. A large dataset from our centre, based on the first BCM measurement made on haemodialysis patients after the technology was introduced into the unit, showed a significant inverse association between BMI and BCM-measured OH at clinically defined target weight (Lindley and Keane, 2014). Elsewhere, both pre- and post-dialysis OH have been shown to be negatively associated with BMI (Antlanger et al., 2013, Ribitsch et al., 2012).

The observed variation in BCM-measured OH at target weight is likely to be multifactorial. Patients with high BMI could be reluctant to increase target weights particularly if they are eligible for transplant listing if they achieve or remain below a specified BMI. It is also plausible that there are physiological factors involved. Although adipose tissue contains a much lower proportion of water than lean tissue, most of the water is extracellular so patients with high BMI may have a reserve of extracellular water that can be recruited into the circulation and effectively buffer larger ultrafiltration volumes.

5.3 Methods

5.3.1 Subjects

The study population consisted of three groups of subjects:

- A large dataset from a previous study provides a group of healthy subjects with normal renal function from the general population with average BMI (Wieskotten et al., 2013), which will be referred to as healthy subjects with normal BMI.

- A second group of healthy subjects with normal renal function who have high BMI were recruited from outpatient clinics for bariatric surgery patients within the local Hospital, referred to as healthy subjects with high BMI.

- Finally, a group of haemodialysis patients with high BMI were recruited from within the two hospital and six satellite haemodialysis units under the care of a large Teaching Hospital.

The healthy subjects with high BMI were greater than 18 years of age and had no history of renal failure. Renal function was assessed with an estimated glomerular filtration rate (eGFR), calculated by the local laboratory with the Modification of Diet in Renal Disease (MDRD) 4 parameter formula (Levey et al., 1999) using the most recent creatinine measurement for the individual (not being more than one month from the date of BCM measurement). Normal renal function was defined as an eGFR of greater than 60 ml/min/1.73m^2. BMI was calculated from measurement of height and weight at the time of data collection and patients with a BMI greater than 30 kg/m^2 were considered for inclusion.

The dialysis patients with high BMI were greater than 18 years of age, had been treated with haemodialysis for more than 3 months and had a BMI greater than 30 kg/m^2 calculated from the patients recorded height and their normally hydrated weight as defined by BCM.

5.3.2 Sample size

The sample of healthy subjects with normal BMI consisted of 1296 subjects with a mean BCM-measured OH of -0.1 litres and a standard deviation of 1.1 litres. Assuming a similar standard deviation for BCM-measured OH in healthy subjects with high BMI, recruiting 20 subjects will detect a difference of approximately 0.6 litres at the level of 5% type I error with 80% power.

The association between BCM-measured OH and BMI in haemodialysis is already established (Lindley and Keane, 2014, Ribitsch et al., 2012, Antlanger et al., 2013). A convenience sample of ten haemodialysis patients was based on the estimated prevalence of eligible patients in the study time-frame and is used for descriptive purposes only.

5.3.3 Procedure

Data for healthy subjects with normal BMI came from a previously reported study (Wieskotten et al., 2013).

Healthy subjects with high BMI had a standard and 8-lead BCM measurement as described in chapter 3. A visual assessment of body shape was made, based on changes in limb cross sectional area (CSA) from proximal to distal ends of the limb as described in figure 5-1.
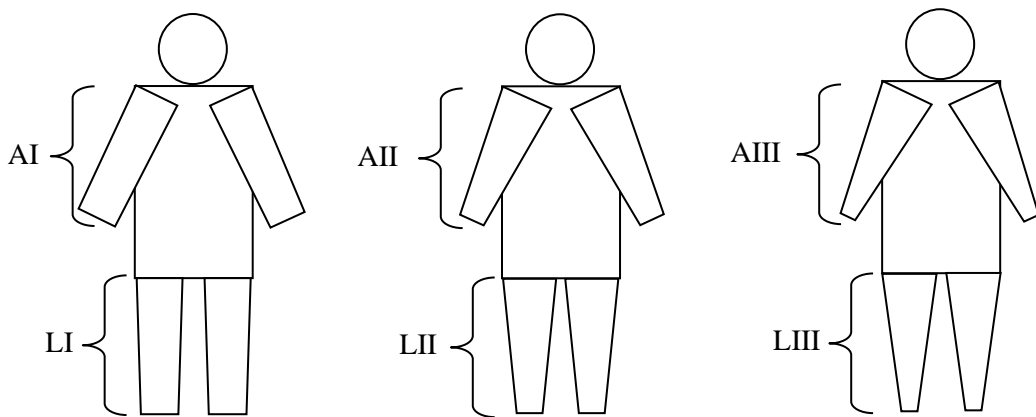
Figure 5-1: Visual assessment of the asymmetry of the limbs. Numerals increase with greater disparity between upper and lower CSA; A refers to the arms and L to the legs.

	AI	AII	AIII
LI	Low	Low*/Medium	Medium/High*
LII	Low*/Medium	Medium/High*	High
LIII	Medium/High*	High	High

Table 5-1: Calculating shape factors for analysis from the visual assessments. Control subjects were split into three groups (low, medium and high) whereas HD subjects were split between low and high as indicated by *.

Dialysis patients had a standard and an 8-lead BCM measurement made pre- and post-dialysis on a mid-week session as described in chapter 3. Ultrafiltration volume, blood pressure and any symptoms experienced were recorded. RBV monitoring was performed where possible as outlined in chapter 3, to identify patients' capacity for vascular refilling. For dialysis patients, shape factor was split into two categories rather than three, due to low numbers.

5.3.4 Statistical analysis

Continuous data was graphically assessed for whether it appeared to come from a normal distribution by plotting histograms. BCM-measured OH was described by mean (standard deviation) for normally distributed data and median (range) for non-normal data. A two sided Student's t-test was used to compare BCM-measured OH in healthy subjects with normal BMI and healthy subjects with high BMI.

The effect of shape was investigated using the Kruskal-Wallis test in the healthy subjects with high BMI and the Mann-Whitney test in dialysis patients. Comparisons were displayed using box-plots.

Haemodialysis patients' refilling capacity was categorised as adequate or insufficient based on the pre-dialysis BCM-measured OH, UF volume and the percentage blood volume drop as described in chapter 3.

5.4 Results

5.4.1 Patient characteristics

The characteristics of the patient groups can be seen in table 5-2.

	Healthy subjects with normal BMI	Healthy subjects with high BMI	Haemodialysis patients
Number	1327	20	10
Age, years	44 (17)	47 (12)	48 (37 to 73)
Sex (% male)	47%	25%	80%
BMI, kg/m^2	26 (5)	41 (7)*	40 (35 to 51)
Shape factor: - low	-	9	7
- medium	-	6	-
- high	-	5	3
OH, l. For HD: pre	-0.1 (1.1)	-0.1 (1.7)	1.8 (-1.9 to 4.6)
post	-	-	-1.8 (-3.8 to 0.5)
LTM (kg)	45 (12)	33 (10)*	49 (29 to 72))
ATM (kg)	24 (11)	84 (23)*	66 (46 to 107)
Ultrafiltration volume (litres)	-	-	2.2 (1.1 to 3.5)
IDFG (litres)	-	-	1.6 (0.4 to 3.0)
BP change (mmHg): systolic	-	-	4 (-30 to 48)
diastolic	-	-	3 (-15 to 28)
Albumin (g/L)	-	-	40 (33 to 43)

Table 5-2: Patient characteristics. Data are mean (sd) for normal data and median (range) otherwise. IDFG is the intradialytic fluid gain that preceded this session. Significant difference between healthy subject groups is indicated by *.

5.4.2 Subjects with normal renal function

Mean BCM-measured OH for healthy subjects with high BMI was -0.1 litres which was not different from the mean BCM-measured OH from healthy subjects with normal BMI (p=0.7; 95% CI: -0.3 to 0.5). Increasing the shape factor - that is a greater difference in limb circumference from proximal to distal ends - was associated with a lower BCM-measured OH (p=0.004) and an increasing BMI (p<0.001). This is shown in the upper chart of figure 5-2.
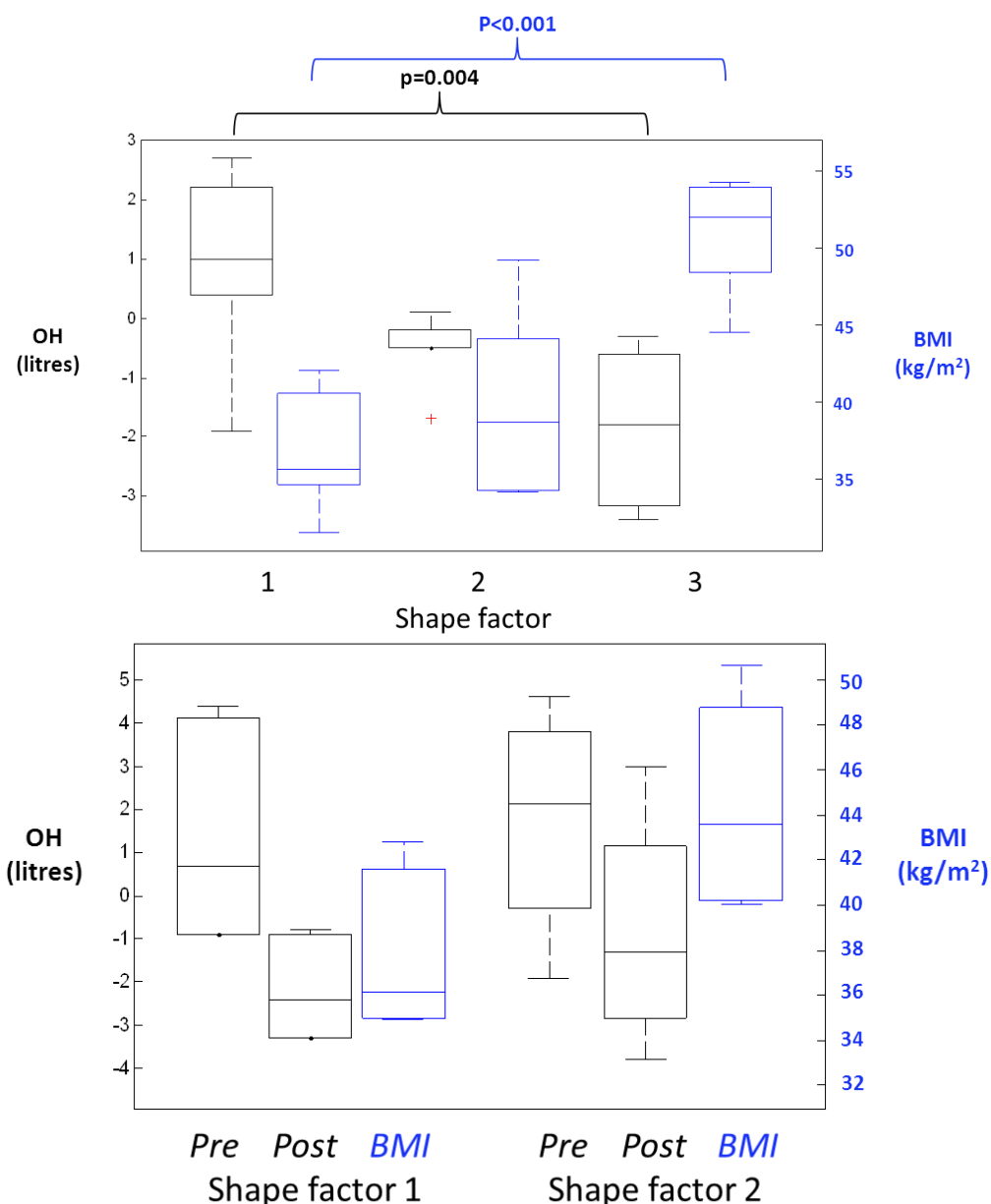
Figure 5-2: Association between shape factor and BCM-measured OH (shown in black) and BMI (shown in blue) for healthy subjects with high BMI (above) and dialysis patients with high BMI pre- and post-dialysis (below). Differences between shape factor groups were non-significant unless indicated.

5.4.3 HD patients

The, median BCM-measured OH values for dialysis patients were 1.8 and -1.8 litres pre- and post-dialysis respectively. The shape factor was not associated with pre- or post-dialysis BCM-measured OH ($p>0.5$; fig. 5-2). RBV was recorded for seven of the dialysis patients with high BMI (fig. 5-3) and 6 of these, including all patients finishing dialysis dehydrated, were deemed as having adequate refill. Median systolic and diastolic blood pressure changes were 13 and 3 mmHg, further supporting that refill was maintained (table 5-2).
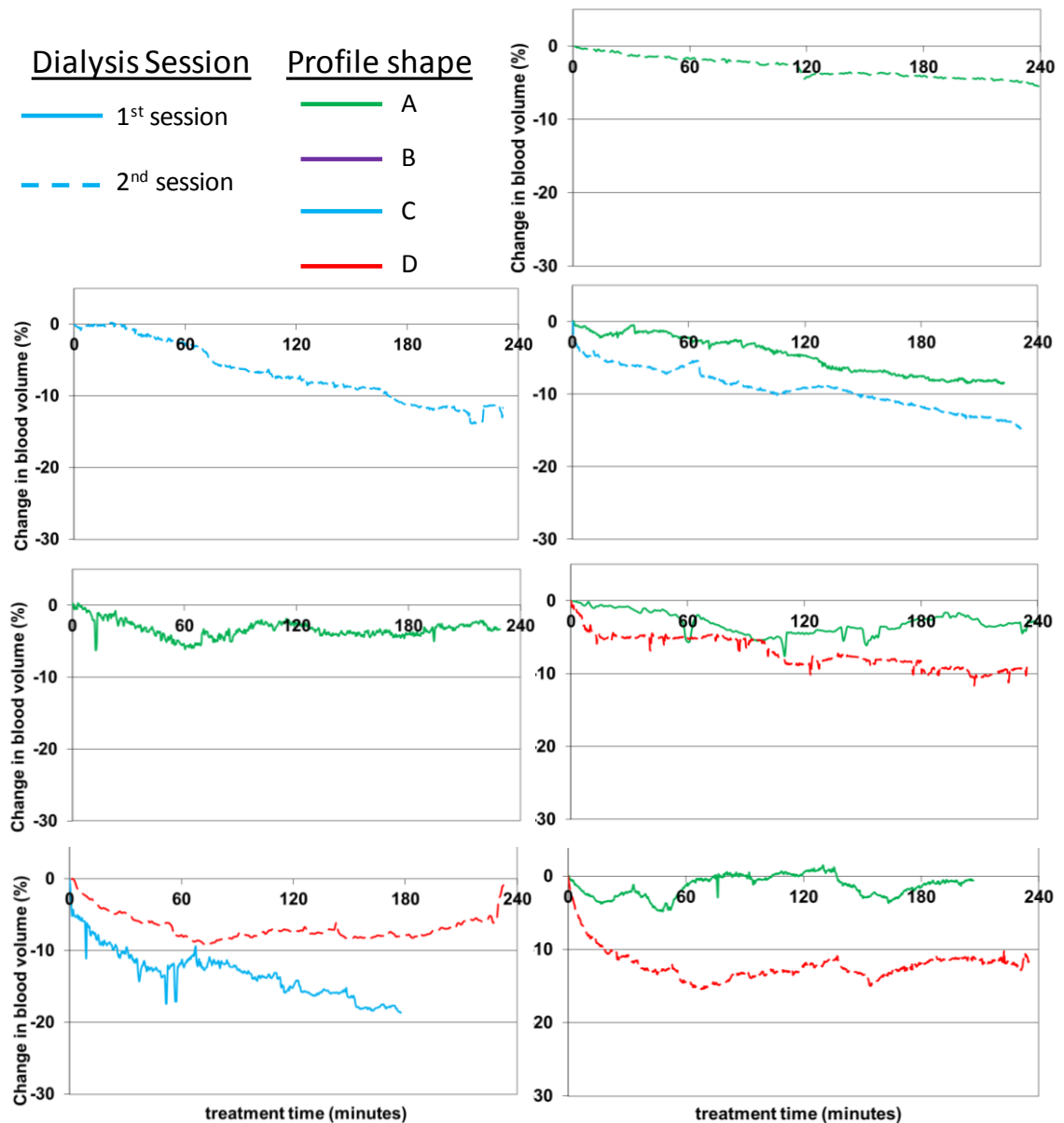
Figure 5-3: RBV curves for HD patients with high BMI. Solid lines are from HD session 1 and dashed lines from the repeat measurement session, HD session 2. Colours reflect different shapes of blood volume curve as defined in chapter 3. Not all patients had RBV measurements on session 1 and/or session 2.

5.5 Discussion

Previous studies around this topic have clearly shown associations between BMI and fluid status in haemodialysis patients. These results suggest that this is not related to a systematic bias in the BCM measurements and is due to treatment related factors, such as patient behaviour, physiology and the way the patients are managed.

The BCM assumes that the body is a series of segments which are represented as individual cylinders of uniform conducting material and the electrical properties of the segments are heavily dependent on the shape and composition of the tissue being measured (Foster and Lukaski, 1996). At extremes of body habitus, the shape of individual segments and the distribution of major body tissues such as lean and adipose tissue influence the measured electrical impedance. The BCM models include a factor to help correct for such effects (Moissl et al., 2006), which assumes that BMI is a surrogate measure of differences in shape and amount of adipose tissue. This correction has been shown to improve fluid estimation by BIS at relatively normal BMI, but there was a lack of validation of this BMI-based correction at high BMI.

Carter et al. investigated the effect of high BMI on measures of fluid by BIS (Carter et al., 2005). However, the results did not consider the accuracy of absolute volumes but rather dialysis-induced changes in ECF and the discrepancies observed highlight the dependence on distribution of body tissues and fluid. This is related to the fact that arms and legs are shaped very differently compared to the trunk and to each other (Foster and Lukaski, 1996) and so localised changes in body composition have different effects on a whole body measurement depending on which limb it occurs in. As such, whether adipose tissue increases are central visceral or peripheral fat accumulation and whether excess fluid removal occurs uniformly from all segments are important. The differences observed in the study by Carter et al. are likely to be related to the dialysis induced effect on fluid distribution, as the authors explain (Carter et al., 2005).

These results for healthy subjects with high BMI showed BCM-measured OH measurements that are consistent with healthy controls with normal BMI. There was no independent measure of the distribution of adipose tissue and the method of assessing shape was crude (in an attempt to minimise intrusion on participants). A significant

association between shape factor and BCM-measured OH in healthy subjects with high BMI was demonstrated which suggests that BCM-measured OH may be under-estimated in subjects with shape factor 3. However, this shape was also associated with higher BMI so BMI may be a confounding factor in the relationship between shape factor and BCM-measured OH. Further investigation of the effect of limb shape and a measure of the distribution of fat, such as waist-to-hip ratio, is warranted.

Haemodialysis patients with high BMI had the tendency to tolerate removal of large fluid volumes, finishing dialysis with extremely negative values of OH (up to -3.8 litres observed in this study). None of the sessions monitored during the study exhibited any acute symptoms of IDH and the RBV results suggest that the patients were able to tolerate dehydration while still being able to recruit fluid from the interstitium into the circulation (fig 5-3). It is possible there may be a "buffering" effect from extracellular fluid in the increased adipose tissue compartment (Waki et al., 1991). This suggests that reducing target weights in this patient group may be less likely to provoke symptoms and could help control excess fluid in patients with low residual renal function and large IDFGs.

5.6 Limitations

This study was limited in design by the lack of a gold standard measure of fluid status. The use of control subjects with normal renal function allowed an estimation of what the BCM would measure in subjects of similar body habitus were their kidney's functioning.

The assessment of body shape in this study was crude. The shape of limbs is likely to be one of the most important factors in framing measurement uncertainty with any whole-body bioimpedance devices and warrants further investigation. However, this study was an assessment of the clinical significance of measurements at the extremes of body habitus rather than an assessment of the underlying mechanisms involved.

5.7 Conclusion and implications for practice

In summary, there was no evidence of a systematic bias in BCM-measured hydration status in subjects with high BMI. In dialysis patients, significant levels of fluid

depletion were observed post-dialysis but RBV suggested refilling was maintained and blood pressure was stable. These patients may have a reserve of ECF to buffer ultrafiltration and it is possible that this feeds into the 'reverse epidemiology' association between BMI and survival in haemodialysis patients. Although further work is needed to fully describe the clinical significance of this observation and help to optimise and individualise BCM based fluid management strategies, this study suggests that low post-dialysis BCM-measured OH should be managed as real fluid depletion which should be limited to avoid compromising residual renal function.

6 **Characterising body composition monitor measurements of fluid status in malnourished subjects**

6.1 Objective

There is a demonstrated association between haemodialysis patients' fluid status and body habitus, suggesting patients with malnutrition finish dialysis overloaded. It is not clear to what extent this is related to differences in the prescription and delivery of haemodialysis treatment or whether it is commonplace for BCM to indicate fluid overload in malnourished individuals who have normal renal function. To be able to differentiate between these two effects, this study made BCM measurements in malnourished subjects with normal renal function as well as haemodialysis patients.

Research question 3: _Is the presence of malnutrition associated with BCM-measured fluid overload?_

6.2 Introduction

As discussed in chapter 5.1, studies have demonstrated an association between BMI and pre- and post- dialysis BCM-measured OH (Wizemann et al., 2009, Antlanger et al., 2013, Ribitsch et al., 2012) as well as between BMI and BCM-measured OH at clinically defined target weight (Lindley and Keane, 2014). In cases of malnutrition, overhydration defined by ECF to body weight ratio has been shown to correlate with subjective global assessment (SGA), a validated tool to aid interpretation of malnutrition risk in haemodialysis patients (Garagarza et al., 2013). Furthermore, in peritoneal dialysis patients, longitudinal increases in overhydration measured as ECF/TBW have been associated with worsening nutritional state as measured by SGA and by hand-grip measures (Cheng et al., 2005).

Again, in parallel with patients with high BMI, the observed variation in OH at target weight is likely to be multifactorial. Malnourished patients are likely to be encouraged to improve dietary intake and avoid weight loss, which may lead to a preference to

maintain or increase target weight. Smaller and malnourished patients lack the adipose tissue reserve that may be able to buffer larger ultrafiltration volumes as described in chapter 5.

There has been extensive validation of BCM-measured OH in subjects with normal renal function (Wabel et al., 2009) but the validation cohort were a generally healthy cohort with a limited BMI range (Ribitsch et al., 2012) and so it is of interest to investigate malnourished subjects with normal renal function. The BCM model has been applied to a malnourished indigenous population from Columbia and given values of OH proportional to the degree of malnutrition, getting as high as 6.2 litres (Chamney et al., 2007). However, this was a re-analysis of the original data which used dilution based estimates of ECF and ICF and was based on a population of indigenous Columbians from 1978 (Barac-Nieto et al., 1978). There is a need to corroborate these findings using BCM-measured fluid volumes and using a population more representative of current haemodialysis populations.

Patients admitted to the wards for the care of the elderly provided a suitable population for the study. They are assessed for malnutrition and renal function using eGFR, and are monitored to ensure that their fluid intake is sufficient to ensure they are not dehydrated. In order to compare malnourished subjects recruited from this population with subjects without malnutrition, a subgroup was extracted from the database of measurements used in the validation study for the BCM which matched for age range.

6.3 Methods

6.3.1 Subjects

The study population consisted of three groups of subjects:

- Healthy subjects with unclassified body habitus: Subjects with normal renal function aged 80 years old or over whose measurements were used in the validation study for the BCM (Wieskotten et al., 2013).
- Healthy subjects with malnutrition: Subjects with normal renal function came from an elderly care ward and were clinically defined as malnourished. (Note that in this context 'healthy' refers only to renal function)

- Haemodialysis patients with malnutrition: Haemodialysis patients from within the two hospital and six satellite haemodialysis units under the care of a large Teaching Hospital defined as malnourished by specialist dietetic input

The healthy subjects with unclassified body habitus were greater than 80 years of age, had no history of renal failure or cardiovascular comorbidities and were drinking freely.

In the healthy subjects with malnutrition, renal function was assessed using the eGFR, calculated by the local laboratory with the MDRD 4 parameter formula (Levey et al., 1999) based on the most recent creatinine measurement for the individual (not being more than one month from the date of BCM measurement). Normal renal function was defined as an eGFR of greater than 45 ml/min/1.73m^2 for subjects greater than 60, to account for the expected reduction in eGFR with aging (Glassock and Winearls, 2009). Definition of malnutrition was based on the Malnutrition Universal Screening Tool (MUST) (Kondrup et al., 2003). BMI was calculated from measurement of height and weight at the time of data collection. Subjects were drinking freely and had no history of renal failure.

The malnourished dialysis patients were greater than 18 years of age and had been on dialysis for at least 3 months to allow time for fluid imbalance on presentation to be corrected. Defining malnutrition using MUST is not validated for haemodialysis patients. A specialist dietitian identified patients for this cohort based on the following criteria:

- SGA score greater than 3 (Enia et al., 1993);
- Presence of two of the three core nutritional variables associated with malnutrition (unintentional weight loss, BMI less than 20 kg/m^2 and reduced dietary intake);
- Scoring greater than 2 on a novel malnutrition screening tool (LTHT) developed locally (Bowra et al., 2015).

BMI was calculated from the patients recorded height and their normally hydrated weight as defined by BCM.

6.3.2 Sample size

The sample of healthy subjects with unclassified body habitus consisted of 39 subjects with a mean BCM-measured OH of 1.3 litres and a standard deviation of 1.1 litres. Assuming a similar standard deviation for measurements of BCM-measured OH in malnourished healthy subjects, recruiting 20 subjects will allow a difference of approximately 0.9 litres to be measured at the level of 5% type I error with 80% power.

The association between BCM-measured OH and BMI in haemodialysis is already established (Lindley and Keane, 2014, Ribitsch et al., 2012, Antlanger et al., 2013). A convenience sample of five haemodialysis patients was based on the estimated prevalence of eligible patients in the study time-frame and is used for descriptive purposes only.

6.3.3 Procedure

Data for healthy subjects with unclassified body habitus came from a previously reported study (Wieskotten et al., 2013) using a standard BCM device measuring across the hand-to-foot path.

Malnourished subjects with normal renal function had a standard and 8-lead BCM measurement as described in chapter 3.

Malnourished haemodialysis patients had a standard and an 8-lead BCM measurement made pre- and post-dialysis on a midweek session as described in chapter 3. Ultrafiltration volume, blood pressure and any symptoms experienced were recorded.

6.3.4 Statistical analysis

Continuous data was graphically assessed for whether it appeared to come from a normal distribution by plotting histograms. BCM-measured OH was described by mean (standard deviation) for normally distributed data and median (range) for non-normal data. A two sided Student's t-test was used to compare BCM-measured OH in healthy subjects with unclassified body habitus and malnourished healthy subjects.

6.4 Results

The characteristics of the patient groups can be seen in table 6-1. The mean BCM-measured OH for malnourished healthy subjects was 1.3 litres. Although this was significantly greater than the mean for the whole population of healthy subjects with unclassified body habitus used to validate the BCM (mean BCM-measured OH = -0.1 litres), it was not different from the elderly subgroup aged over 80 years (p=0.5).

Although the inclusion criteria for malnourished dialysis patients did not include age, the five patients recruited were aged between 68 and 72 years. The post-dialysis BCM-measured OH for this group ranged from -0.1 to 4.5 litres, with a median of 1.8 litres.

	Healthy subjects (unclassified)	Healthy subjects (malnourished)	Haemodialysis patients (malnourished)
Number	39	20	5
Age, years	87 (5)	87 (5)	75 (68 to 82)
Sex (% male)	33 (%)	40%	60%
BMI, kg/m²	23 (4)	19 (7)*	20 (16 to 22)
OH, l. For HD: pre	1.3 (1.1)	1.3 (1.1)	2.5 (0.6 to 5.9)
post	-	-	1.8 (-0.1 to 4.5)
LTM (kg)	31 (7)	26 (7)*	27 (17 to 29)
ATM (kg)	32 (9)	22 (20)*	25 (13 to 32)
Ultrafiltration volume (litres)	-	-	1.6 (0.7 to 2.5)
IDFG (litres)	-	-	1.6 (0.2 to 2.3)
BP change (mmHg): systolic	-	-	13 (-13 to 21)
Diastolic	-	-	3 (-23 to 24)
Albumin (g/L)	-	-	36 (27 to 43)

Table 6-1: Patient characteristics. Data are mean (sd) for normal data and median (range) otherwise. IDFG is the intradialytic fluid gain that preceded this session. Significant difference between healthy subjects is indicated by *.

6.5 Discussion

These results suggest that some degree of fluid overload as measured by BCM is common in elderly subjects irrespective of whether they come from the general population or from a cohort with a clinical diagnosis of malnutrition.

The presence of oedema and expanded fluid volumes is relatively common in protein deficient states of malnutrition (Alberda et al., 2006) and has been linked to hypoproteinemia and leaky capillaries (Guirao et al., 1994, Fiorotto and Coward, 1979). BCM-measured OH has been shown to be associated with the severity of malnutrition

by SGA in subjects with normal renal function, but despite being statistically significant the effect sizes were very low and clinically not of great importance (Wieskotten et al., 2008). However, this study was not designed to assess this association and the numbers of subjects with high degrees of malnutrition appear to be low. It is also interesting to note that one patient from the group of healthy subjects with high BMI recruited for chapter 5 had bariatric surgery during the study and BCM measurements before and after surgery indicated a measured increase in OH of 3 litres over a 3 month period. This may have been the consequence of nutritional defects following bariatric surgery as reported elsewhere (Dalcanale et al., 2010) which has previously been associated with expanded extracellular fluid and fluid overload (Mazariegos et al., 1992, Leone et al., 2000).

Although the BCM models include a factor to adjust OH for age, measurements in the subgroup of healthy subjects aged over 80 indicates that the correction is insufficient at this age. Further inspection of the database suggests a tendency to develop increased BCM-measured OH at ages above 70 years of age (Wieskotten et al., 2013). This systematic increase in BCM-measured OH could be explained by changes in body composition that affect the average hydration of lean and/or adipose tissue, the fundamental constants in the BCM model (Chamney et al., 2007). Variation in the constants could result from changes in the proportion of the different tissues that make up the total lean and adipose tissue or from changes in the ECF and ICF content of the tissue at normal hydration. Measurements of FFM using DEXA and body cell mass using total body potassium counting have suggested that there is a normal increase in the hydration of lean tissue with age (Gallagher et al., 1996). There is also a trend of increasing number of comorbidities with age (Tomson et al., 2007) and an association between number of comorbidities and BCM-measured OH (Antlanger et al., 2013), which suggests that comorbidities are another important variable when trying to explain these relationships. A possible explanation of this is an increase in the fat within muscle, intermuscular and intramuscular adipose tissue (IMAT). Age, disease, injury, inactivity and obesity are all associated with increased levels of IMAT (Addison et al., 2014). If IMAT has a higher ECF content than subcutaneous or other visceral fat, this ECF will be interpreted as over-hydration when IMAT levels are higher than expected by the BCM model.

If BCM-measured OH is associated with IMAT, the increase in IMAT associated with muscle wastage would be expected to lead to a higher BCM-measured OH in malnourished subjects. However, for elderly subjects without renal failure BCM-measured OH was not significantly different in the group with malnutrition defined by the MUST score and the unselected group who would be expected to have a range of nutritional states. To obtain a conclusive answer to the original research question would require the collection of BCM-measured OH in a younger cohort with a diagnosis of malnutrition and without any concomitant disease, injury or restriction on activity which are likely to confound the results. Such a cohort would be difficult to find and unrepresentative of the haemodialysis population.

The small sample of haemodialysis patients classified as malnourished in this study had BCM-measured OH when at their target weight that is consistent with previously reported data for patients with low BMI (Lindley and Keane, 2014). To some extent, the wide variation in their post-dialysis BCM-measured OH may be explained by the differences in the many factors associated with age and fluid status.

If some or all of the measured excess fluid is actually confined within muscle it will not increase cardiovascular risk, as with intravascular excess fluid. Consequently, attempts to reduce target weights in these patients to levels of 'normal' hydration as measured by BCM or below may not be necessary and indeed may be excessive with the potential to cause IDH episodes. In the absence of experimental measurements of the actual ECF and ICF in IMAT and in subcutaneous and visceral adipose tissue, it should be possible to establish whether or not the measured excess fluid is able to transfer into the circulation and lead to harmful excessive intravascular volumes. Further investigations making use of absolute and relative blood volume measurements can help to explore the degree of BCM-measured OH that can be considered 'normal' for these subjects and what information is required for individualised fluid management.

6.6 Limitations

As described in section 5.6, the lack of a gold-standard assessment of OH limited this study.

The sample of malnourished control subjects was predominantly elderly, which was related to the hospital wards that were used to recruit these subjects. Ultimately, the effect of malnutrition on BCM-measured OH could not be distinguished from the association between age and BCM-measured OH in subjects greater than around 70 years of age. However, recruiting younger malnourished subjects poses practical and ethical issues.

6.7 Conclusion and implications for practice

Although the study failed to answer the original research, the data collected and the subgroup analysis of the BCM validation study indicate that the presence of excess fluid as measured by BCM is common in elderly subjects. There will be some differences depending on individual comorbidities, however in general, older, and more comorbid patients, and patients with muscle wastage due to inactivity, should not be expected to achieve a post-dialysis fluid status as low as younger, fitter, more active patients. Fluid management strategies using BCM-measured OH that are designed for use across all patients may be inappropriate in these individuals. A patient with left-sided heart failure will usually need to achieve the lowest tolerable hydration post-dialysis to avoid breathlessness during the interdialytic period. In this case, the BCM-measured OH is mainly used to guide patients back to target after weight gain or loss. For other patients, especially those with a low IDFG, as if often the case for those with low BMI or poor nutritional state (Kalantar-Zadeh et al., 2009), a degree of BCM-measured overhydration post-dialysis may not be a problem, whereas IDH is known to be a risk. Use of intravascular blood volume measurements with RBV should help confirm to what degree this excess fluid is confined to the tissues or is recruited to the circulation. There is a real need for further work to help individualise fluid management in the cohorts discussed here.

7 Managing localised oedema in haemodialysis: can it be monitored by BCM and mobilised by simple interventions

7.1 Objective

Patients with heavily localised oedema present a problem both to the use of BCM for guiding fluid management and to achieving prescribed ultrafiltration goals during treatment. To inform management strategies, there is a need for a better understanding of the effect of localised fluid on different BCM measurement paths and of whether it is possible to monitor changes in localised oedema during haemodialysis. For successful implementation of these strategies, there is a need for better understanding of the ability to mobilise and remove oedematous fluid during haemodialysis. This preliminary study addressed these two knowledge gaps and additionally evaluated the efficacy of two simple interventions for mobilising localised fluid into the circulation by assessing tolerance of ultrafiltration and the reduction in localised fluid.

Research question 4: (a) Can BCM measurements be used to assess and monitor localised oedema and (b) Can easily administered, non-invasive interventions mobilise persistent localised oedema so that it can be recruited into the circulation?

7.2 Introduction

7.2.1 Fluid accumulation in haemodialysis patients

Haemodialysis is an intermittent replacement of kidney function which is based on several treatment sessions separated by longer periods in which toxins and fluid will accumulate in the body to different degrees depending on an individual's residual renal function.

The fluid in the intracellular compartment remains relatively stable and the excess fluid accumulation is predominantly located in the ECF, including in the plasma volume (Fisch and Spiegel, 1996). However, the plasma volume has a finite capacity so the

majority of the excess fluid accumulates in the interstitium, where the skin and subcutaneous tissue initially act as a reservoir (Gunga et al., 1994) and not until excess fluid becomes around 10% of body weight is clinically perceptible oedema manifested (Mees, 1995).

Disruption to the normal mechanisms involved in fluid homeostasis, including poor lymphatics, damaged microvascular membranes and elevated hydrostatic pressures can cause localised oedema formation. These localised accumulations often present in the lower limbs, due to the effect of gravity, and can cause problems for both the use of BCM as a diagnostic tool and to the management of fluid status.

7.2.2 Localised oedema and bioimpedance

Localised fluid accumulations are most likely to occur in the arms and legs because of the limited drainage capacity (Board and Harlow, 2002). As the arms and legs have significantly smaller cross sectional areas than the trunk, localised changes in the impedance in these segments can disproportionately affect whole-body bioimpedance, leading to measurements made across oedematous limbs which indicate unrealistically high ultrafiltration volumes for patients.

It is also much more likely that measurements across oedematous tissue will suffer from artefacts, causing the display of an error message or inaccurate results. There are a number of reasons for this. The swollen tissue is more likely to cause a short circuit for the measurement signal between the legs or at the underarm. The tissue viability issues relating to oedematous tissue can cause sores, broken skin or use of heavy moisturiser on the site of electrode placement, all of which are issues.

The effect of measuring across different paths in subjects with localised oedema has not been well described. This information can feed into individualised fluid management strategies to be used alongside clinical assessments which can help to avoid inappropriate decision making in these subjects.

7.2.3 Mobilisation of localised oedema during haemodialysis

To understand how best to manage the fluid status in patients with localised oedema, there is a need to examine whether the established risk factors that drive prescription of target weights are applicable in this group. In particular, the evidence relating to the association between fluid overload and cardiovascular risk, and between excessive fluid removal and multiple end-organ damage, may not be directly applicable to this population.

The cardiovascular risk associated with fluid excess is primarily due to left ventricular hypertrophy and arterial disease (Meeus et al., 2000). Although there are many factors involved in these processes, both are significantly associated with fluid overload (Seibert et al., 2013, Lin et al., 2005). Left ventricular hypertrophy is an adaptation associated with increased work in response to volume and pressure overload (Meeus et al., 2000). Hypervolemia is associated with arterial dilation and hypertrophy of the vessels, leading to reduced compliance (London et al., 1996), and these have been linked to all cause mortality in haemodialysis patients (Blacher et al., 1998). Without knowing the refilling characteristics of oedematous tissue it is not clear to what extent localised fluid is involved in arterial dilation, volume and pressure overload and other processes involved in the cardiac and arterial damage. As such, the influence of localised, extravascular fluid overload on cardiovascular risk is not known.

At the other extreme, risk factors associated with excessive fluid removal are primarily related to intravascular fluid depletion and poor perfusion of end-organs (McIntyre, 2010). An improved understanding of the mobilisation of localised oedema is key to managing these subjects in such a way that intravascular fluid depletion is avoided.

7.2.4 Improving mobilisation from localised oedema

As discussed in section 2.1.2.4, oedema is the result of capillary filtration rate exceeding lymphatic drainage and it manifests as localised fluid accumulation. Established physical therapies for treating oedema include compression hosiery, intermittent pneumatic compression (IPC), massage, exercise and elevation (Lymphology, 2003, Ogawa, 2012). Compression hosiery and elevation are part of routine care for haemodialysis patients with oedema, while massage alone is deemed to be ineffective

and potentially harmful (Lymphology, 2003). Exercise and IPC are not part of routine management of oedema in haemodialysis patients and provide further opportunities to mobilise fluid in these patients.

IPC involves the cycling of positive pressure to the limb via the inflation of a cuff, sleeve or boot, increasing the hydrostatic pressure of the interstitial fluid. This leads to an increased arterio-venous pressure gradient, increased arterial flow, cardiac venous return and refilling of the blood volume (Tai et al., 2013, Chen et al., 2001). IPC has been applied to haemodialysis patients previously. A case series has described how four patients who regularly suffered from cramp were given IPC during dialysis which completely stopped the symptoms in all cases (Ahsan et al., 2004). A randomised control trial of IPC for preserving central blood volume (CBV) during haemodialysis (Tai et al., 2013), stratified patients by intradialytic hypotension-prone status, finding no effect on preservation of CBV but a significant impact on the volume of fluid removed during dialysis.

Exercise can improve fluid mobilisation through activation of the muscle pump improving venous and lymphatic return. The movement of the limbs creates pressure changes in the tissue, providing a rhythmic opening of the lymphatics and increasing lymph transport (Partsch, 2008). Exercise also increases capillary filtration in exercising tissue, which raises the interstitial pressure and drives lymph flow. The lymphatics which encircle muscles are subjected to arterial pulsations due to increased cardiac output during exercise and also the contracting muscle fibers help to propel fluid away from exercising muscle (Korthuis, 2011). The lymph vessels are lined with smooth muscle cells and have one-way valves which aid the flow of lymph to the central circulation (Korthuis, 2011).

There has been little published on the effect of exercise on microvascular fluid exchange. Olszewski showed that during two hours of ergometer cycling there was an 83% increase in lymph flow - ascribed to the muscle pump driving lymph flow and an increase in skin lymph formation due to the exercise - and that a two hour period of venous stasis caused by limiting the muscle pump reduced lymph flow by 50% (Olszewski et al., 1977). Stick et al. looked at reductions in venous pressure due to muscle pump activation and showed that ergometer exercise decreased venous pressures

(Stick et al., 1993a). A more passive approach to activation of the muscle pump is the use of electrical stimulation to mimic the action potentials associated with muscle contraction. Plantar stimulation of the calf in haemodialysis patients with consistent intradialytic hypotensive episodes improved vascular refilling and achievement of ultrafiltration targets (Madhavan et al., 2009). In chronic heart failure patients, daily stimulation of the calf muscle reduced oedema by on average 0.5 kg over one month (Pierce and McLeod, 2009). Stimulation of lower leg muscles minimised the increase in lower limb volume after motionless standing, illustrating the activation of the muscle pump in the stationary position (W Man et al., 2003).

7.2.5 Study aims

This study aimed to provide better evidence for making and interpreting BCM measurements and to evaluate simple interventions for improving fluid mobilisation in patients with localised oedema. This was based upon three principle objectives:

- Characterise differences in BCM measurement paths in subjects with localised lower-limb oedema.
- To evaluate whether localised lower-limb oedema is mobilised during haemodialysis.
- To evaluate whether simple interventions can improve fluid mobilisation from localised lower-limb oedema during haemodialysis.

The results will feed into fluid management strategies for this group of patients.

7.3 Methods
7.3.1 Study design

This study was designed as a preliminary investigation into three knowledge gaps in the management of fluid status in subjects with localised oedema, without intending to explore the mechanistic effects involved. This objective was consistent with the overarching aim of this thesis to improve the evidence base underpinning routine BCM based fluid management.

A feasibility cross-over study design allowed the potential of the interventions to mobilise localised fluid to be evaluated while providing observational data to address the other two objectives. The cross-over design is particularly suited to this study in a

population with a chronic condition. For pilot or feasibility studies, a sample size of 12 has been suggested as a minimum target for parallel arm trials (Julious, 2005). The same study demonstrates that, for cross over studies, the gain in precision for a unit increase in sample size at around 6 subjects in cross over studies is similar to parallel arm trials with 12 subjects. As such, 6 was taken as the target sample size for this study.

7.3.2 Participants

Participants were recruited from the haemodialysis units of a large Teaching Hospitals Trust. Eligible patients had lower limb oedema as defined by pitting and were identified by staff responsible for their care as having problems with routine fluid management.

Exclusion criteria included having less than 3 months haemodialysis vintage, being less than 18 years of age, the inability to be weighed, amputations and the presence of metallic implants.

To achieve the planned sample size, units were approached successively and nurses, dietitians and doctors responsible for care in each unit were asked to identify individuals who had evident lower limb oedema. These patients were then screened against the inclusion/exclusion criteria and approached if appropriate.

7.3.3 Study protocol

The study period ran for 16 weeks as described in figure 7-1 and 7-2. Monitoring and interventions took place on the middle session of the week. Two weeks of baseline measurements and two weeks of follow up measurements where participants received treatment as normal formed the control period. In between, the two arms alternately had compression followed by stimulation or, stimulation followed by compression, with concurrent monitoring. The intervention periods were followed by a two week washout period. Participants were randomised to one of the two arms, A and B, by use of concealed envelopes.

During the IPC phase, participants received compression of the lower limbs using a Flowtron Hydroven 3 (Arjo-Huntleigh, Bedfordshire UK). The compression sleeve was placed around the calves of both legs, with the bottom of the sleeves positioned around

the participants' ankles. The amplitude of the contractions was set to 40 mmHg based on manufacturer's recommendations. Patients were encouraged to use the devices for as much of the haemodialysis session as possible and adherence was recorded.
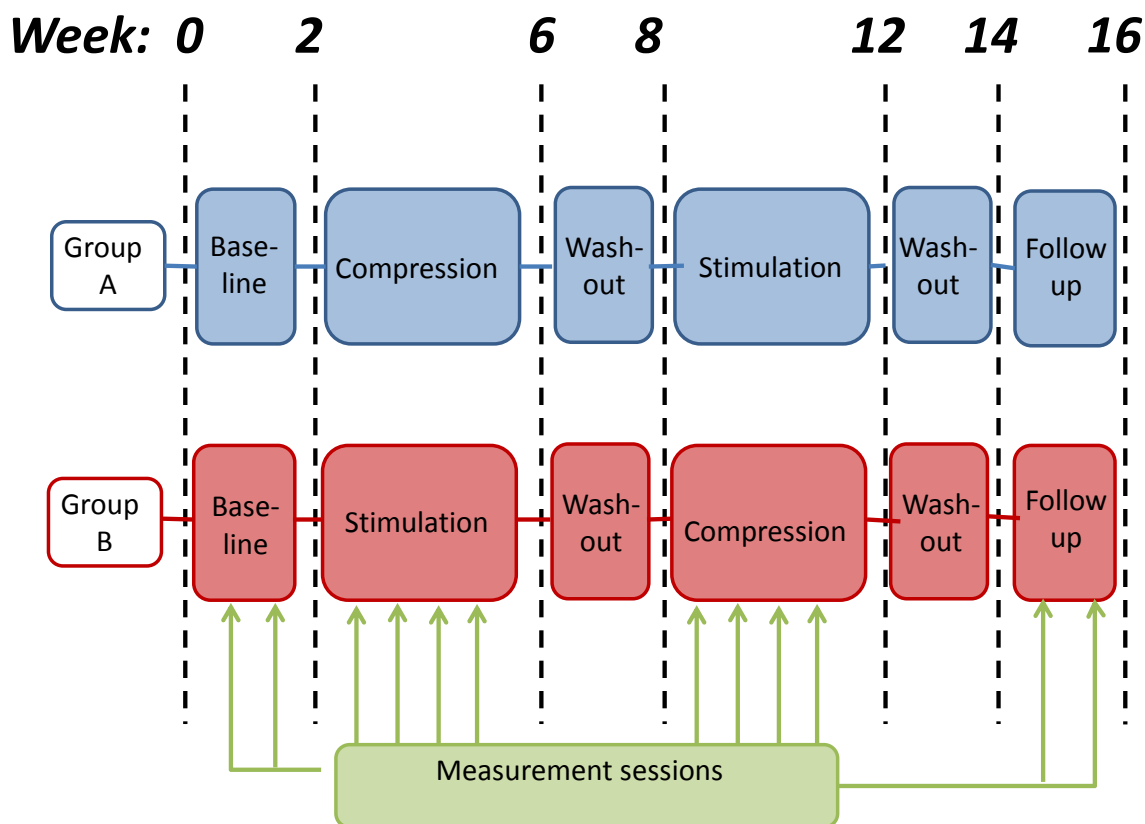
Figure 7-1: Study protocol

During the stimulation phase, participants were encouraged to use the device for two sessions of 30 minutes of stimulation, not being during the first hour of dialysis. The primary device used to deliver stimulation was the Circulation Booster (Actegy Health, Bracknell, UK), which consists of a plate with two pads that can be placed under a subjects' feet. It produces a series of phases of stimulation, which target different muscle groups in the lower leg. It has a remote control to change signal amplitude and is very user friendly. Where this device was not suitable, the more flexible Neurotrac Sports XL (Verity Medical, Hampshire, UK) was used. This is based on applying adhesive electrodes above specific muscle groups on the lower leg and applying a signal via a battery operated control box. Electrodes were placed as per manufacturer's guidance to target the calf muscles and a pre-programmed stimulation cycle that was designed to improve blood flow. For both devices, the strength of the stimulations was slowly increased until the participant starts to feel some form of discomfort and then the

setting was reduced by two units. The participant could control the strength of the contractions at all times and could reduce this if they felt any discomfort.

At each measurement session, a number of observations were recorded:

- Standard and 8-lead BCM measurements were made pre- and post-dialysis
- The Crit-line blood volume monitor was used to measure RBV change and refilling capacity as described in chapter 3
- The extent of oedema was recorded pre- and post-dialysis by measuring lower-limb circumference at a reproducible location on the lower calf.
- Patient symptoms
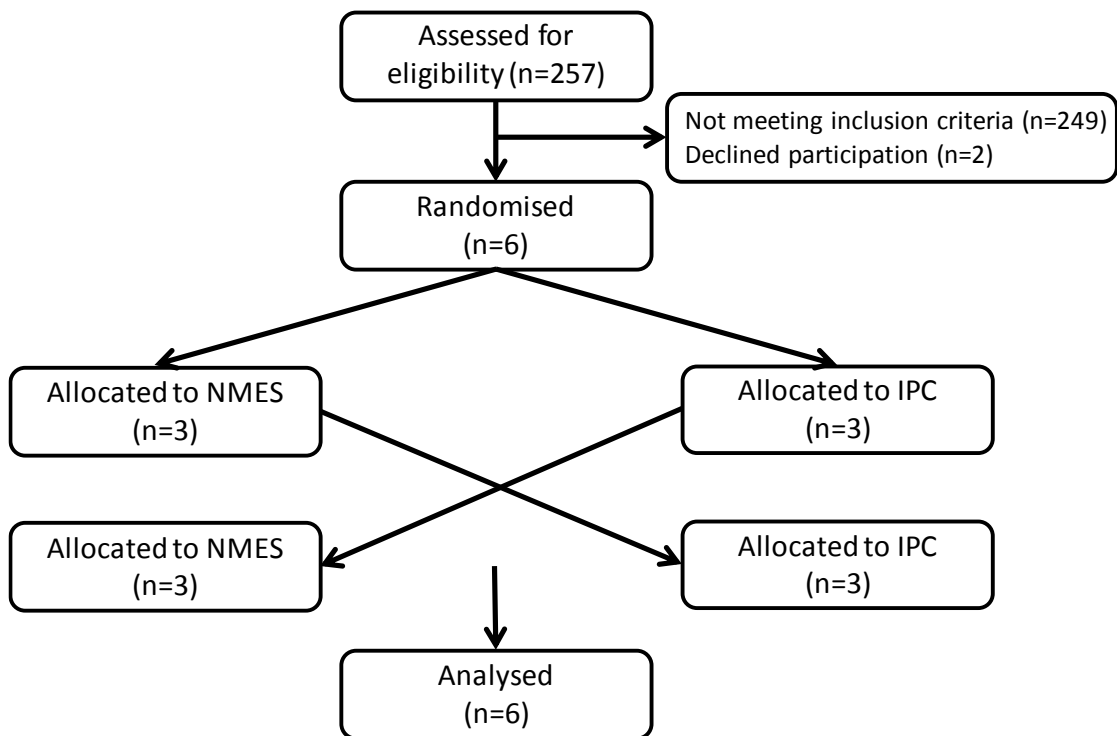
Figure 7-2: Study flow diagram

7.3.4 Outcome measures

7.3.4.1 Characterising BCM measurements in patients with localised oedema

Reliability of BCM measurements in these patients was assessed by reviewing all measurements for artefacts and calculating the proportion of measurements that were artefact free. Significant artefacts included measurements that had:

- divergence of data points from the fitted curve
- a curve with no clear parabolic shape
- abnormally high reactance

and that also had unexpected body composition values.

The results from chapter 4 describe minimal differences in BCM-measured OH between the main whole body paths. Given the similarities between the two sides and the cross measurements, to characterise BCM-measured OH in patients with localised oedema it was sufficient simply to characterise the difference between hand-to-foot measurements across the effected region and hand-to-hand measurements. This difference in OH was called the oedema bias and was calculated as described in equation 7-1.

Equation 7-1: $\qquad oedema\ bias = OH_{Hand\ to\ foot} - OH_{Hand\ to\ hand}$

7.3.4.2 Mobilisation of lower limb oedema for ultrafiltration during haemodialysis

The ability to mobilise the lower limb oedema was measured by:

Reduction in limb circumference – The extent of oedema was assessed pre- and post-dialysis by circumferential measure of the oedema at a reproducible point close to the site of most prominent oedema.

Vascular refilling capacity – Absolute change in RBV and a classification of refilling (see chapter 3) were recorded.

Fluid shift – The difference between the change in BCM-measured OH using the hand-to-hand path (ΔH_H) and the change in BCM-measured OH using the standard whole-body path (ΔH_F) during dialysis (equation 7-2).

Equation 7-2 $\qquad Fluid\ shift = \Delta H\_F - \Delta H\_H$

7.3.4.3 Interventions for improving fluid mobilisation from localised oedema

The potential of each intervention as a therapy was assessed by:

Rate of recruitment – The rate of recruitment was based on the proportion of patients screened that were suitable for inclusion and consented to the study.

Adherence to the interventions – Adherence of the participants to the interventions was measured using the fractional time of prescribed treatment that was carried out over the sessions of interest. For compression, this meant the proportion of the total treatment time during the four sessions that compression was used was recorded. For stimulation, this was the proportion of the hour of prescribed treatment from each study session.

Post-dialysis fluid status – BCM-measured OH from the post-dialysis measurement.

Patient symptoms – Any interventions given for treatment of IDH were recorded. Patients were asked at the end of every session if they experienced any symptoms related to IDH (cramping, dizziness, nausea or excessive tiredness). If any of these markers were present, the session was classed as a symptomatic session.

Adverse events – Any adverse events that occurred during the interventions were recorded.

Results were summarised with descriptive statistics.

7.4 Results

7.4.1 Included subjects

Subject	Age (years)	Sex	Comorbidities	Primary renal diagnosis	RRT vintage (months)	Ethnicity
1	70	M	N/a	CKD (unknown)	30	White
2	81	F	Malignancy	Traumatic or surgical renal loss	48	White
3	59	F	Smoking Cerebral palsy	Pyelonephritis	99	White
4	45	F	N/a	Pyelonephritis	65	White
5	57	M	Heart failure Diabetes Neuropathic ulcers	Diabetic neuropathy	21	Black
6	58	F	Myocardial infarction Claudication	Renovascular disease	22	White

Table 7-1: Patient characteristics

The characteristics of the patients included in the study can be seen in table 7-1. Even with relatively strict inclusion criteria, it can be seen that the sample includes patients with very different comorbidities and individual needs. Only two of the patients were fully ambulatory, with three predominantly using wheelchairs and one subject unable to move the lower limbs at all.

7.4.2 Characterising BCM measurements in patients with localised oedema

Considering the primary 6 whole body paths measured using the 8-lead BCM, at least one of the Cole plots had clear artefacts in 12% of measurements. The prevalence of artefacts for each subject individually can be seen in table 7-2, along with suggested sources of the artefact.

There was a consistent bias when measuring hand-to-foot compared to hand-to-hand in subjects with localised oedema, both pre- and post-dialysis (table 7-3). The mean bias from the 6 subjects for each phase and for all phases were tested against a null of zero difference and each case was significant other than one subject with missing data.

Subject	Prevalence of artefacts	Proposed source of artefacts
1	17%	Heavy use of moisturiser; flaky skin.
2	12%	Use of moisturiser; high BMI leading to 'short circuit' effects between the legs and under the arms.
3	33%	Use of moisturiser; poor mobility secondary to cerebral palsy leading to 'short circuit' effects between the legs and under the arms.
4	0%	N/a
5	21%	Poor foot care; significant tissue viability issues; previous foot ulcers and scarring.
6	0%	N/a

Table 7-2: Details of the prevalence of artefacts on 8-lead BCM measurements

Phase	Measure	1	2	3	4	5	6	All subjects
Control	oedema bias (Pre) (litres)	1.9	1.0	1.2	1.5	1.4	1.1	1.4*
	oedema bias (Post) (litres)	1.8	1.4	1.2	1.6	0.4	0.7	1.1*
	fluid shift (litres)	0.1	-0.3	1.0	0.0	0.8	0.2	0.3
IPC	oedema bias (Pre) (litres)	1.9	2.5	1.4	2.0	0.8	1.2	1.7*
	oedema bias (Post) (litres)	1.7	3.8	0.3	2.1	2.2	0.9	2.0*
	fluid shift (litres)	0.6	-1.3	1.1	0.1	-0.6	0.2	0.0
Stimulation	oedema bias (Pre) (litres)	1.6	2.1		2.7			2.1*
	oedema bias (Post) (litres)	0.3	1.7		2.4			1.4
	fluid shift (litres)	1.0	0.8		0.3			0.7
All phases	oedema bias (Pre) (litres)	1.7	1.9	1.1	2.3	0.9	1.2	1.6*
	oedema bias (Post) (litres)	1.3	2.3	1.8	2.2	0.3	0.8	1.6*
	fluid shift (litres)	0.7	-0.3	1.1	0.2	0.1	0.4	0.3

Table 7-3: Pre- and post-dialysis oedema bias is defined in equation 7-1. This gives an indication of the extent of the localised fluid in the lower limb. Fluid shift is defined in equation 7-2 and gives an indication of the extent of mobilisation of fluid during dialysis from the localised oedema into the blood volume. Values are the mean of the four measurement sessions. Statistical significance is indicated by *

7.4.3 Mobilisation of lower limb oedema for ultrafiltration during haemodialysis

Reduction in limb circumference – There was a moderate change in limb circumference in the control phase, with a median reduction of 8.5 mm and a range from 2 to 28 mm. During both interventional phases there was a trend for a greater reduction in limb circumference, with a median 4 mm increase in the stimulation phase (range -3 to 6mm) and a median 6mm increase (range -4 to 13 mm) in the compression phase compared to the control phase. The median absolute reduction was greatest in the compression phase at 14 mm.

Vascular refilling capacity – Only three of the six participants were monitored for vascular refilling capacity. A recall of all Crit-line monitors on 21st July 2015 (MHRA, 2015) meant that vascular refilling could not be defined in subjects 5 and 6. Subject 4 was being treated with pre-dilution haemodiafiltration which interferes with the blood line before the cuvette used for RBV detection. In the three subjects who were monitored, good refilling was observed (as defined in chapter 3) in all measured sessions.

Fluid distribution changes – Fluid distribution changes can be indicated by considering the relative changes in BCM-measured OH in both the hand-to-foot path (ΔH_F) and the hand-to-hand path (ΔH_H). There was no significant difference between ΔH_F and ΔH_H across the subjects for any phase individually or all phases together, indicating there was no greater mobilisation of fluid for ultrafiltration from the lower limbs than across the upper body (table 7-3).

7.4.4 Interventions for improving fluid mobilisation from oedematous tissue

Rate of recruitment – Figure 6-2 describes the number of patients at each stage of the study. Three units were used to recruit the 6 participants: two satellite dialysis units and the main hospital unit (evening shifts were not included for practical reasons). 257 patients were considered for inclusion, based on the population of these units. Of these, 8 were identified as meeting the inclusion criteria, two of which declined to take part in the study and 6 of whom gave informed consent and were randomised. This gave a recruitment rate of around 2% of prevalent haemodialysis patients in the units considered.

Adherence to the interventions – All 6 participants completed the compression phase. Adherence varied between 50%, in one individual, up to 100% in three of the six participants. None of the participants found the intervention uncomfortable or painful. Only three of the six participants completed the stimulation period. One of the participants moved away during the trial before this period. Two of the participants, had comorbid conditions that prevented effective use of the stimulators. In the case of subject 3, the cerebral palsy and kyphoscoliosis left the lower limbs in such a condition that it was impossible to place the feet on the pads of the Circulation Booster. For the NeuroTrac, issues with skin integrity and the heavy use of moisturisers prevented

sufficiently good contact with the disposable electrodes and no stimulation was evident. Subject 6 had undergone surgery on one foot which again prevented full contact with the Circulation Booster pads and neuropathic ulcers on the leg prevented effective placement of disposable electrodes.

Post-dialysis fluid status – There was a large range of values for fluid status in the cohort, from 4.4 litres fluid depleted up to 2.1 litres overloaded. The median post-dialysis OH was -0.8 litres in the control phase and increased in both stimulation and compression phases by 0.4 and 1.1 litres respectively.

Symptoms – Two of the subjects were symptom free in the control phase, two experienced symptoms in half of the sessions, one in a quarter of sessions and one in three quarters. The frequency of having symptomatic sessions was reduced in both intervention phases: with stimulation, two patients were less symptomatic and one had similar results to the control period; and with compression, five patients had similar results to the control phase and one had reduced frequency of symptoms.

Adverse events – There were no adverse events.

		Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Median
Control	Post-HD OH (l)	-4.4	-1.6	2.1	0.1	-0.3	-1.3	-0.8
	Δcirc (mm)	2	28	15	5	9	8	8.5
	Refilling	100%	100%	100%				100%
	Symptoms	50%	25%	75%	0%	0%	50%	38%
NMES	Adherence	75%	90%		100%			90%
	Post-HD OH (l)	-4.6 (+0.2)	-0.4 (+1.2)		0.1 (0)			-0.4 (+04)
	Δcirc (mm)	8 (6)	32 (4)		2 (-3)			8 (4)
	Refilling	100% (0%)	100% (0%)					100% (0%)
	Symptoms	25% (-25%)	0% (-25%)		100% (0%)			25% (-25%)
IPC	Adherence %)	100%	90%	50%	100%	100%	80%	95%
	Post-HD OH (l)	-3.8 (+0.6)	0.2 (+1.8)	1.2 (-0.9)	1.1 (+1)	0.4 (0.7)	-1.2 (+0.1)	0.3 (1.1)
	Δcirc (mm)	14 (12)	28 (-4)	28 (13)	11 (6)	9 (0)	13 (5)	14 (6)
	Refilling	100% (0%)	100% (0%)	100% (0%)				
	Symptoms	50% (0%)	25% (0%)	50% (-25%)	0% (0%)	0% (0%)	0% (0%)	38% (-13%)

Table 7-4: Study results. Δcirc refers to reduction in calf circumference over dialysis. Numbers in brackets show the effect size, being the difference with the control period.

7.5 Discussion

7.5.1 Characterising BCM measurements in patients with localised oedema

The characterisation of BCM measurement configurations in chapter 4 show that in healthy controls and haemodialysis patients without localised fluid accumulation, measurements from hand-to-hand give comparable results to the standard measurement path. Similar analysis in this group of patients with localised lower limb oedema displays a significant disparity between BCM-measured OH using the reference path and the hand-to-hand path.

Based on the lack of clear evidence from this study that localised fluid can be mobilised, the difference between the two measurement paths could potentially be used to set target weights that maximise the removal of oedematous fluid from the lower limbs without making the upper body too fluid depleted. Given that localised fluid is not preferentially removed, monitoring the body segments that have lower fluid status will give better protection against potential perfusion defects, IDH and end organ damage. However, as there are significant differences between patients with this indication, hand-to-foot measurements can be used to monitor and maximise the relative reduction in lower limb volumes when the upper body does not appear to be fluid depleted. The use of RBV measurements in combination with BCM may also allow monitoring of the plasma volume to avoid symptomatic fluid depletion.

7.5.2 Mobilisation of lower limb oedema for ultrafiltration during haemodialysis

Oedema is a standard indicator of fluid overload that traditionally would lead to a reduction in target weight to try and reduce the oedema (Charra et al., 1996). However, it is not known to what extent localised oedema is accessible during haemodialysis or to what extent it influences cardiovascular risk factors. If mobilisation of oedematous fluid is poor, ultrafiltration as a method of reducing the extent of oedema could put patients at risk of IDH.

For interpretation of the BCM results and individualising fluid management strategies in this patient group, it is important that a better idea of the refilling characteristics of localised oedema is established. Plasma refilling is difficult to directly assess, but information on refilling capacity was obtained indirectly from three other assessments.

The circumferential change in the lower limb showed a reduction in limb circumference in all phases but the extent of the reductions observed was moderate. Haemodialysis patients have been shown to have 14 mm change in calf circumference simply from sleeping and remaining supine for an extended period (Elias et al., 2012) and stable, non-oedematous patients have lower limb circumference reductions typically above 10mm during a standard haemodialysis session (Zhu et al., 2011). This suggests that despite, in some cases, patients dialysing to target weights that leave them significantly fluid depleted or symptomatic, there was no indication of notable changes in the extent of lower limb oedema.

Assessment of vascular refilling capacity was hampered by the recall of the Crit-line device, meaning there was a lack of data. Of the patients that were monitored, none of the dialysis sessions indicated a drop in blood volume that would be defined as suggesting a lack of vascular refilling as defined in chapter 3. Subject 1 tended to be managed to finish dialysis with significant fluid depletion, suffered severe cramping on occasions and regularly achieved ultrafiltration volumes above 3 litres, yet did not have any RBV charts classified as having poor refilling. Subject 3, on the other hand was managed to finish dialysis with a degree of fluid overload, suffered regularly from cramp and had characteristic 'A' shaped RBV curves at all sessions. This is potentially linked to comorbidities and inability to move her lower limbs. It is hard to form conclusions from this limited dataset and further categorisation of RBV in these patients is warranted.

The difference between hand-to-foot and hand-to hand BCM measurements give an indication of the relative reduction in excess fluid in the lower half of the body compared to the upper body and is an important consideration when interpreting BCM measurements on subjects with localised fluid. The results here that there is a minimal difference in measurements across the two paths suggest that there is a lack of significant mobilisation of fluid from the localised oedema.

The results here are not able to demonstrate a clear and noticeable mobilisation of lower limb fluid for ultrafiltration during haemodialysis. However it must be acknowledged that the study is exploratory in nature and has a diverse cohort of subjects so cannot categorically define this capacity. Basing fluid management strategies on hand-to-hand BCM measurements may be advisable in the first instance to reduce the risk of IDH, but additional hand-to-foot measurements can allow the identification of preferential removal of fluid from the lower limbs in an individual case and alteration of management accordingly.

7.5.3 Assessing the feasibility of IPC and neuromuscular stimulation for mobilisation of lower limb oedema

The issue of haemodynamic stability in haemodialysis is a poorly understood area. Methods of monitoring haemodynamics have been developed but very few interventions have been proposed. The use of compression devices has been trialled as a method of improving central blood volume, but with inconclusive results (Tai et al., 2013). Cool dialysate and midodrine have been shown to improve haemodynamic stability (Hoeben et al., 2002), but this is related more to compensatory mechanisms of the cardiovascular system than the mobilisation of excess fluid. Anti-embolism stockings have been shown to have some effects on haemodynamic variables early in haemodialysis sessions, but with no clear improvement over the treatment as a whole (Kim et al., 2010).

This study assessed the feasibility of compression or stimulation as methods of mobilising localised lower limb oedema by improving venous and lymphatic return. The outcomes around feasibility are discussed here.

Rate of recruitment – The rate of recruitment was low at around 2% of the local population. Previous reports have estimated around 23% of haemodialysis patients as having pitting oedema (Agarwal et al., 2008) while it is estimated that up to a 30% of HD treatments include IDH (Palmer and Henrich, 2008). There are a number of possible explanations for this disparity. Firstly, the study was undertaken in a unit with a particular interest in avoiding excessive dehydration and where BCM has been part of routine care for a number of years. Also, the identification of IDH was not based on BP

measurements but on nursing staff recording episodes of IDH, which may not always happen. Previous interventional studies have been able to recruit patients into studies based on suffering IDH in 30% or more sessions over one month (Tai et al., 2013). In the department in which the research was carried out, using the recorded metrics for IDH, only 13% of subjects would meet these particular inclusion criteria.

Adherence to the interventions – Adherence to the interventions was good. During the compression phase, half of the patients were happy to wear the cuffs throughout treatment. Other patients liked to have some breaks, when the cuffs started to itch or get warm. During the stimulation phase, adherence to the prescribed time was only slightly lower. Most participants experienced a strange sensation but none reported it to be uncomfortable. However, for two patients, no response - in terms of patient sensation or visual observation of muscle movement was possible from either stimulator. Patients found both interventions to be minimally burdensome and in fact of the six patients recruited, one patient purchased a compression device and one patient purchased a stimulation device for home use because of the benefits they perceived from the devices.

Clinical outcome measures - The study highlighted issues that need to be considered when designing trials with outcome measures relating to fluid status. BCM measurements are rarely made for routine management with the frequency that they were undertaken here for recording outcome measures, so blinding is important. However, this is also ethically challenging. In this study, there was no blinding and the results were used to alter target weights when felt there was an indication. This is likely to result in fewer IDH episodes and less chance of patients target weight becoming inappropriate. The results also highlighted the diurnal variations in lower limb oedema that can impact upon outcome measures. During the transition from compression to stimulation with subject 4, the patient changed from having dialysis in the afternoons to being treated in the morning. Given that fluid accumulates in the lower limbs during the day, as patients are more likely to be upright, and then reduces at night when supine, the extent of the oedema was much reduced during the stimulation phase and this was reflected by a lower reduction in limb circumference than the control period.

7.6 Limitations

There are a number of limitations to this study. This study was also limited by issues around availability of blood volume monitor chambers part way through the study. However, in the patients that were monitored, there were no instances of poor refilling observed which, as discussed, may be related to the high degree of care the patients received in relation to fluid management.

This study did not investigate or optimise the application of the compression device for the purpose of this trial. The devices used in the trial are intended for use in preventing deep vein thrombosis. There are other devices that encompass the whole leg and that have difference settings to maximise fluid removal. However, these devices are much less commonly available and much more expensive. The simple device was chosen as a pragmatic solution that could be reproduced easily in most centres, due to the ease of use, patient acceptability and prevalence of the devices across the country. However, it has been shown that different settings on the device have significantly different effects (Grieveson, 2003). The fact that the region of compression was just the calves may mean that the fluid does not move adequately out of the region of intervention towards non-swollen tissues, for maximum vascular refilling (Olszewski et al., 2011).

A better assessment of IDH would have given a more sensitive outcome measure for the trial. One method of doing this is repeated blood pressure measurements throughout treatment, although this can be of some discomfort to the patients.

7.7 Conclusion and implications for practice

Use of the BCM in patients with localised oedema allows the potential for better tailoring of target weights to best reduce the morbidity associated with oedema and normalise fluid status without provoking IDH. Standard measurement paths give a biased assessment of whole-body fluid status and achievable ultrafiltration volumes and there is no clear evidence that all this fluid is readily mobilised into the vascular space. However, even within this small group of six subjects, there was a great degree of variability in tolerance of ultrafiltration, comorbidities and ability to obtain valid BCM measurements amongst other things. This highlights the need for an individualised

approach to BCM based fluid management in these subjects. Post-dialysis hand-to-hand measurements and RBV monitoring can be used to ensure the upper body does not become fluid depleted and hand-to-foot measurements pre- and post-dialysis can help to guide the extent to which lower limb oedema can be mobilised. An understanding of the differences in BCM measurement path and timings can allow users to adapt to the clinical situation and have confidence in reporting the results.

Clear feasibility for a study to categorically assess the interventions for improving the process of fluid removal on haemodialysis has not been demonstrated. The low prevalence of patients meeting the inclusion criteria would mean a study would have to be multi-centre, and necessitate standardisation of centre-to-centre practice pattern differences. The majority of patients eligible were happy to consent, which may be related to the morbidity associated with lower limb oedema and patients' desire to intervene.

8 Conclusion

8.1 Unmet clinical need

This research aimed to address the need for a greater evidence base to underpin the development of bioimpedance and RBV monitoring beyond the research setting and into routine care. For this, they must become practical and effective tools that can be used to provide individualised fluid management across the haemodialysis population.

The results have characterised the uncertainties associated with measuring outside the standard protocol suggesting that, despite the many practical and clinical complications common in haemodialysis units, BCM can obtain useful diagnostic information in most scenarios. The results inform the development of individualised approach to interpretation of BCM measurements in certain patient groups and the need for further research to dissect the associations of fluid overload and fluid depletion with mortality. The combined use of RBV and BCM has indicated that existing management strategies based on RBV may need to be altered, as the 'flat-line' ('A') curve that has been assumed to indicate fluid overload has been observed in many patients who are normally hydrated or fluid depleted based on the BCM measurements.

The findings provide both a grounding for development of measurement protocols to improve fluid management strategies and a reference for further research aimed at describing the underlying mechanisms.

8.2 Research questions

Here the main conclusions from each research question are summarised and implications for clinical practice outlined.

Research question 1: *Can characterisation of alternative measurement paths and timing for the BCM, and the use of simultaneous RBV monitoring, lead to more individualised and effective fluid management?*

With use of an 8-lead BCM, measurements across all configurations of electrodes placed at the wrist and ankle allowed the characterisation of BCM measurements in relation to the standard path. Measurements made on control subjects with normal renal

function – where euhydration and no perturbations from normal fluid distributions were assumed – showed that measurements on all of the primary whole body paths other than foot-to-foot gave similar fluid status. 8-lead BCM measurements in haemodialysis patients pre-dialysis, showed similar patterns except for a small increase in BCM-measured fluid overload associated with the presence of vascular access. Finally, measurements made post-dialysis demonstrated that there was minimal bias compared to pre-dialysis measurements and that post-dialysis measurements should not be ruled out. Having a reference for the variation in BCM-measured OH using alternative electrode configurations and timings will have a significant impact on the flexibility and inclusivity of routine BCM measurements. Importantly, BCM measurements can be made after dialysis sessions during which patients have experienced severe symptoms allowing excessive fluid depletion to be identified and the patient to be reassured that a simple increase in their target weight will prevent recurrence.

The simultaneous measurement of BCM and RBV measurements gave a new insight into some of the fundamental assumptions of RBV monitoring for fluid management. It is widely accepted that a flat-line ('A') curve suggests the need for a reduction in target weight. While this approach has been shown to reduce weight and improve blood pressure in interventional studies (Agarwal et al., 2009),the sole robust clinical trial investigating the use RBV found higher mortality in the RBV managed group than in standard care (Reddan et al., 2005). There has been no good explanation for these unexpected findings but based on the results presented here, it is possible that reducing target weights based on RBV flat-lines increases fluid depletion in these patients, which is associated with a number of deleterious effects including loss of residual renal function (Jansen et al., 2002) and cardiovascular stress (McIntyre, 2010). This highlights the complexity of RBV measurements used for fluid management and the urgent need for further studies in this area. Of particular benefit would be a better understanding of how whole-body and segmental BCM-measured fluid volumes, RBV and absolute blood volume are related to risk factors and how these factors differ between patient groups.

Research question 2: Is the tendency for high BMI haemodialysis patients to finish dialysis fluid depleted according to the BCM associated with an artefact in the

mathematical model or with systematic differences in the prescription and delivery of treatment?

Observational studies have demonstrated a clear link between post-dialysis fluid depletion as defined by BCM and high BMI and this has raised doubt about the validity of BCM in subjects with high BMI. However, these studies are unable to define whether the observations relate to systematic bias in the BCM modelling or are treatment related effects.

BCM measurements on high BMI subjects with normal renal function showed these subjects to be normally hydrated suggesting the BCM models are valid in subjects with BMI up to 50 kg/m^2. The fluid depletion observed in very large haemodialysis patients is likely to be related to a combination of factors, including patient's desire to minimise weight and a large ECF volume that can buffer plasma volume changes. Indeed, RBV measurements on high BMI dialysis patients show that refilling was maintained, despite high levels of fluid depletion.

Research question 3: Is the presence of malnutrition associated with BCM-measured fluid overload?

Mirroring the association between high BMI and fluid depletion, observational data suggest that low BMI is associated with post-dialysis fluid overload. In this study it was shown that a degree of excess fluid as measured by BCM is common in elderly subjects with normal renal function irrespective of whether they were classified as malnourished or of unclassified body habitus. Without recruiting a cohort of younger malnourished subjects with normal renal function, it is not possible to define the effect of malnutrition on BCM-measured OH isolated from confounding factors. However, the results support the tailoring of fluid management strategies in patient with increasing combinations of ageing, malnutrition, comorbidities and frailty to account for the fact that a degree of excess fluid as measured by BCM appears to be common in these groups.

Research question 4: (a) Can BCM measurements be used to assess and monitor localised fluid oedema and (b) Can easily administered, non-invasive interventions mobilise persistent localised oedema so that it can be recruited into the circulation?

Standard hand-to-foot measurement paths should not be used in isolation for managing patients with localised oedema. These patients can have differing needs and no single approach to measurement and interpretation of results is sufficient. By using standard and alternative measurement paths and, if appropriate both pre- and post-dialysis measurements, users should be able to optimise any possible reduction in oedema while minimising the risk of fluid depletion to other parts of the body.

The feasibility of IPC and neuromuscular stimulation as interventions for increasing refilling and improving both fluid removal and patient experience during dialysis was assessed. A conclusive trial to evaluate these devices would likely be difficult to design and deliver, mainly due to the difference in the presentation and response of patients with localised oedema. However the interventions were well tolerated and two of the six patients in the feasibility study wanted to purchase the devices as they perceived a benefit.

8.3 Further work

The practicality of measurements with BCM and the potential for use across the haemodialysis population has been demonstrated. This focus on measurement should be followed up by work that develops the interpretation of BCM results and the evidence base for management strategies. A number of interventional studies, including randomised controlled trials and prospective cohort studies, have showed that using BCM to guide populations to target OH values has improved hard outcomes, including fluid status, blood pressure, arterial stiffness and survival (Onofriescu et al., 2014, Onofriescu et al., 2012, Moissl et al., 2013). However, these are based on universal strategies and neglect personalised approaches.

To better understand the mechanisms behind the morbidity and mortality associated with both fluid overload and fluid depletion, intravascular fluid status is likely to be important. BCM and RBV are complementary measurements - BCM provides tissue hydration information and quantifies fluid status, while RBV gives information on intravascular fluid changes and can help patients avoid IDH. But there is also a need for an absolute measure of blood volume for assessment of intravascular hydration. Recent work has demonstrated a simple method for measuring absolute blood volume (ABV)

from a dialysis machine and a RBV measurement device. Simultaneous assessments of BCM, RBV and ABV would allow a more complete picture of the characteristics of fluid accumulation and removal in dialysis patients and potentially develop the conclusions from this piece of work.

Training materials for BCM use in the large proportion of dialysis patients with special needs are lacking and this work can form the basis of practical training tools. More focussed research using information from BCM and RBV is clearly needed to improve methods of interpretation of RBV data.

8.4 Summary

This study provides important information to support flexible and individualised routine use of BCM for fluid management in haemodialysis. Based on manufacturer's guidance, a significant subset of patients would be excluded from measurement or measurements would give inappropriate results. These data show that alternative measurement approaches are valid and supports individualised interpretation of BCM-measured OH. Further work is needed to better understand how to use RBV measurements in routine practice.

9 Appendix

9.1 8-lead BCM measurement specification

Path	Current	Current	Voltage	Voltage	Segments measured
1	Right hand	Right foot	Right hand	Right foot	Right arm, Right trunk, Right leg
2	Left hand	Left foot	Left hand	Left foot	Left arm, Left trunk, Left leg
3	Right hand	Left foot	Right hand	Left foot	Right arm, R-L trunk, Left leg
4	Left hand	Right foot	Left hand	Right foot	Left arm, L-R trunk, Right leg
5	Right hand	Left hand	Right hand	Left hand	Right arm, Top trunk, Left arm
6	Right foot	Left foot	Right foot	Left foot	Right leg, Low trunk, Left leg
7	Right hand	Right foot	Right hand	Left foot	Right arm + Right trunk
8	Left hand	Left foot	Left hand	Right foot	Left arm + Left trunk
9	Right hand	Right foot	Left hand	Right foot	Right leg + Right trunk
10	Left hand	Left foot	Right hand	Left foot	Left leg + Left trunk
11	Right hand	Left hand	Right hand	Right foot	Right arm only
12	Right hand	Left hand	Left hand	Left foot	Left arm only
13	Right foot	Left foot	Right hand	Right foot	Right leg only
14	Right foot	Left foot	Left hand	Left foot	Left leg only
15	Right hand	Right foot	Left hand	Left foot	Right trunk only
16	Left hand	Left foot	Right hand	Right foot	Left trunk only
17	L&R hand	L&R foot	L&R hand	L&R foot	Full whole body

9.2 Mixed regression model assumption checks

Section 4-4 presented results from mixed regression models investigating OH, lean tissue and adipose tissue. Here, the results from checks of the models investigation lean tissue (figure 9-1) and adipose tissue (figure 9-2) are presented. Visual inspection of the residual plots suggested random scatter. Although Q-Q plots showed some dispersion from normality; sensitivity analyses were done by checking the outliers for errors, but no clear problems with the data were found.
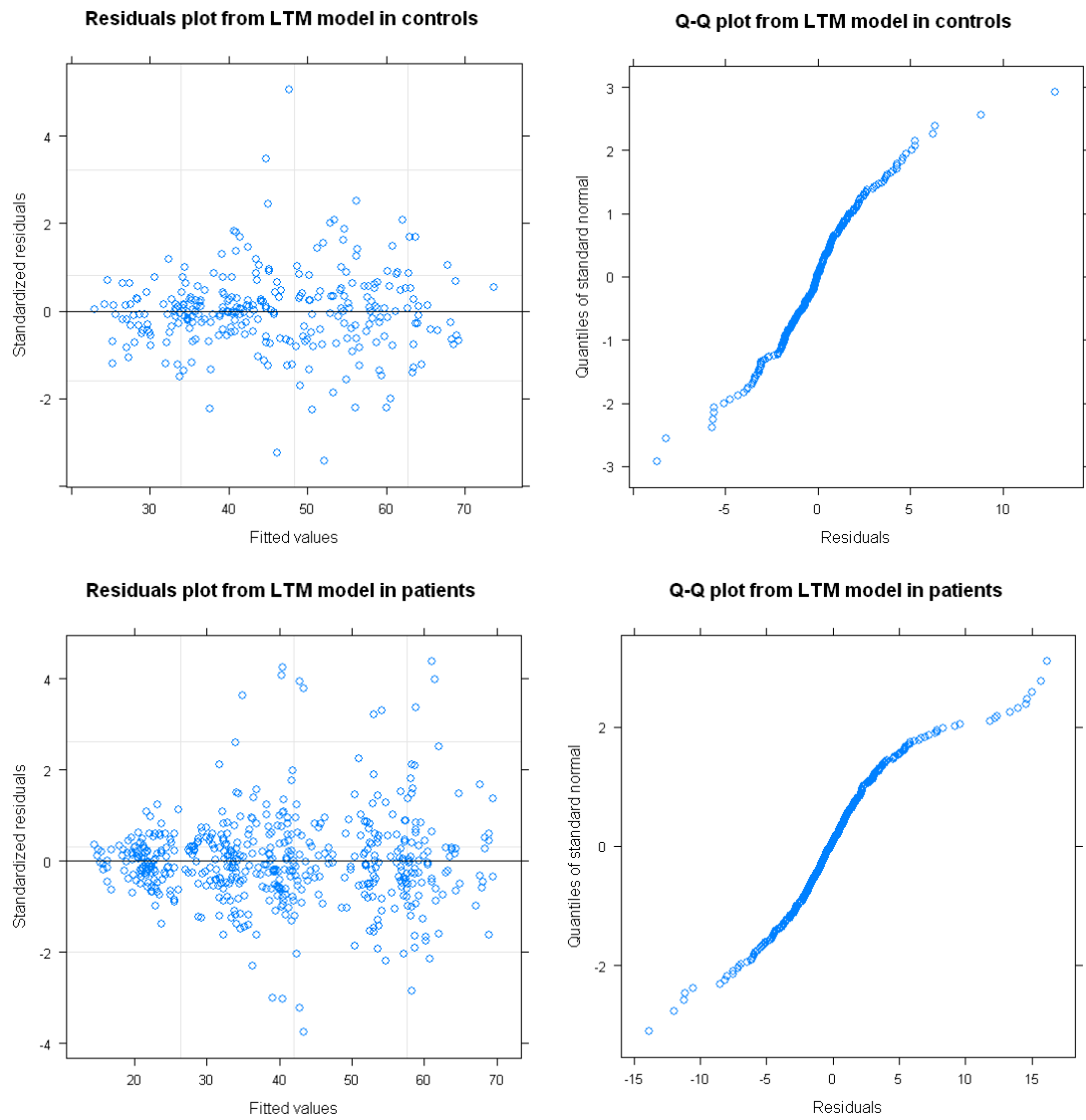
Figure 9-1: Plot of residuals (left) and a 'Q-Q' plot of the residuals (right) for mixed-effects model of LTM in healthy controls (above) and haemodialysis patients (below)
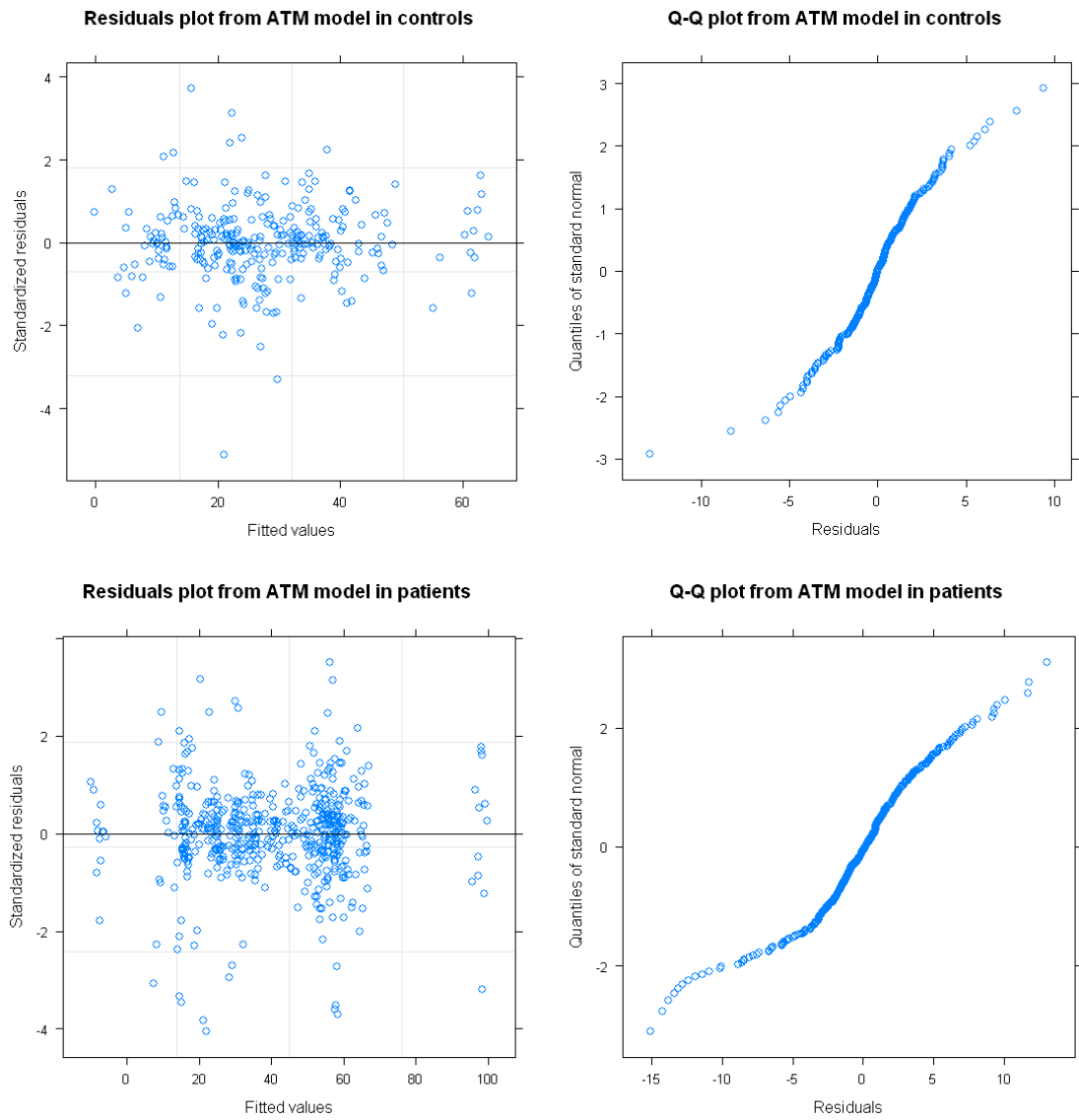
Figure 9-2: Plot of residuals (left) and a 'Q-Q' plot of the residuals (right) for mixed-effects model of ATM in healthy controls (above) and haemodialysis patients (below)

9.3 Repeatability of regression model

Repeat measurements were made for 36 of the 48 dialysis patients to assess the reproducibility of the regression model. The analysis was done in exactly the same way and the results can be seen in tables 9-1 to 9-3. The model had lower power than the primary analysis, which can explain why some of the results that were significant in the primary analysis are no longer significant here. However, the trends are consistent and the effect sizes similar, suggesting good reproducibility of the model.

	Measurement Path	OH (litres)	Bias (litres)	p-value	Approx. 95% CI
Pre-dialysis	Non-VA side	1.6	-	0.19	-0.20 to 3.3
	VA-side	-	0.01	0.02	-0.45 to 0.48
	Cross1	-	0.07	0.91	-0.39 to 0.54
	Cross2	-	-0.12	0.02	0.59 to 0.34
	Arms	-	-0.51	0.23	-0.55 to 0.13
	Legs	-	1.6	<0.01	1.1 to 2.1
Post-dialysis	Non-VA side	-0.33	-	0.45	-0.81 to 0.26
	VA-side	-	0.31	0.37	-0.35 to 0.97
	Cross1	-	0.01	0.98	-0.65 to 0.67
	Cross2	-	0.37	0.28	-0.29 to 1.0
	Arms	-	0.52	0.13	-0.13 to 1.2
	Legs	-	-0.43	0.20	-1.1 to 0.22

Table 9-1: Repeat OH measurements for dialysis patients. Reference path is non-VA side. Data are presented for a 60 year old female, where the adjustment for age was 0.06 per year (p=0.6; 95% CI: -0.02 to 0.03) and for sex was -0.38 for male (p=0.4; 95% CI: -1.3 to 0.5)

	Measurement Path	LTM (kg)	Bias (kg)	p-value	Approx. 95% CI
Pre-dialysis	Non-VA side	23	-	<0.01	5 to 41
	VA-side	-	1.0	0.45	-0.81 to 2.8
	Cross1	-	-0.12	0.61	-1.9 to 1.7
	Cross2	-	1.3	0.12	-0.5 to 3.1
	Arms	-	1.7	0.08	-0.13 to 3.5
	Legs	-	1.9	0.04	0.06 to 3.7
Post-dialysis	Non-VA side	22	-	<0.01	20 to 24
	VA-side	-	-0.81	0.69	-3.4 to 1.8
	Cross1	-	-0.03	0.90	-2.6 to 2.5
	Cross2	-	-0.94	0.41	-3.5 to 1.6
	Arms	-	-0.36	0.82	-2.9 to 2.2
	Legs	-	-0.44	0.89	-3.0 to 2.1

Table 9-2: Repeat measurements for LTM with dialysis patients. Reference path is non-VA side. Data are presented for a 60 year old female, where the adjustment for age was -0.16 per year (p=0.25; 95% CI: -0.44 to 0.11) and for sex was -2.6 for male (p=0.6; 95% CI: -12 to 6.8)

	Measurement Path	ATM (kg)	Bias (kg)	p-value	Approx. 95% CI
Pre-dialysis	Non-VA side	36	-	<0.01	11 to 60
	VA-side	-	-1.0	0.26	-2.8 to 0.7
	Cross1	-	0.05	0.98	-1.7 to 1.8
	Cross2	-	-1.1	0.20	-2.9 to 0.6
	Arms	-	-1.1	0.21	-2.9 to 0.6
	Legs	-	3.6	<0.01	-5.3 to -1.8
Post-dialysis	Non-VA side	37	-	<0.01	12 to 61
	VA-side	-	0.49	0.70	-2.0 to 2.9
	Cross1	-	0.02	0.99	-2.4 to 2.5
	Cross2	-	0.56	0.66	-1.9 to 3.0
	Arms	-	-0.18	0.88	-2.6 to 2.3
	Legs	-	0.90	0.48	-1.6 to 3.4

Table 9-3: Repeat measurements ATM model results for dialysis patients. Reference path is non-VA side. Data are presented for a 60 year old female, where the adjustment for age was 0.06 per year (p=0.8; 95% CI: -0.14 to 0.58) and for sex was 6.6 for male (p=0.3; 95% CI: -9.0 to 14)

9.4 Complete RBV results

Table 9-4 presents the complete set of RBV results for all 48 patients and over both sessions. Where a shape factor is presented in brackets, this is the result of the sensitivity analysis.

Session 1				Session 2			
Pre-HD OH	UF vol.	Refilling	Shape	Pre-HD OH	UF vol.	Refilling	Shape
-0.7	1.5	1	A (C)	3.8	3.4	0	C
3	1.4	1	D	-	-	-	-
4.4	2.1	0	D	4.5	2.7	0	D
-1.4	0.7	1	D	0.4	2.5	1	D
-	-	-	-	-	-	-	-
3.2	3	0	D (C)	-0.1	2.6	0	C
1.6	1.5	0	C	-	-	-	-
-0.5	0.4	1	C	-0.8	0.9	1	A
1.4	3.3	1	C	2.5	3.8	1	C
1	2.2	1	D (C)	-	-	-	-
1.1	1.3	0	D	0.5	0.6	0	D
0.9	1.7	1	D	1.6	1.6	1	D
2	3.1	1	A (D)	-	-	-	-
-1	0.5	0	C	-0.6	0.8	1	A (C)
0.5	1.4	1	D	0.8	2.4	1	D
1.5	1.7	0	C	3.4	2.5	0	D
-0.9	1.5	1	A (D)	0.7	1.8	1	C
2.2	3.5	1	C	-	-	-	-
2.6	0.6	1	D	0.7	0.4	0	D
2.4	2.1	0	C	1.4	1.9	0	C
				0.7	1.1	1	D
-0.8	1.1	1	A	-1.2	0.9	1	D
3.2	1.4	1	A	0.9	1.9	1	C
0.6	1.3	0	D	-0.9	0.6	1	A
-	-	-	-	-0.6	1.0	1	A (B)
2	1.7	1	A (B)	1.9	2.8	1	A (C)
-	-	-	-	1.4	2.6	1	A
1.8	2.9	1	A (C)	1.4	2.6	1	D (C)
-	-	-	-	2.8	4	1	C
-0.7	0.7	1	A	-0.7	1.2	1	A (C)
-	-	-	-	2.9	3.0	1	C
1.5	1.2	0	D	-	-	-	-
4	3.2	1	D	5.6	3.2	0	D
1.1	0.8	0	D	1	1.2	1	A (C)
0.7	1.6	1	D	-0.8	1.5	0	D
4.1	3	1	C	5.8	4	1	C
0.9	2.4	1	C	0.1	1.6	1	C
2.1	2.1	1	A	2.1	2.4	1	D (C)
2.7	2.7	1	B	-	-	-	-
0.5	2	1	C	0.7	1.2	1	B
0.4	1.2	1	A	1.8	1.1	1	A (C)
1.1	1.7	1	A (C)	0.9	1.4	1	D
2.1	1.4	1	A (B)	0.9	0.4	1	A (C)
3.3	3	1	A (c)	-	-	-	-
3.8	3	1	D	1.9	2.6	1	D
2.8	1.4	1	C	-	-	-	-
3.9	3.5	1	C	-	-	-	-
-	-	-	-	4.1	2.5	0	C

Table 9-4: Complete RBV results

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