ENERGY EXPENDITURE IN KIDNEY FAILURE: IMPLICATIONS FOR MANAGEMENT

SIVAKUMAR SRIDHARAN

A thesis submitted in partial fulfilment of the requirements of the University of Hertfordshire for the degree of Doctor of Philosophy

School of Life and Medical Sciences

September 2014

To Archana and Abhinaya

for the endless joy and love you have given me in the toughest of times

To My Parents

for the immense support and love you have given me in all aspects of my life

Index

Acknowledgements

The work in this thesis would not have been possible without the invaluable guidance of my supervisors Prof. Ken Farrington, Dr Justin Roberts and Dr Enric Vilar. My supervisors have been a great source of support throughout my studies. I would like to express my gratitude to Prof Farrington, who not only believed in me and gave me this opportunity for research, but also has provided with endless motivation and inspiration throughout my time working with him. I would like to thank Dr Justin Roberts for his support and encouragement during my research period. Special thanks are due for Dr Enric Vilar for his advice and support in both academic and practical study-related issues.

I would like to express my heartfelt gratitude for my family who have provided me with love and encouragement. I would like to express special thanks to my wife Archana, who has provided me with invaluable support and encouragement during difficult times. I would like to thank my parents for their love and support in all aspects of my life.

I would like to thank my research lab colleagues Dr Jonathan Wong and Jocelyn Berdeprado. I am grateful for their support and friendship. I would also like to thank my colleagues at the University of Hertfordshire Dr David Wellsted and Dr Kirsten Rennie for their advice on statistical and methodological issues. I would like to thank Jade Collard for her support in analysing study data. I would also like to particularly thank Dr Kenneth Smith for the invaluable advice in study designing and help in analysing the samples related to the protein turnover study.

None of the work here would have been possible without the financial support of the Lister Nephrology Research Fund and I would like to express my special thanks to them. I would also like to acknowledge and thank British Renal Society and British Kidney Patient Association for providing two research project grants for this work.

Finally, I owe a great deal of thanks to the patients who have participated in the studies. I am grateful for the efforts and kindness of these patients without whom this work would not have been possible.

Collaborations

Two studies reported in defence of the thesis were undertaken in collaboration with two other researchers.

The study data for the retrospective analysis examining the relationship between energy expenditure and urea generation rate (chapter 4) was obtained from an existing dataset. The study assessments were originally performed by Dr Vilar as part of his PhD (Dr Enric Vilar, PhD, University of Hertfordshire – degree awarded Feb 2012). However, the modelling of urea generation rate and the analysis of study data presented in this thesis were carried out by me. The results presented in chapter 4 have not been reported previously as part of Dr Vilar's PhD. Dr Vilar is one of my clinical supervisors for the work presented here and the data from Dr Vilar's study was used with his consent.

The study of total energy expenditure using doubly labelled water method (chapters 6 and 7) was carried out in 40 patients in collaboration with Dr Jonathan Wong, renal research fellow at Lister hospital. Dr Wong was involved in seeking ethics approval, patient recruitment and performing study assessments for some patients in the study. My role in the study included designing the study and writing the protocol, contributing to the ethics committee application, patient recruitment, conducting study procedures, data collection, analysis and presentation. Dr Vilar also conducted a similar study on 40 patients as part of his PhD. As the study protocol was the same for these two studies, the results presented in this thesis include data from all the 80 patients.

I would like to acknowledge the role of Dr Enric Vilar and Dr Jonathan Wong in the two studies mentioned above and thank them for their collaboration. The contributions of collaborators and myself for the studies in this thesis are outlined in the table below.

Extent of Contribution of collaborators for the studies in this thesis

Chapters	Contributors	Extent of contribution
Chapter 4	Enric Vilar	Writing up protocol, Ethics application, patient recruitment and conducting study assessments
	Sivakumar Sridharan (myself)	Urea Kinetic modelling, data analysis and writing up of the chapter
Chapter 5	Sivakumar Sridharan (myself)	Conducted the study in its entirety (protocol writing, Ethics application, patient recruitment, conducting study assessments, data analysis and writing up results)
Chapters 6 and 7	Jonathan Wong	Ethics application, helping with patient recruitment and conducting study assessments for some subjects
	Sivakumar Sridharan (myself)	Writing up protocol, Ethics application, patient recruitment, conducting study assessments, data analysis and writing up
Chapter 8	Sivakumar Sridharan (myself)	Conducted the study in its entirety
Chapter 9	Sivakumar Sridharan (myself)	Conducted the study in its entirety
Chapters 10-12	Sivakumar Sridharan (myself)	Conducted the study in its entirety

Thesis

Metabolic activity influences uraemic toxin generation and impacts on patient survival in end-stage renal disease. Management of patients with advanced kidney disease can be optimised by taking into account energy expenditure and physical activity.

Sivakumar Sridharan (2014)

Abstract

Renal replacement therapy, in the form of dialysis or transplantation, is the cornerstone of management for end-stage renal disease. UK renal registry shows nearly half of those needing renal replacement therapy are treated by dialysis – predominantly by haemodialysis. Patients on renal replacement therapy have increased mortality risk compared to age matched general population. Moreover, some specific subgroups of patients on haemodialysis have increased risk of mortality than expected. The survival benefit seen in women in the general population is attenuated resulting in similar survival for men and women on haemodialysis therapy. In addition, obese individuals and those of non-Caucasian origin have better survival outcome. Though the underlying reason for these findings is not clear and is likely to be multi-factorial, it has been hypothesised that this paradox could be due to the current practice of normalising dialysis dose to total body water. A number of metabolic factors – body surface area, resting energy expenditure and total energy expenditure – have been proposed as alternative to total body water for scaling dialysis dose.

There were two overarching aims of this work – one was to study the effect of declining renal function on resting and total energy expenditure and to study the influence of various energy expenditure measures on uraemic toxin generation. The second was to study the impact on survival outcome of using these alternate parameters for normalising dialysis dose and to derive dialysis dose adjustments based on these metabolic parameters. In order to study these aims, studies were designed to explore different aspects of energy expenditure measures along with a longitudinal study to examine the impact of these parameters on survival outcome.

The relationship between energy metabolism, body composition and uraemic toxin generation was studied with a retrospective analysis of 166 haemodialysis patients in whom urea generation rate was used as surrogate marker of uraemic toxin generation. It was found that total energy expenditure and fat-free mass predicted uraemic toxin generation after adjustment for other relevant variables. This study provided the preliminary data which was useful in designing further studies for this work.

The effect of renal function on resting and total energy expenditure was studied in 80 patients with varying stages of chronic kidney disease who were not on renal replacement therapy. Resting and total energy expenditures were measured directly using gold-standard methods. It was found that declining renal function did not have a significant influence on either of these measures. This supports the hypothesis that metabolic rate is the driving force for glomerular filtration rate and not vice-versa. The directly measured energy expenditure measures were also found to have a moderately strong relationship with urea generation rate in these patients not on renal replacement therapy.

The impact of physical activity on uraemic toxin generation, and thereby dialysis requirement, was studied in a prospective cross-sectional study of 120 haemodialysis patients in whom the physical activity was measured by an accelerometer device. Results from the study showed physical activity level to be a significant predictor of uraemic toxin generation after adjustment for gender and body size differences. This study results stressed the importance of adjusting dialysis dose based on individual's physical activity level.

To study the impact of using metabolic factors as normalising parameter for scaling dialysis dose on survival outcome, a large-scale longitudinal study was conducted with 1500 maintenance haemodialysis patients recruited for the study. Dialysis dose-related parameters and survival outcomes were collected at baseline and at various time points during the follow-up period of 18 months. Study results were analysed in two parts - the theoretical basis for using these metabolic factors as scaling parameters was explored which showed that current minimum target dialysis dose risks under-dialysis in certain subgroups of patients and using these alternative parameters may provide a more equivalent dialysis dose across individuals of different body sizes and gender.

With these results arguing for potential use of the alternative parameters, the impact on survival of using them were examined. It was found that all three parameters performed better than the current parameter (total body water) with regards to predicting mortality. Total energy expenditure was found to be the best parameter with the lowest hazard ratio for risk of death. The study data was also analysed to derive an algorithm for adjustment of minimum target dialysis dose based on body

size and physical activity level. This newly derived minimum dose target was also shown to impact on survival with those underdialysed based on this criteria having poorer survival outcomes.

To understand the impact of whole body protein turnover on resting energy expenditure and uraemic toxin generation, a cross-sectional study was conducted on 12 patients with advanced CKD – 6 each in pre-dialysis CKD and haemodialysis group. It was found that haemodialysis patients had higher rate of protein turnover compared to pre-dialysis patients. Whole body protein turnover was found to contribute significantly to resting energy expenditure and had a moderately strong relationship with urea generation rate.

In the course of these studies, two questionnaire tools have been validated for use for clinical and research purposes – one is a self-report comorbidity questionnaire and the other, the Recent Physical Activity Questionnaire. The comorbidity questionnaire was developed as part of this work and was validated against Charlson Comorbidity Index. The Recent Physical Activity Questionnaire was validated for physical activity data collection and energy expenditure calculation against the gold-standard doubly labelled water method.

In conclusion, it has been demonstrated that metabolic factors such as body surface area, resting energy expenditure and total energy expenditure are more closely related to uraemic toxin generation compared to total body water. It has also been demonstrated that physical activity contributes to metabolic waste production and may necessitate changes in dialysis requirement. It has been shown that these metabolic factors, when used as scaling parameter for dialysis dosing, may predict survival better than the current parameter in use. The algorithm for dialysis dose adjustment and the questionnaires validated in this work have provided novel tools for further research studies and clinical practice.

The central hypothesis of this work is that some metabolic factors may be better markers of uraemic toxin generation compared to total body water. It is hypothesised that modifications in dialysis practice based on these factors may improve the quality of haemodialysis and favourably impact on survival outcome for patients with end-stage renal disease. The work presented here largely supports this hypothesis.

List of Tables

List of Figures

Abbreviations

ADH	Antidiuretic hormone
BMR	Basal Metabolic Rate
BSA	Body Surface Area
CCI	Charlson Comorbidity Index
CSCS	Composite Self-report Comorbidity Score
CKD	Chronic Kidney Disease
DLW	Doubly Labelled Water
ECW	Extracellular Water
ESRD	End-stage Renal Disease
FFM	Fat-free Mass
GFR	Glomerular Filtration Rate
HD	Haemodialysis
HDF	Haemodiafiltration
HMRO	High Metabolic Rate Organs
ICW	Intracellular Water
KIC	Keto-isocaproate
MET	Metabolic Equivalent of Task
PAEE	Physical Activity related Energy Expenditure
PCR	Protein:Creatinine ratio
PD	Peritoneal Dialysis
PTH	Parathyroid hormone

RPAQ	Recent Physical Activity Questionnaire
REE	Resting Energy Expenditure
RQ	Respiratory Quotient
RR	Relative Risk
RRF	Residual Renal Function
RRT	Renal Replacement Therapy
TEE	Total Energy Expenditure
VCO_2	Carbon dioxide production rate
VO_2	Oxygen consumption rate
WBPT	Whole Body Protein Turnover

PhD Related Publications and Presentations

Publications

Sridharan, S., Berdeprado, J., Vilar, E., Roberts, J., Farrington, K (2014). A self-report comorbidity questionnaire for haemodialysis patients. BMC Nephrol, 15(1): 134

Sridharan, S., Vilar, E., Berdeprado, J., Farrington, K (2013). Energy metabolism, body composition and urea generation rate in hemodialysis patients. Hemodial Int, 17(4): 502-509

Presentations

Sridharan, S., Vilar, E., Davenport, A., Ashman, N., Almond, M., Banerjee, A., Roberts, J., Farrington, K. Evidence for a gender-specific minimum haemodialysis dose. UK Kidney Week, Glasgow, UK. April/May 2014 – Oral presentation

Sridharan, S., Davenport, A., Vilar, E., Roberts, J., Farrington, K. Relationship of physical activity and urea generation rate in haemodialysis patients. UK Kidney Week, Glasgow, UK. April/May 2014 – Oral presentation

Sridharan, S., Berdeprado, J., Ashman, N., Davenport, A., Almond, M., Banerjee, A., Vilar, E., Roberts, J., Farrington, K. Risk of under-dialysis: Use of scaling parameters reflecting metabolic activity. ASN Kidney Week, Atlanta, USA. November 2013 – Oral presentation

Sridharan, S., Berdeprado, J., Rennie, K L., Ashman, N., Davenport, A., Almond, M., Banerjee, A., Vilar, E., Roberts, J., Farrington, K. Predictors of habitual physical activity in haemodialysis patients. ASN Kidney Week, Atlanta, USA. November 2013 – Poster presentation

Sridharan, S., Berdeprado, J., Rennie, K L., Almond, M., Davenport, A., Ashman, N., Banerjee, A., Vilar, E., Roberts, J., Farrington, K. Ethnic differences in

habitual physical activity levels in haemodialysis patients. BRS Conference, Manchester, UK. May 2013 – Oral presentation

Sridharan, S., Berdeprado, J., Vilar, E., Almond, M., Davenport, A., Ashman, N., Banerjee, A., Roberts, J., Farrington, K. Comparison of a novel predictive equation for resting energy expenditure in haemodialysis patients with existing general equations. BRS Conference, Manchester, UK. May 2013 – Poster presentation (Awarded best poster of the group)

Sridharan, S., Berdeprado, J., Vilar, E., Farrington, K. Determinants of energy expenditure during physical activity in haemodialysis patients. ASN Kidney Week, San Diego, USA. November 2012 – Poster presentation

Sridharan, S., Berdeprado, J., Farrington, K. Reliability of self-report comorbidity questionnaire in haemodialysis patients. ASN Kidney Week, San Diego, USA. November 2012 – Poster presentation

Sridharan, S., Vilar, E., Farrington, K. Relationship between metabolic rate and urea generation rate in haemodialysis patients. Renal Association Conference, Gateshead, UK. June 2012 – Oral presentation

Table of Contents

List of Appendices

Thesis Structure and Prelude

This work is organised into four main sections to defend the thesis offered.

Following the introductory chapter on kidneys and kidney failure, the first section provides a detailed review of relevant literature on dialysis dosing. The section focuses specifically on inadequacies of current dialysis dosing practice and examines the potential role of metabolic factors in haemodialysis therapy.

The second section describes general methodology relevant to all the studies presented in this work and provides information on calculation of various parameters used in the studies.

The third section describes the clinical studies used in defence of the thesis offered. These empirical studies could be broadly divided into those focusing on the relationship of metabolic factors to uraemic toxin generation and those examining the impact of the metabolic factors on survival outcome.

The fourth section focuses on overall conclusions derived from the studies reported in this work and suggests recommendations for future work.

Chapter 1

1. Introduction: End Stage Renal Disease

1.1 The Kidneys and Kidney failure

This introductory chapter has been divided into two sections. The first section will provide an overview of functions of the kidneys and about chronic kidney disease, its measurement and consequences on various organ systems. The second section will discuss the options for replacement of kidney function in patients with kidney failure.

1.1.1 Functions of the Kidney

Humans normally have two kidneys which are located in the posterior part of the abdomen. The size of the kidneys is dependant on body size with the typical range being 10 to 13 cm. Healthy kidneys weigh approximately 150g each. The kidneys are highly vascular and receive nearly 25% of the cardiac output (approximately 1250ml/minute of blood) (Marieb & Hoehn, 2014). This high proportion of cardiac output delivery is needed for the kidneys to perform its functions effectively. The primary functions of the kidneys are excretion of metabolic waste products, regulation of body water and maintaining electrolyte and acid-base balance. They also perform a number of secondary, albeit essential, functions. The kidneys play an important role in regulation of blood pressure through secretion of a hormone called renin. They also secrete erythropoietin, a hormone which regulates red blood cell production. Kidneys produce metabolically active form of Vitamin-D (1,25-dihydroxy cholecalciferol) which regulates bone mineral metabolism in conjunction with parathyroid hormone. Kidneys also play a major role in protein and amino acid metabolism through the synthesis, degradation, filtration, reabsorption and urinary excretion of various amino acids.

Each kidney has two sections - an outer cortex and a deeper medulla. The functional unit of the kidney is a nephron and each kidney has approximately a million of them. Each nephron is comprised of a renal corpuscle located in the cortex of the kidney and a tubular segment that occupies both cortex and medullary portions of the kidney. The renal tubules consist of primary convoluted tubule, loop of Henle and

distal convoluted tubule and drains into collecting ducts. The renal corpuscle is the initial filtering unit and consists of glomerulus within Bowman's capsule. Glomerulus consists of a tuft of capillaries from which the blood is filtered through different barriers before the final glomerular filtrate reaches the Bowman's space. These capillaries have fenestrations in their walls which measure approximately 200-500 Å. These fenestrae allow many of the metabolic waste products to be filtered. The filtrate from the capillary fenestrae passes through the glomerular basement membrane surrounding the capillary walls. The other side of the basement membrane is surrounded by cells called podocytes. These podocytes have foot processes with filtration slits through which the filtrate has to pass through to reach Bowman's space. These 3 layers – capillary wall, basement membrane and podocyte foot processes form an effective barrier and prevent filtration of red blood cells, leucocytes and platelets and also the serum proteins such as albumin and globulin into the urinary space.

The driving force of this filtration (ultrafiltration) process is the hydrostatic pressure within the glomerulus. The filtrate entering the Bowman's space is thereby termed ultrafiltrate. Glomerular filtration rate (GFR) is the volume of ultrafiltrate filtered through the glomerular capillaries into Bowman's space per unit of time and is commonly used as a marker of renal function. The normal GFR is approximately 100-125 ml/min/1.73 m^2 body surface area in adult men and women (Marieb & Hoehn, 2014). This means around 150 Litres of ultrafiltrate is generated daily. This high volume of ultrafiltration necessitates significant reabsorption in the various tubular segments. The proximal convoluted tubule reabsorbs nearly 60-70% of the filtrate by active reabsorption of sodium ions and passive chloride ion transport along with a number of other substances such as glucose and amino acids. The remainder of the filtrate passes down the loop of Henle.

Loop of Henle, with its descending and ascending limbs, extends between cortex and medulla. The filtrate passing into the loop of Henle is processed by a countercurrent multiplier mechanism which enables the kidneys to recirculate electrolytes and trap it in the medulla surrounding the loop of Henle. The descending loop is permeable to water but impermeable to solutes which makes the filtrate hypertonic as it passes through this segment. The ascending loop is impermeable to water but sodium, chloride and potassium ions are actively transported in this

segment from the tubular lumen into the surrounding medulla. This in turn provides a positive feedback loop and the consequence of this is that the osmolality is much higher in the renal medulla compared to the cortex. This osmotic gradient is vital to enable reabsorption of water in the collecting ducts.

Filtrate from the loop of Henle passes into the distal convoluted tubule where further reabsorption of sodium and chloride ions occurs. Parathyroid hormone (PTH) acts on this segment to increase calcium reabsorption through the tubules. Aldosterone is a mineralocorticoid hormone secreted in the adrenal cortex in response to a number of factors including increase in serum potassium and renin concentration. Aldosterone acts on the distal segment to increase active reabsorption of sodium and with it, the passive diffusion of water. There is also active secretion of potassium and hydrogen ions in the distal part of distal convoluted tubule along with reabsorption of bicarbonate which is essential for maintaining acid-base balance. The filtrate finally passes into the collecting ducts where further active reabsorption of water occurs through the influence of Antidiuretic hormone (ADH). The final outcome of these complex processes is not only the formation of urine containing the waste products of metabolism but also maintenance of fluid, acid-base and electrolyte balance.

The kidneys also perform important endocrine functions through secretion of various hormones. Renin is secreted from the juxtaglomerulus apparatus in the kidneys, which plays an important role in regulation of blood pressure and salt and water retention. Kidneys secrete erythropoietin which acts on the bone marrow to stimulate red blood cell production. Erythropoietin production is stimulated by reduced levels of oxygen. Kidneys also perform an essential final step in the production of active metabolite of Vitamin D (1,25-dihydroxy vitamin D) by hydroxylating the circulating form of vitamin D (25-hydroxy vitamin D). This active metabolite acts in conjunction with PTH to regulate bone mineral metabolism and to maintain serum calcium and phosphorus levels.

1.1.2 Chronic Kidney Disease and its measurement

Chronic kidney disease (CKD) is a general term for heterogeneous disorders where there is kidney damage and reduction in kidney function. CKD is a worldwide health problem and has got wide ranging health and financial implications in the long term. Guidelines to diagnose, investigate and manage CKD have been published by the

United States National Kidney Foundation in 2002 ("K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification," 2002) and more recently by KDIGO in 2013 ("KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease," 2013). These guidelines define CKD as structural or functional abnormality of kidney function persisting for ≥ 3 months with or without reduced GFR. The K/DOQI stages of CKD are shown in **Error! Reference source not found.**.

Table 1-1: K/DOQI Stages of Chronic Kidney Disease

Stages	Description	GFR (ml/min/1.73 m^2 BSA)
1	Kidney damage with normal or increased GFR	≥90
2	Kidney damage with mild reduction in GFR	60-89
3	Moderate reduction in GFR	30-59
4	Severe reduction in GFR	15-29
5	Kidney failure	<15 (or dialysis)

Though patients with stage 1 kidney disease have normal GFR, they ought to have other markers of structural or functional kidney damage such as proteinuria to be included in the CKD classification. Further stages of CKD are defined by progressively reduced levels of GFR.

The markers of kidney damage include pathological abnormalities detected by histology, structural abnormalities detected by imaging, electrolyte abnormalities due to tubular disorders, abnormalities in blood such as raised urea and creatinine and abnormalities in urine such as presence of blood and protein. Proteinuria is presence of significant amounts of protein such as albumin in urine (albuminuria). Proteinuria is regarded as key marker of kidney damage and has been shown to be a significant prognostic indicator for progression of CKD and cardiovascular mortality (Jafar et al., 2001; Keane & Eknoyan, 1999; Ruggenenti, Perna, Mosconi, Pisoni, & Remuzzi,

1998). Traditionally, the amount of proteinuria has been calculated through a 24-hour urine collection. In current clinical practice, this is calculated from a spot urine sample by the comparison of urine protein concentration to creatinine level and is termed protein:creatinine ratio (PCR). Proteinuria is generally due to glomerular injury although it could be due to excessive synthesis of specific proteins in conditions such as myeloma. Increased levels of proteinuria are associated with increased risk of progression of CKD and when conditions such as hypertension co-exist, the risk is even higher (Jafar et al., 2003). Proteinuria is predominantly albuminuria and hence, the potential of albuminuria as an early marker of kidney damage is being explored in clinical studies. Microalbuminuria has been shown to be an indicator of incipient diabetic nephropathy (Chiarelli, Verrotti, Mohn, & Morgese, 1997; Mogensen, 1987) and there is considerable focus on initiating treatment in early stages of microalbuminuria detection.

Glomerular Filtration Rate (GFR) is the measure that is used to define level of kidney function. GFR is dependant on several factors such as age, sex, body size and metabolic rate. The most accurate method of measuring GFR is through Inulin clearance. Inulin is a polysaccharide that is freely filtered at the glomerulus and is neither reabsorbed nor secreted by the tubules. Because of the expensive and cumbersome nature of studies involving Inulin, alternative reliable methods have been devised for GFR measurement using markers such as iohexol (Brändström et al., 1998; Olsson, Aulie, Sveen, & Andrew, 1983) and radioactive isotopes such as ^{125}I-iothalamate and ^{99m}Tc-DTPA (Perrone et al., 1990). These methods are time-consuming and are not routinely used in clinical practice.

Creatinine clearance is the method that can be used in clinical practice for measurement of GFR. This method entails 24-hour urine collection along with a blood test to measure creatinine levels in urine and serum. Creatinine clearance is then calculated using the following formula:

$$Creatinine\ Clearance = \frac{Urine\ creatinine\ concentration \times Urine\ volume}{Serum\ creatinine\ concentration \times timeperiod\ of\ collection}$$

Creatinine is an endogenous substance generated as a result of muscle metabolism and as such, is dependant on muscle mass of the individual. Hence, for results to be

comparable between individuals of different body size, creatinine clearance is usually corrected to body surface area of 1.73 m^2 (Stevens, Coresh, Greene, & Levey, 2006). Creatinine is freely filtered in the glomerulus but is also actively secreted by the proximal tubules into the tubular lumen (van Olden, Krediet, Struijk, & Arisz, 1996). As a result, it overestimates GFR by around 20% (van Olden et al., 1996). An alternative method is to measure urinary urea clearance in a similar fashion. Urea clearance underestimates GFR by 20% and hence, some authors suggest calculating the average of urea and creatinine clearance as a measure of GFR (Traynor, Mactier, Geddes, & Fox, 2006). The variations in the assays used for creatinine estimation and the problem of inaccurate 24-hour urine collection complicate this methodology further.

In view of the practical difficulties with the methods described above, there is a need for simpler methods to estimate GFR in clinical practice. Serum creatinine alone can be used as a measure of renal function but the serum level is affected by a number of factors such as age, sex, diet, ethnicity and muscle mass (Stevens et al., 2006; Jones et al., 1998; Levey, 1990). In order to relate serum creatinine to GFR, two predictive equations are in common use – Cockcroft–Gault equation (Cockcroft & Gault, 1976) and the Modification of Diet in Renal Disease (MDRD) study equation (Levey et al., 1999) which are shown below.

Cockcroft-Gault equation:

$$Creatinine\ clearance\ (ml/min) = \frac{(140 - age) \times weight\ (kg) \times (1.23\ if\ male, 1.04\ if\ female)}{Serum\ creatinine\ (\mu mol/L)}$$

Abbreviated 4-variable MDRD equation:

$$eGFR = 186 \times \left(\frac{creatinine}{88.4}\right)^{-1.154} \times (age)^{-0.208} \times (0.742\ if\ female) \times (1.210\ if\ black)$$

where creatinine is measured in μmol/L.

It is worth noting that the former equation measures creatinine clearance while the latter measures estimated GFR (eGFR). Also, there is no correction for body weight in MDRD formula. Both these formulae have been shown to be less accurate for

individuals without CKD (Stevens et al., 2006; Poggio, Wang, Greene, Van Lente, & Hall, 2005). Nevertheless, MDRD formula is now commonly used in routine clinical practice to aid recognition of chronic kidney disease in combination with the K/DOQI stages of CKD mentioned above.

1.1.3 Progression of Chronic Kidney Disease

The rate of CKD progression varies widely on the basis of population studied. The rate of decline of GFR has been found to be associated with a number of factors in many clinical studies – these include hypertension, presence of proteinuria, glycaemic control in those with diabetes and use of medications such as Angiotensin Converting Enzyme inhibitors (ACEI) or Angiotensin receptor blockers (ARB) to control proteinuria.

Diabetes Mellitus is the single most common cause of end-stage renal disease (ESRD) in UK ("UK Renal Registry - The Sixteenth Annual Report," 2013) with nearly 26% of incident ESRD patients having diabetic renal disease. There is strong evidence to suggest that tight glycaemic control in patients with diabetes mellitus can prevent or slow down the progression of kidney disease ("The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group," 1993). The Diabetes Control and Complications Trial was a randomised controlled study in which type 1 diabetics were randomised to either conventional or intensive glucose control group (Newman et al., 2005). The study showed that intensive glucose control reduced the occurrence of microalbuminuria by 39% and that of albuminuria by 54%. Similarly, United Kingdom Prospective Diabetes Study (UKPDS) also showed a reduction in the incidence of microalbuminuria and of decline in renal function with intensive glycaemic control ("Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group," 1998).

Presence of proteinuria is a significant factor in the progression of renal disease irrespective of the underlying aetiology. A systematic review conducted by Newman et al (2005) showed that in the presence of microalbuminuria, the relative risk (RR) of developing ESRD was 4.8 for patients with type 1 Diabetes Mellitus and 3.6 for

those with type 2 diabetes (Newman et al., 2005). RR of developing clinical proteinuria was also higher at 7.5 for patients with either types of diabetes. The Irbesartan Diabetic Nephropathy Trial randomised hypertensive patients with diabetic nephropathy to receive either irbesartan, amlodipine or placebo with primary composite outcome as doubling of serum creatinine, development of ESRD or death (Lewis et al., 2001). This study showed that irbesartan was effective in slowing the progression of the renal disease with 33% reduction in the risk of CKD progression. ACEIs have also been shown to have similar effects on progression of renal disease and proteinuria (Lewis & Lewis, 2003; Brenner et al., 2001; Laffel, McGill, & Gans, 1995). Subsequent clinical studies also confirmed the reno-protective effects of ARBs and ACEIs in hypertensive and diabetic patients (Izuhara et al., 2005; Wright et al., 2002; "Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group," 1998). It is now common practise that ARBs and ACEIs are routinely prescribed for patients with diabetic nephropathy to reduce proteinuria and limit the progression of renal disease. Hypertension is another important factor contributing to the progression of renal disease. Several studies in patients with and without diabetes have clearly shown that better blood pressure control is associated with reduced incidence of microalbuminuria and reduced rate of renal function decline ("Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia)," 1997; Hunsicker et al., 1997; Klahr et al., 1994; Lewis, Hunsicker, Bain, & Rohde, 1993).

1.1.4 Effects of CKD on Organ Systems

Progressive renal impairment has adverse effects on many organ systems. Early stages of CKD are usually asymptomatic with little biochemical abnormalities. As CKD progresses, the serum concentrations of various metabolites, collectively termed “uraemic solutes”, increase progressively. Whilst many of the adverse effects of CKD are due to the loss of various endocrine and excretory functions of the kidney itself, an increasing number of uraemic solutes are being identified as potential causes of various cardiovascular and immune system defects seen in advanced CKD. The prevalence of various complications according to CKD stages are shown

in Table 1-2 ("KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease," 2013).

Table 1-2: Prevalence of complications of CKD

Complication	GFR Category (ml/min/1.73m^2)			
	60-89	45-59	30-44	<30
Anaemia	4.7%	12.3%	22.7%	51.5%
Hypertension	41.0%	71.8%	78.3%	82.1%
25(OH) Vit D deficiency	9.1%	10.7%		27.2%
Acidosis	8.4%	9.4%	18.1%	31.5%
Hyperphosphataemia	7.4%	9.2%	9.3%	23.0%
Hyperparathyroidism	9.4%	23.0%	44.0%	72.5%

Hypertension is a common complication of CKD and has also an adverse effect on the progression of CKD as discussed above. This is generally managed by a combination of dietary salt restriction and anti-hypertensive medications. Secondary hyperparathyroidism is another complication of CKD. Kidneys convert 1α-hydroxy Vitamin D to its active form 1,25-dihydroxy Vitamin-D, which plays a vital role in regulation of phosphorus and calcium in the blood and in bones. In addition, progression of CKD leads to hyperphosphataemia and hypocalcaemia due to deranged excretory function. All these combine to cause hyperparathyroidism. Secondary hyperparathyroidism has low prevalence in early stages of CKD and is initially asymptomatic. However, its prevalence increases with progression of CKD (Figure 1-1) (Levin et al., 2006) and has various adverse effects on bone mineral metabolism finally culminating in renal osteodystrophy in untreated cases. Secondary hyperparathyroidism is usually treated with 1,25-dihydroxy Vitamin-D replacement, dietary phosphate restriction and oral phosphate-binding medications.

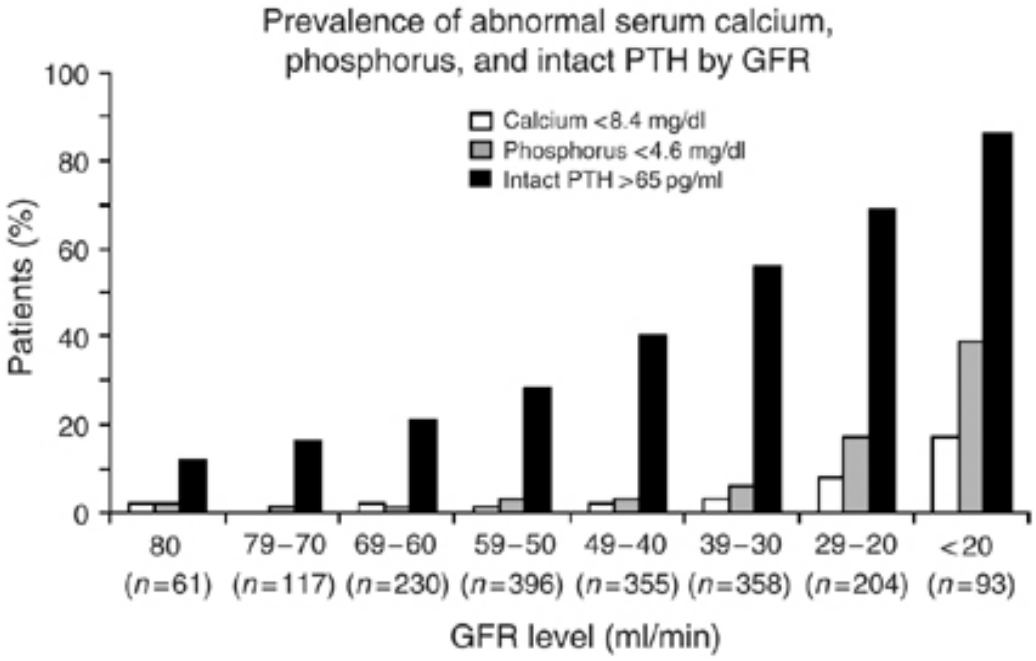


Figure 1-1: Prevalence of abnormal serum calcium, phosphorus and PTH by GFR levels

Reproduced by permission from Macmillan Publishers Ltd: Kidney International, Levin et al, 2006; 71: 31-38, copyright 2007

Anaemia is a common complication of CKD. Although symptomatic anaemia is seen only in CKD stages 4 and 5, the prevalence of anaemia starts increasing even in earlier stages of CKD. Whilst anaemia prevalence is around 4.7% in CKD stage 2, it increases steadily to around 51.5% in CKD stage 5 (Inker, Coresh, Levey, Tonelli, & Muntner, 2011). This is due to progressive reduction in secretion of erythropoietin by the kidneys. The effects of anaemia are quite varied ranging from fatigue and reduction in quality of life to cardiovascular effects such as left ventricular hypertrophy in the long-term. Anaemia has been shown to be associated with increased mortality risk, hospitalisation and reduced quality of life (Foley et al., 2000; Collins, Ma, Xia, & Ebben, 1998). Renal anaemia is treated with recombinant human erythropoietin injections.

Metabolic acidosis is also a complication of advanced CKD with nearly 31% of patients in CKD stage 5 having acidosis. Acidosis is one of the causes for protein energy wasting commonly seen in patients with renal failure. Acidosis has also been

shown to contribute to the progression of CKD and increased mortality risk in CKD patients (Kanda, Ai, Yoshida, Kuriyama, & Shiigai, 2013; Raphael et al., 2013). Acidosis is treated by oral sodium bicarbonate supplementation.

In addition to the above complications due to the direct effect of loss of kidney functions, many other adverse effects are also being increasingly identified due to the actions of accumulated uraemic solutes. The database of uraemic retention solutes continues to increase with nearly 25 new retention solutes added to the initial reported list of 90 solutes (Vanholder et al., 2008; Vanholder et al., 2001) some of which are shown to exert toxic effects on various organ systems. For example, Guanidino compounds have been implicated in neurotoxicity and stimulation of leucocytes to produce pro-inflammatory cytokines leading to atherogenesis (Glorieux, Dhondt, et al., 2004; D'Hooge et al., 2003). Advanced glycation end-products (AGEs), p-cresylsulphate and homocysteine have been shown to stimulate various lymphocyte cells leading to increased oxidative stress and atherogenesis (Schepers et al., 2007; Au-Yeung, Yip, Siow, & O, 2006; Glorieux, Helling, et al., 2004; Vanholder et al., 1995). Moreover, uraemic solutes such as AGE (Rashid, Benchetrit, Fishman, & Bernheim, 2004), asymmetric dimethylarginine (Vallance, Leone, Calver, Collier, & Moncada, 1992), homocysteine (Abahji et al., 2007) and indoxyl sulphate (Dou et al., 2007; Dou et al., 2004) have been shown to cause endothelial dysfunction and inhibition of endothelial proliferation which may precipitate development of atherosclerotic vascular disease. The increasing recognition of the in-vivo effects of these retention solutes help us understand better the mechanisms behind various complications of CKD.

1.2 Treatment of End-Stage Renal Disease

Management of End-stage renal disease is focused on preventing and treating the consequences of renal failure. Many of the complications discussed above are common by the time ESRD is established. Appropriate dietary restrictions are an important part of managing ESRD to prevent hyperkalaemia and hyperphosphataemia. Anaemia, acidosis and hypertension are managed usually by medications. Nevertheless, renal replacement therapy becomes a necessary addition to these treatments.

Renal replacement therapy (RRT) aims to replace the excretory functions of the kidney including excretion of metabolic wastes, maintenance of acid-base and fluid balance. RRT is offered in the form of dialysis or kidney transplantation. The ideal RRT modality for an individual patient is decided based on many clinical and social factors. Clinical factors such as comorbid conditions, frailty and age need to be considered in the decision-making process. A detailed assessment of social factors needs to be carried out to determine suitability for home-based therapies. A subset of patients will not be suitable for dialysis or transplantation and the choice of treatment in those individuals is conservative management. Conservative management involves continuing support to ESRD patients and aims to provide a good quality of life by means of medical and psychological interventions.

Dialysis is the most commonly used renal replacement therapy (Grassmann, Gioberge, Moeller, & Brown, 2005). Broadly, there are two dialysis modalities – haemodialysis and peritoneal dialysis. Although the principles are similar in these modalities, their clinical application and patient selection are different.

1.2.1 Haemodialysis

Haemodialysis (HD) is administered commonly as a hospital-based therapy although home haemodialysis is now increasingly being made available in many centres. HD involves routing the blood from the patient through the HD circuit and returning it to the patient. The dialyser in the HD circuit is the artificial kidney where removal of fluid and uraemic solutes takes place. The uraemic solutes are removed from patient's blood through diffusion and osmosis in the dialyser. Dialyser is a synthetic semi-permeable membrane with separate compartments for flow of blood and dialysis fluid. A schematic representation of HD circuit is shown in Figure 1-2.

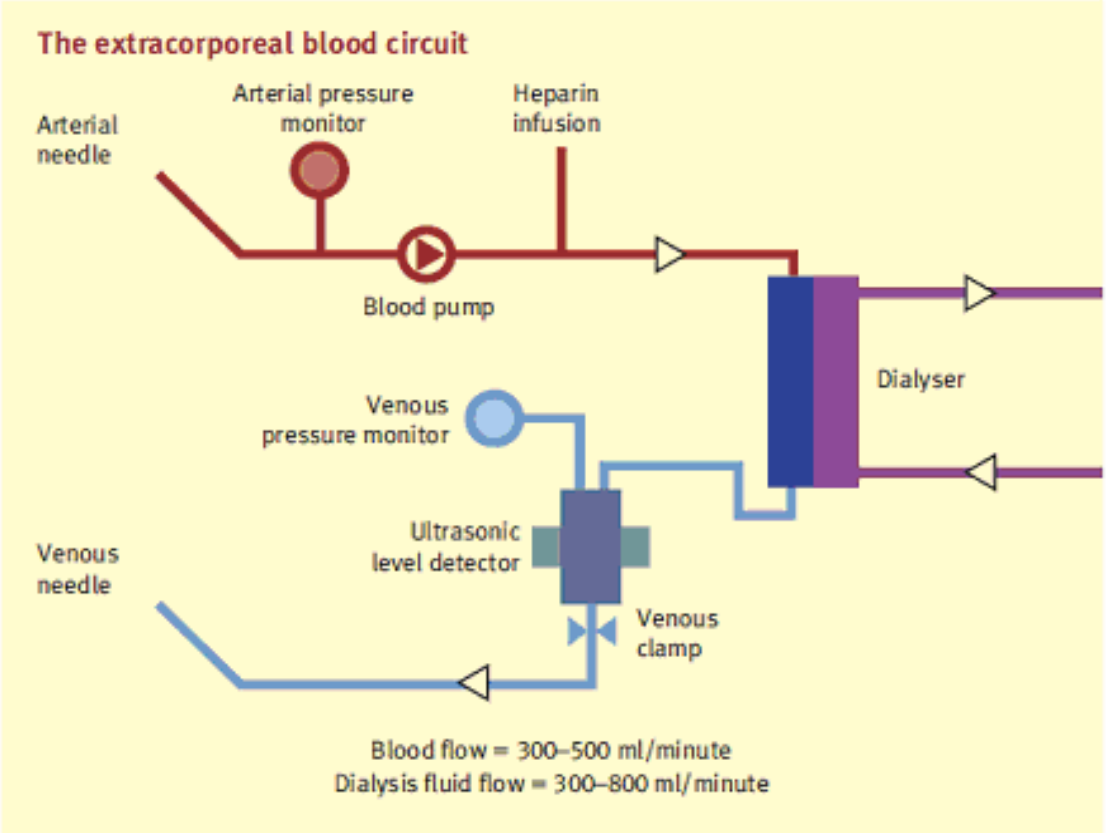


Figure 1-2: Haemodialysis Circuit

Reproduced with permission from Elsevier Ltd: Sivalingam M, Farrington K. Haemodialysis. Medicine 2007; 35(8): 461-465.

The preferred access for HD is an arterio-venous fistula (AVF) which is fashioned surgically either near the wrist or the elbow by joining a superficial vein to an artery. Two needles are inserted into the AVF – one to draw the blood away from the patient ("A-needle") and the other to return it to the patient ("V-needle"). Blood from the A-needle flows through the blood compartment of the dialyser where it interacts with the dialysate compartment through minute pores in the dialyser membrane. It is through these pores the exchange of solutes and fluid take place. The composition of the dialysate fluid is devised for optimal removal of uraemic solutes and correction of acidosis. Solutes such as urea, creatinine, potassium and phosphate that are high in patient's blood move to the dialysate compartment as bicarbonate moves from the dialysate into patient's blood. A schematic representation of solute movement within the dialyser is shown in Figure 1-3.

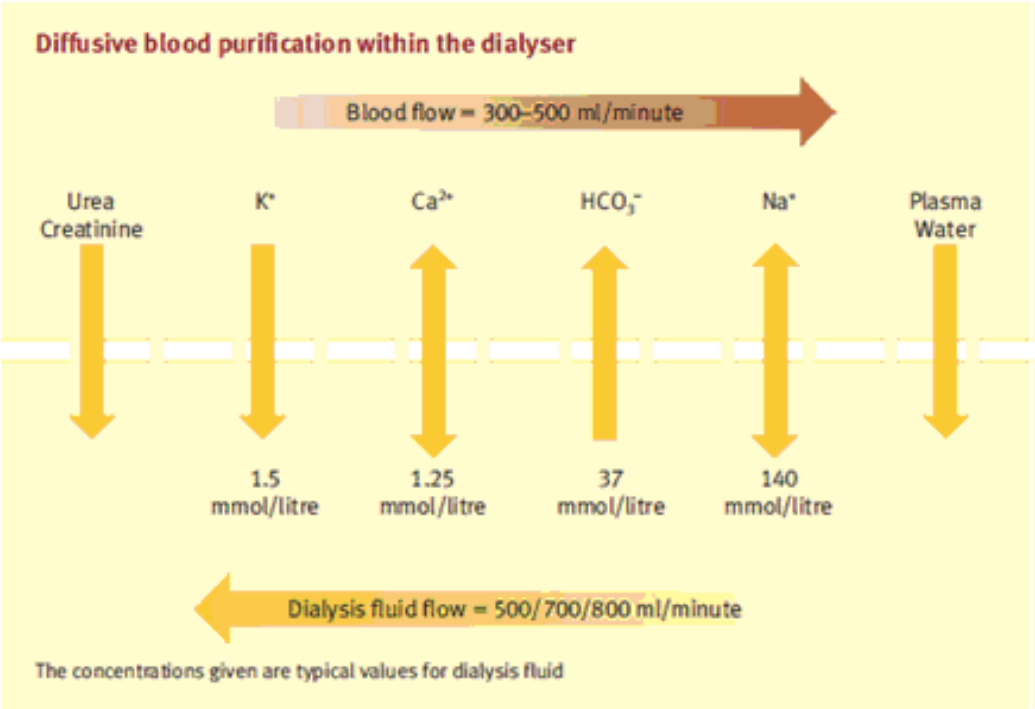


Figure 1-3: Solute and fluid movement within the dialyser

Reproduced with permission from Elsevier Ltd: Sivalingam M, Farrington K. Haemodialysis. Medicine 2007; 35(8): 461-465.

While small molecules such as urea and creatinine are removed easily by conventional “low-flux” HD, much larger uraemic solutes (i.e. middle molecules) such as β2-microglobulin and Cystatin C are not removed by this method. To enhance removal of these middle molecules, the pore size of the dialyser needs to be increased thereby providing a “high-flux” haemodialysis. HD is predominantly a diffusive treatment. Haemofiltration is a convective form of treatment and has been combined with haemodialysis to develop Haemodiafiltration (HDF). HDF using a high-flux dialyser provides better middle molecule clearance and causes less haemodynamic instability in patients.

Haemodialysis is commonly provided in hospital setting, typically for 3 days a week for 3-4 hours per session. There has been a surging interest in home haemodialysis in the recent years. Typically, a home HD patient will dialyse 4-5 times a week for shorter times per session. The technological advancement in home HD delivery has led to portable dialysis machines which provide reliable dialysis to patients. Home

HD patients have less dietary restrictions compared to those having in-centre HD and may provide better quality of life.

1.2.2 Peritoneal Dialysis

Peritoneal dialysis (PD) employs the same principles of diffusion and osmosis but the dialysis is not performed outside the body like HD. Instead, the peritoneal membrane in the abdomen is used as the semi-permeable membrane for dialysis. A peritoneal dialysis catheter is inserted in between the two layers of the peritoneal membrane. An external connecting tube is used to fill in and drain the dialysis fluid from the peritoneal cavity. The patients fills the peritoneal cavity with the dialysis fluid which is different in composition to that used in HD and leave it to 'dwell' in the cavity for a few hours before draining it out and exchanging it with another bag of dialysis fluid. Similar to HD, the process of diffusion and convection takes place between the dialysate in the peritoneal cavity and the blood flowing in the capillaries of the peritoneal membrane.

The advantage of PD is that it is home-based and causes less intrusion to patient's everyday activities compared to HD. Patients could choose to dialyse in one of the two ways - Continuous Ambulatory Peritoneal Dialysis (CAPD) or Automated Peritoneal dialysis (APD). CAPD is typically carried out during the day in which the patient does the fluid exchanges manually. APD is usually carried out at night with the help of an automated machine which performs the fluid exchanges at specified intervals. As it is a home-based therapy, it is imperative that patients opting to do PD have appropriate provisions at home and have a reasonable level of physical and mental functioning capacity.

Two potentially fatal complications of PD are PD peritonitis and Encapsulating Peritoneal Sclerosis (EPS). PD peritonitis is an infection of the peritoneal membrane. This is usually precipitated by failing to follow sterile precautions before doing a PD fluid exchange. Rarely, this could also be caused by contaminated PD fluid. Most PD peritonitis can be treated successfully with antibiotics if diagnosed early. In non-responsive cases, PD catheter is removed and the patient is required to do HD. In a minority of patients, a severe PD peritonitis may be fatal. EPS is widespread thickening of peritoneal membrane leading to frequent episodes of bowel obstruction due to adhesions. This is mostly seen in patients who have been on PD for many

years or in those who have had multiple episodes of PD peritonitis. EPS is a progressive condition in majority of patients leading to multiple abdominal surgeries and poor nutrition with high mortality risk.

1.2.3 Transplantation

Renal transplantation is the preferred form of RRT. A well functioning transplant replaces the lost kidney function and reverses many of the complications associated with ESRD. Transplantation involves a kidney donor – either a living donor or a cadaveric donor. Cadaveric kidney donation could be from a “non-heart beating” donor or a “heart-beating, brain-dead donor”. In order to maximise the benefit of a transplant and to minimise the risks associated with immunosuppression post-transplant, most centres employ rigorous criteria before accepting a CKD patient for transplant. A potential transplant recipient is expected to have good cardiac and functional status. Many ESRD patients are elderly and frail and hence, are not suitable for transplantation. Traditionally, transplant is only carried out between ABO-compatible and HLA-matched individuals. With the advent of very effective immunosuppressants, HLA-mismatch and even ABO-incompatibility are not seen as absolute barriers to transplantation at present.

Following a transplant, patients are required to take immunosuppressive medications to avoid episodes of transplant rejection. Infective complications due to immunosuppression are common in post-transplant period and have to be diagnosed early and treated aggressively. Nevertheless, the benefits of transplantation, which include increased survival and better quality of life, outweigh the risks as long as the transplant recipients are appropriately selected.

Chapter 2

2. A Review on Haemodialysis Dose

2.1 Haemodialysis dose

Kidneys play a central role in maintaining the body's internal milieu. Excretion of metabolic waste products, generated as a result of a myriad of metabolic processes, is a primary function. In kidney failure, metabolic waste products accumulate and exert pathological effects on many organ systems. Many such uraemic toxins are end-products of body protein metabolism. To maintain life in advanced kidney failure, these uraemic toxins must be removed by dialysis. Judging minimal requirements for dialysis is difficult. It might be expected that requirements should be related to the rate of metabolic waste production i.e. metabolic rate. Current practice however, adjusts dialysis requirement to body water volume. This may have a number of unintended consequences. This chapter discusses the basis and disadvantages of the current dialysis practice along with evidence base for potential alternative scaling measures for dialysis dosing.

2.1.1 Dialysis dose based on urea clearance

Urea was identified in early days as one of the many uraemic toxins whose serum levels are significantly elevated in renal failure. It was also recognised as a useful marker of dialysis clearance. As it is a small molecule, it is easily cleared by the kidneys and through dialysis. It is also an end-product of protein metabolism and could be used as a marker of whole body protein turnover. Moreover, it is inexpensive and easy to quantify in laboratory settings.

The current practice of deciding dialysis dose is based on urea clearance, a concept that was developed through a number of clinical studies. Teschan and colleagues conducted a study to examine the adequacy of dialysis (Teschan, Ginn, Bourne, & Ward, 1977). They proposed a minimum dialysis urea nitrogen clearance of 3000ml/week/litre of body water (equivalent to a Kt/V of 1). During an experimental period lasting for 6 months, patients were underdialysed with a total dialytic urea nitrogen clearance of 2000ml/week/litre of body water (equivalent to a Kt/V of 0.66).

The researchers found that patients exhibited subclinical EEG abnormalities and behavioural test scores, when they were underdialysed and these changes normalised on returning to baseline dialysis dose.

Subsequently, the National Cooperative Dialysis Study (NCDS) was conducted in an attempt to define adequate dose of dialysis. NCDS was the first randomised controlled trial examining the effect of dialysis dose on clinical outcomes (Lowrie, Laird, Parker, & Sargent, 1981). The study was designed to evaluate the clinical effects of different dialysis prescriptions. It was a 2x2 factorial design with 151 patients randomised to be included in one of the four study groups. The treatment groups were based on time-averaged concentration of urea (TAC_{urea}) – high or low concentration (pre-dialysis Blood Urea Nitrogen of 60-80 or 110-130 mg/dL) and on the duration of dialysis – long or short duration (4.5-5 hr or 2.5-3.5 hr). Outcome measures studied were hospitalisation rates and withdrawal from the study which included death (treatment failure). The follow-up was for less than a year. The study was stopped as the treatment failure rates were significantly higher in groups with high TAC_{urea} (groups II and IV in Figure 2-1) compared to groups with low TAC_{urea} (groups I and III in Figure 2-1). Although dialysis time was not a predictor of treatment failure, hospitalisation rates were higher in the group with high TAC_{urea} receiving short dialysis. It is worth noting that the dialysis dose received by patients in the high TAC_{urea} group were very low (average Kt/V 0.45) and this may have contributed to poor outcomes. Nevertheless, this study demonstrated the important association between urea clearance and short-term survival for haemodialysis patients.

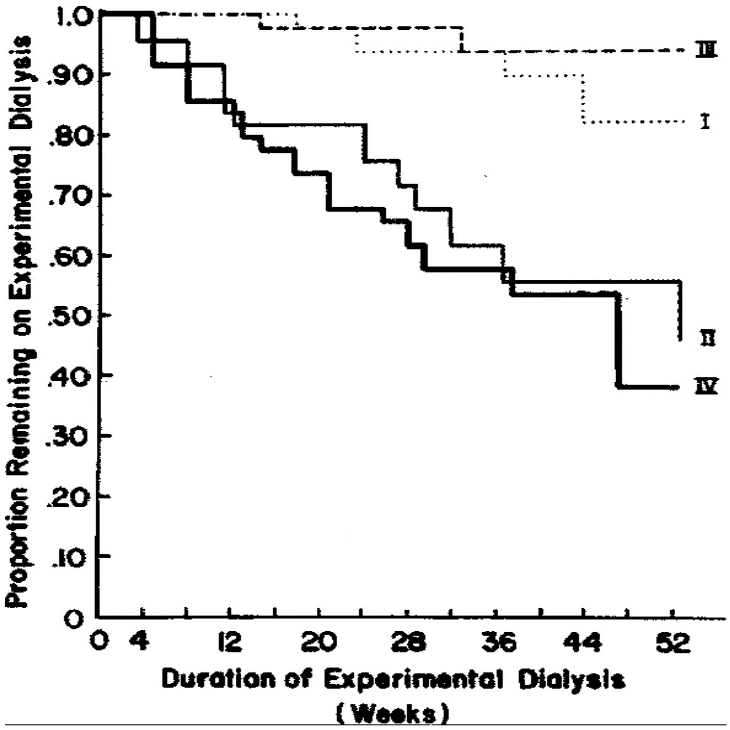


Figure 2-1: Proportion of Patients Not Withdrawn for Medical Reasons or Death in NCDS

Reproduced with permission from Lowrie et al. N Engl J Med 1981; 305:1176-1181, Copyright Massachusetts Medical Society.

Gotch and Sargent (1985) followed this up with a mechanistic analysis of the NCDS study (Gotch & Sargent, 1985). They introduced the concept of measuring dialysis dose by the dimensionless parameter Kt/V, where K is the dialyser urea clearance, t is the treatment time and V is the total body water volume which is the volume of distribution of urea. The mechanistic analysis showed that Kt/V < 0.8 was associated with higher risk of treatment failure irrespective of the nutritional status of the patients. The authors concluded that a fully adequate dialysis can be provided with a Kt/V of 1.0.

A number of studies carried out subsequently showed a consistent relationship between reduced mortality and higher Kt/V values. Marshall et al (2006) suggested that a combination of higher Kt/V dose (≥1.3) and longer dialysis duration (≥4.5 hours) will provide maximal survival benefit (Marshall, Byrne, Kerr, & McDonald, 2006). Other studies have also demonstrated survival benefits with Kt/V of greater than 1.0 (Shinzato et al., 1997; Charra et al., 1992). However, there were practical difficulties in calculation of Kt/V. As in-vivo dialyser clearance of urea was lower than

in-vitro clearance, the calculation of K (conventionally done using in-vitro clearance) resulted in overestimation of Kt/V by around 15-20% (Hakim, 1990). Also, the documentation of the dialysis duration and estimation of V are prone to clerical errors which may result in inaccurate Kt/V calculation.

In light of the benefits shown with higher doses of Kt/V from observational and prospective studies, the HEMO study was designed to test whether increasing dialysis dose will affect survival. The HEMO study was a randomised controlled trial to examine the effect of increasing dialysis dose or using a high-flux dialyser on survival (Eknoyan et al., 2002). This study randomised patients in a 2x2 factorial design to receive low- or high-flux haemodialysis and standard (urea-reduction ratio of 65% or a single-pool Kt/V of 1.25) or high dose dialysis (urea-reduction ratio of 75% or a single-pool Kt/V of 1.65). Patients who died or transplanted during the study were replaced with new recruits, thereby increasing the power of the study. The primary outcome was death from any cause and the main secondary outcome was rate of hospitalisation not related to vascular access. There was no difference in all-cause mortality between the different dose or membrane-flux groups. This study concluded that increasing the sessional dialysis dose beyond a single-pool Kt/V of 1.25 did not confer survival benefit in haemodialysis patients which forms the basis for many of the current guidelines for minimum dialysis dose requirements.

2.1.2 Inclusion of residual renal function to dialysis dose

The dialysis dose measurement discussed above relates only to the urea clearance achieved by dialysis. Most patients have reasonable amount of urine output at the time of dialysis initiation and have considerable urea clearance through the residual renal function (RRF). Although most centres prescribe and measure dialysis dose solely based on dialysis urea clearance, there is an alternative approach termed “incremental haemodialysis”. In this approach, total target urea clearance is calculated as the sum of dialysis clearance and clearance from residual renal function. As RRF declines with time, dialysis dose is titrated up to achieve target urea clearance value.

Incremental haemodialysis was first introduced in 1985 by Bonomini et al (Bonomini, Feletti, Scolari, & Stefoni, 1985). The researchers compared 82 patients dialysing twice weekly but had early initiation of dialysis (at a mean creatinine clearance of 11

ml/min) to 308 patients who started dialysis at a mean creatinine clearance of 2-5 ml/min. They showed that patients who had incremental haemodialysis had better 12-year crude mortality and tended to preserve RRF better. Preserving RRF through incremental haemodialysis has been shown to be associated with reduced mortality (Vilar, Wellsted, Chandna, Greenwood, & Farrington, 2009) and better phosphate and anaemia control (Penne et al., 2011). Though the mechanisms underlying the survival benefit associated with preserving RRF are not clear, it is likely that it may be due to better clearance of middle molecule and protein-bound uraemic solutes by the native kidneys.

The urea clearance from RRF is continuous but this is not the case with haemodialysis which is intermittent. Hence, to measure total urea clearance, either the dialysis clearance (K_d) needs to be converted to an equivalent continuous measure or the residual renal function (K_r) needs to be changed to an equivalent intermittent clearance. Different approaches have been proposed for this conversion. The NKF-K/DOQI guideline recommends using standardised Kt/V developed by Frank Gotch (Gotch, 1998) in which the sessional Kt/V is converted to an equivalent weekly urea clearance. The European Best Practice Guidelines recommend using the Casino-Lopez equivalent renal urea clearance ("Measurement of renal function. European Best Practice Guidelines for Haemodialysis," 2002; Casino & Lopez, 1996). A simpler approach is to inflate K_r by a factor to add it to K_d. As renal clearance is continuous, it is more efficient than dialysis clearance and this inflating factor will adjust for the higher efficiency of the renal clearance.

There are some practical difficulties in measuring residual renal function. RRF is measured through timed urine collection. As RRF declines with time, it has to be measured at regular intervals so that appropriate adjustments can be made to dialysis prescription. Due to the low-volume of urine output in ESRD patients, the timed urine collection has to be carried out through the entire inter-dialytic period. This can be inconvenient to patients and may lead to incomplete urine collection resulting in inaccurate RRF calculation. Alternative novel methods to measure RRF using markers such as Cystatin C are being explored by many researchers (Lindsay, Huang, & Filler, 2010; Hoek, Korevaar, Dekker, Boeschoten, & Krediet, 2007).

2.2 Deficiencies in haemodialysis dose quantification methods

2.2.1 Is Urea Clearance the correct marker for dialysis adequacy?

The two randomised controlled trials on dialysis dosing – NCDS and HEMO study – used small molecule clearance (Kt/V_{urea}) to define the study outcomes. Both these studies did not show survival benefit for longer dialysis treatment time. Knowledge about the kinetics of uraemic toxins of various molecular weights has expanded over the last decade. Urea was considered a surrogate marker of small molecule clearance in the above studies. It has now been shown that the kinetics of small molecule toxins such as guanidine compounds and methylamine are different to urea (Ponda et al., 2010; Eloot et al., 2007; Eloot et al., 2005) and hence, the clearance of these substances may not be similar to urea during standard haemodialysis. It has also been acknowledged by other authors that urea is a poor surrogate marker of removal of other toxic uraemic compounds(Depner, 2001).

There has been growing interest in middle molecule clearance as a measure of dialysis dose. HEMO study did not show overall survival benefit with the use of high-flux membranes which augment middle-molecule clearance as measured by β2-microglobulin clearance in the study. The HEMO study data was subsequently re-analysed to examine whether there was survival benefit in sub-groups of patients in different groups. The sub-group of patients whose dialysis duration was >3.7 years at the time of recruitment had 32% reduction in all-cause mortality on high-flux haemodialysis (Cheung et al., 2003). The researchers also noted 37% reduction in cardiac deaths in the same long duration dialysis group on high-flux therapy, although none of the secondary outcomes reached statistical significance after adjustment for multiple comparisons. This may be due to the inadequate power of the study to detect such small differences. Although, no obvious reason was identified to explain the interaction between dialysis vintage and flux, it is likely that this may be due to better clearance of middle molecules. This suggestion is supported by the study finding that patients on long-term dialysis who had been receiving low-flux dialysis previously had a 41% reduction in all-cause mortality when dialysed using high-flux dialysers. This sub-group analysis, therefore, suggested there might be potential long-term survival benefit from enhanced middle molecule clearance through high-flux dialysis.

The Membrane Permeability Outcome (MPO) Study was a prospective randomised controlled trial to compare the impact of dialyser membrane permeability, and thereby the middle molecule clearance, on survival in HD patients with normal or low albumin with a minimum dialysis dose (single-pool Kt/V) of 1.2 (Locatelli et al., 1999). Although there was no significant survival benefit associated with use of dialysers with high membrane permeability in the study population as a whole, the sub-group of patients with low albumin had significant survival benefit with 37% reduction in mortality risk with use of high-flux membranes (Locatelli et al., 2009).

Evidence from observational studies had also shown other benefits associated with middle-molecule clearance such as reduction in the risk of dialysis-related amyloidosis (Küchle, Fricke, Held, & Schiffl, 1996; van Ypersele de Strihou, Jadoul, Malghem, Maldague, & Jamart, 1991). These data suggest there is potentially a role for middle molecule clearance in defining the minimum dialysis requirements alongside urea clearance. Nevertheless, the kinetics and metabolism of many of these molecules have not been mapped and urea remains the best possible surrogate marker of uraemic toxins at least in the small molecular weight category.

2.2.2 The Denominator Problem: Body Water

The above mentioned disadvantages of using urea clearance to define minimum dialysis requirement is also compounded by other problems. Dialysis adequacy as expressed by Kt/V_{urea} normalises the urea clearance to the volume of distribution of urea (V). This method is based on the assumption that uraemic toxin production is a function of distribution volume. Hence, the dialysis dose here is normalised not on the basis of the rate of production of the toxin (urea in this instance) but on the distribution volume of the toxin. Such use of distribution volume to normalise dialysis dose poses certain problems.

V, sometimes referred to as Watson volume, is normally estimated using Watson formula (Watson, Watson, & Batt, 1980). This volume is derived from anthropometric measures of weight, height and age. For any given age, weight and height, the estimated V is lower in women than men. Similarly, patients with low body mass will be estimated to have low V values. Hence, if the dialysis dose is scaled based on V, this may result in the prescribed standard dialysis dose being inadequate in relation to the rate of uraemic toxin production in females and males with low body mass

index. Needless to say, this sub-optimal dialysis will impact on the survival of these patient groups and indeed, several studies have shown mortality differences in women and patients with low body mass index on haemodialysis. These studies are discussed in the following section.

2.2.3 Survival Differences in ESRD patients on Haemodialysis

Since the publication of HEMO study, a number of researchers have investigated dialysis dose as a predictor of survival in different groups of haemodialysis patients. Survival differences among patients of different gender, body size and body composition have been explored in various studies. A post-hoc analysis of the HEMO study showed that women randomised to the higher dialysis dose group had lower risk of death (Figure 2-2) compared to those receiving standard dialysis dose with a 19% reduction in mortality (Depner et al., 2004). Also in the high dose group, patients with larger body size had 10% reduction in mortality when body size was expressed as body mass index (BMI) and 12% reduction in mortality risk when expressed as body surface area (BSA) compared to those in the standard dose group. These mortality differences persisted even after adjustment for gender differences. The study concluded that there were significant gender and body size interactions with regards to dialysis dose and further studies were needed to explore the benefit of higher dialysis dose in specific groups of patients.

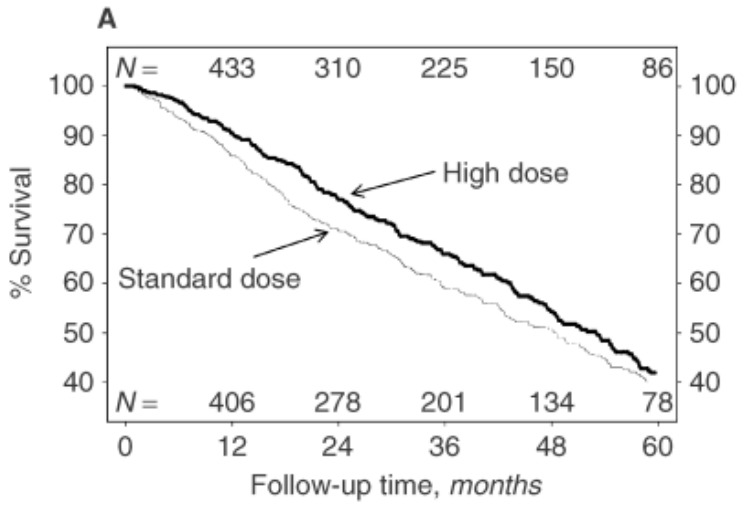


Figure 2-2: Kaplan Meier curve showing time to death in women by randomised treatment group in HEMO study

These gender and body size differences suggest that using V as the denominator to scale dialysis dose may disadvantage specific groups of patients. In fact, analysis of distribution of Watson volume (V) ranges of the study participants showed significantly lower V values for women compared to men (Figure 2-3). A large observational study of 74,120 US haemodialysis patients and data from Dialysis Outcomes and Practice Patterns Study (DOPPS) showed a 15% lower mortality risk in women who achieved urea reduction ratio (URR) of >75% on dialysis compared to those who received less dialysis dose but similar effect was not seen in men (Port et al., 2004) further strengthening the findings from HEMO study about gender differences in survival among haemodialysis patients.

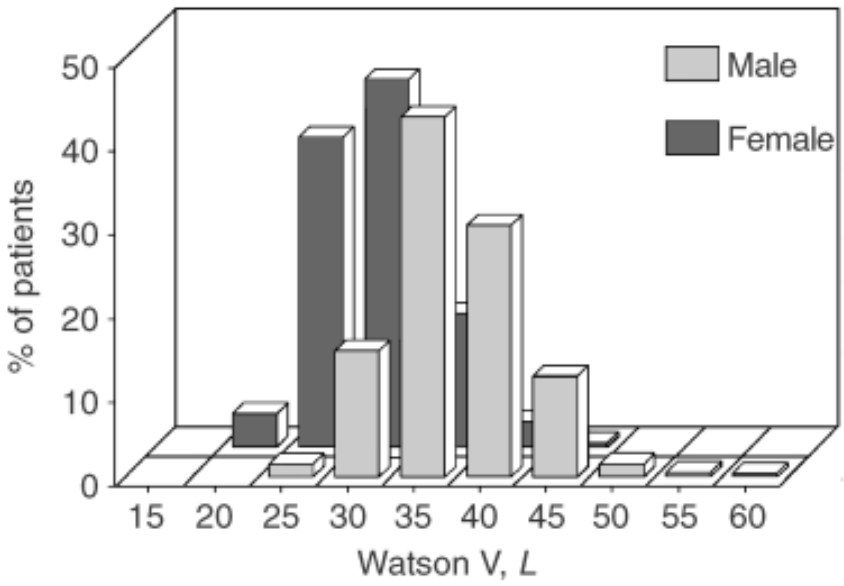


Figure 2-3: Distribution of body water volumes (V) by gender in HEMO study

Reproduced by permission from Macmillan Publishers Ltd: Kidney International, Depner et al, 2004; 65: 1386-1394, copyright 2004

The problem of using V as a normalising factor for dialysis dose becomes clear when the delivered dose is compared with other variables as normalising factor. A study was conducted by Spalding et al (Spalding, Chandna, Davenport, & Farrington, 2008) to compare the delivered dialysis dose by substituting V for other parameters

such as body surface area (BSA) or as a power function of body weight ($W^{0.67}$). This study demonstrated that for any given Kt/V, equivalent dialysis dose using BSA or $W^{0.67}$ would deliver significantly lower doses to women and small men. The researchers showed that the current delivered dose using Kt/V needed to be 16% higher in women and 6% higher in small men compared to larger men.

Apart from the gender differences in delivered dialysis dose and mortality with the use of Kt/V, the relationship between body size measures and mortality in dialysis population was also explored by many investigators. A number of retrospective and prospective observational studies showed that haemodialysis patients with high body mass index (BMI) had a survival advantage and both short-term and long-term mortality risk was significantly lower in this patient group (Port, Ashby, Dhingra, Roys, & Wolfe, 2002; Wolfe et al., 2000; Fleischmann et al., 1999; Leavey, Strawderman, Jones, Port, & Held, 1998). This interaction between BMI and mortality was observed even in patients with extremely high BMI (BMI ≥37) and the survival advantage was significant even after adjustment for serum creatinine and estimated lean body mass (Johansen, Young, Kaysen, & Chertow, 2004). This suggests that there is a survival advantage for obese haemodialysis patients irrespective of their body muscle mass content.

The above findings are in stark contrast to those found in the general population where females have higher life expectancy compared to males. The mortality risk also increases progressively in individuals with higher BMI levels in the general population as shown in Figure 2-4 (Calle, Thun, Petrelli, Rodriguez, & Heath, 1999). The reduced mortality risk noted in haemodialysis patients with regards to gender and body size is strongly suggestive that Kt/V_{urea} underestimates dialysis requirements in women and small men.

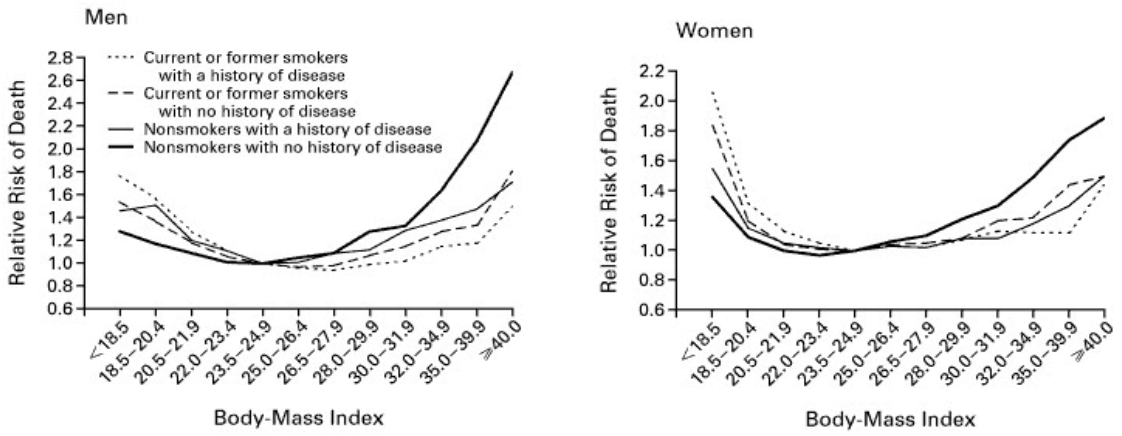


Figure 2-4: Multivariate Relative Risk of death from All Causes among Men and Women

Reproduced with permission from Calle et al. N Engl J Med 1999; 341:1097-1105, Copyright Massachusetts Medical Society.

This phenomenon of reduced mortality risk noted in obese dialysis patients, termed “reverse epidemiology”, led many investigators to explore the mechanisms underlying these differences. As people with higher BMI may receive relatively larger dialysis dose, could dialysis dose be a confounding variable in these analyses? In order to answer this, Levey et al (Leavey et al., 2001) used DOPPS data and showed that the reduced mortality risk persisted in haemodialysis patients with high BMI even after adjustment for dialysis dose, dialysis time and associated comorbid conditions (Figure 2-5). Other studies have also shown that the interaction between BMI and mortality risk was maintained for any given dialysis dose as measured by URR or Kt/V (Port et al., 2002). These studies demonstrated that there may be a partial interaction in survival analyses between dialysis dose and BMI but this alone does not explain the reduced mortality risk in this patient population.

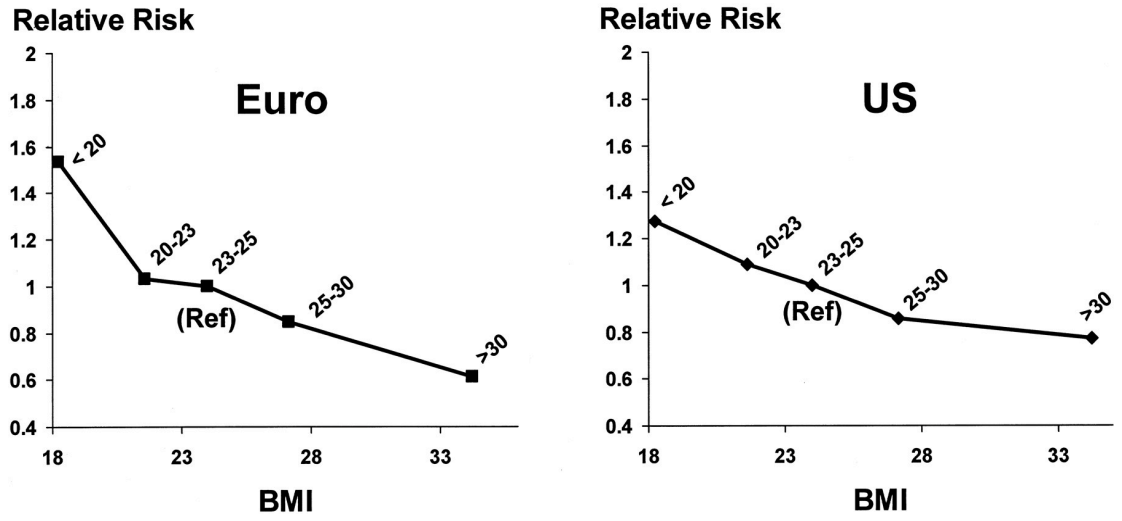


Figure 2-5: Relative mortality risk vs. BMI in Haemodialysis patients in Europe and USA

Leavey et al. Nephrol Dial Transplant 2001; 16: 2386-2394, by permission from European Renal Association-European Dialysis and Transplant Association.

The disadvantage of using BMI as a body size measure is that it does not differentiate between fat mass and muscle mass. Could the phenomenon of reverse epidemiology be related to their body composition? It may be that the survival advantage seen in these patients is limited to those with relatively less fat mass and more muscle mass. The evidence to support this is somewhat conflicting. A retrospective study (Beddhu, Pappas, Ramkumar, & Samore, 2003) conducted to explore the interaction between muscle mass and survival in high BMI patients showed the mortality risk to be higher in patients with low muscle mass (and inferred high fat mass). In this study of more than 70,000 patients with the study data collected through the Medical Evidence Form from the Centers of Medicare and Medicaid, patients with BMI ≥ 25 with low muscle mass (defined by Urine creatinine excretion of >0.55 g/day) had higher risk of all-cause death (Hazard ratio of 1.14) and cardiovascular death (HR 1.19) compared to those with normal BMI. However, analysis of the HEMO study data showed both low muscle mass and low fat mass to be independently associated with higher mortality risk in dialysis patients (Huang et al., 2010). It is worth noting that neither of these studies measured whole body muscle and fat mass directly. Whilst the former study used urine creatinine concentration as a surrogate marker of muscle mass, the latter used anthropometric measurements such as triceps skin-fold thickness and mid-arm muscle

circumference. However, in a study conducted by Kalantar-Zadeh et al in which the fat mass was measured by near infrared interactance, high baseline fat percentage and maintenance of fat mass over time was associated with better survival in dialysis patients with individuals with low body fat having four-fold increase in the Hazard ratio for death compared to those with normal body fat percentage (Kalantar-Zadeh et al., 2006). Nevertheless, the above-mentioned studies did not provide conclusive answers to explain the phenomenon of reverse epidemiology observed in dialysis patients.

2.3 Alternative Measures of Dialysis Dose Quantification

The survival disadvantage, noted in women and small men with the use of urea distribution volume (V) as the scaling parameter, led many authors to suggest that Kt/V is not the best measure of dialysis dose. A number of alternative scaling parameters, instead of V, have been suggested. These include resting energy expenditure (basal metabolic rate), body surface area, high metabolic rate organ mass and liver size (Daugirdas, Levin, et al., 2008). Using total dialysis clearance (Kt) alone without any scaling factor has also been suggested as an alternative (Lowrie, 2000).

Kt, the product of dialyser clearance of the solute and dialysis time, represents the total dialysis dose and is not normalised to V. In 1999, Lowrie et al proposed ‘Kt’ as a measurement index for dialysis dose (Lowrie, Chertow, Lew, Lazarus, & Owen, 1999). The argument in favour of using Kt is that the relationship between Kt and survival is somewhat linear and increasing doses of dialysis as expressed by Kt is always associated with better survival. This is in contrast to the finding that there is a trend toward reduced survival at higher doses of URR or Kt/V demonstrated by some previous studies (Chertow, Owen, Lazarus, Lew, & Lowrie, 1999; McClellan, Soucie, & Flanders, 1998). Also, Kt, unlike Kt/V, is not confounded by nutritional factors. However, there are some factors that argue against applying Kt in the wider clinical practice. Direct calculation of Kt is difficult due to complexities associated with calculation of in-vivo clearance (K). Although initial studies derived Kt by multiplying Kt/V with V, this calculation needs prior determination of Kt/V and V through different equations and increases the workload of dialysis unit staff and as such, is difficult to apply in clinical practice.

The emergence of ionic dialysance measurement resolved this problem. Ionic dialysance measurement entails calculation of conductivity across the dialyser membrane and is similar to the urea clearance (K). Kt obtained by ionic dialysance also has a direct relationship with survival (Lowrie, Li, Ofsthun, & Lazarus, 2004). The ionic dialysance monitors automatically provide Kt values for each dialysis session. The problem in using these monitors is that there are considerable variations in the calculated Kt values depending on the type of monitor that is used (Maduell et al., 2008). This means that dialysis units should be equipped with similar ionic dialysance measuring monitors for the results to be comparable. In addition, as the dialysis dose is not scaled to any body size measure, patients across a wide range of body weights will receive similar dialysis doses irrespective of their nutritional status. Although a method has been described to correct Kt by BSA (Lowrie, Li, Ofsthun, & Lazarus, 2005), there are still no recommended target levels for quantifying dialysis dose using this method. These practical difficulties surrounding the use of Kt as a dialysis dose remains a barrier to its wider application to clinical practice.

As a result of the above issues, there is a need to explore an appropriate normalising factor for dialysis dose scaling. Scaling dialysis dose to body surface area (BSA) is a promising alternative. The advantage in using BSA is that the GFR is routinely adjusted to this parameter in clinical practice and normalising dialysis dose to the same parameter will allow comparison between dialysis dose and residual renal function. In a study carried out in healthy kidney donors, GFR estimated from ^{125}I-iothalamate clearance was scaled to various parameters including BSA, V, metabolic rate and liver size (Daugirdas, Meyer, Greene, Butler, & Poggio, 2009). This study demonstrated that GFR scaled to BSA was similar in men and women whereas the difference in measured GFR between sexes was approximately 14% when scaled to V and 7% with metabolic rate. The authors concluded that BSA was the best parameter for scaling GFR and possibly, for scaling dialysis dose as well.

There is evidence from observational studies that this approach of normalising dialysis dose by BSA would deliver higher dialysis dose to women and small men and may favourably impact on survival in this subgroup of dialysis population. A study of 328 HD patients showed that women and small men will indeed receive higher dialysis doses if the target levels were decided according to Kt/BSA or

Kt/Weight$^{0.67}$ (Spalding et al., 2008). This study showed that to provide similar dialysis dose across all patients with current dialysis dosing practices, the dose for women should be 16% higher compared to large men and at least 6% higher in small men. A retrospective analysis of the HEMO study also found that the BSA adjusted dialysis dose in the HEMO study was significantly lower in women than men which may explain in part the survival benefit observed in women in the high-flux dialysis group (Daugirdas, Greene, Chertow, & Depner, 2010). A study exploring the implications of BSA-based dialysis dose scaling – expressed as surface area normalised Standard Kt/V – showed that this approach will mandate more dialysis for women and small men and slightly lower doses for large men (Daugirdas, Depner, et al., 2008). In a more recent study using the same BSA-based scaling parameter, Ramirez et al (2012) demonstrated that women had better short-term survival compared to men when this approach of dialysis dose scaling was followed (Ramirez et al., 2012). This pattern of survival is similar to that observed in general population.

All these studies support the use of BSA as a scaling parameter and suggest that BSA may reflect better the dialysis requirements compared to body water volume. BSA is closely related to liver size and hence, has an inherent gender-related adjustment (Daugirdas, Levin, et al., 2008). This links BSA to the rate of metabolic waste production and hence, it may be an appropriate parameter for dialysis dose scaling.

If a body size measure such as BSA that is linked to liver size is a better measure of dialysis requirement, then why not use the liver size directly as a scaling parameter? It has indeed been proposed by some investigators that liver mass could be used for scaling dialysis dose. Liver is an important site for metabolism of nitrogenous compounds and could be viewed along with kidney as a paired set of excretory organs working in tandem. High metabolic rate compartment comprises of the visceral organs including the gut and liver is a major component of this. It has been hypothesised that visceral organs generate bulk of uraemic toxins (Kotanko et al., 2007) and hence, scaling dialysis dose to liver mass may provide dialysis based on the rate of uraemic toxin generation. However, there are practical difficulties in this approach. It is not practical to measure liver size in all dialysis patients and hence, it has to be estimated from equations. The problem here is that most of the available equations calculate liver size as a function of BSA (Yoshizumi et al., 2003;

Heinemann, Wischhusen, Püschel, & Rogiers, 1999) and is not gender dependent. Moreover, the data on liver size norms and variability in renal failure patients is scant which hinders the application of this approach to clinical practice at the present time.

2.3.1 Metabolic Rate and Dialysis Dose Measurements

Basal Metabolic Rate (BMR) represents the sum of all the metabolic activities that occur in an organism at rest and as such, is closely related to the rate of metabolic waste production. Using Kt/V_{urea} means that the dialysis dose is scaled to the volume in which the uraemic toxins are distributed rather than the generation rate of these toxins. Morton and Singer (2007) argued that the dialysis dose should be scaled to metabolic rate as it is more closely related to uraemic toxin generation than distribution volume (Morton & Singer, 2007).

Nitrogenous waste products are formed as a result of amino acid and protein metabolism. The primary function of the kidneys is excretion of nitrogenous waste products at a rate proportional to its production in the body. Does this mean that GFR is related to metabolic rate? Singer (2001) argues that GFR increases in proportion to body mass and so does the BMR (Singer, 2001). Based on the review of body functions by White and Seymour (White & Seymour, 2005) in a wide range of animal species, allometric scaling applies to mammals of all sizes. Allometric scaling allows scaling of biological functions, such as GFR or BMR, to body mass according to the equation $y=ax^b$ where 'y' is the biological function of interest, 'a' is the scaling coefficient, 'x' is body mass and 'b' is the exponent. A number of body functions including cardiac output, respiratory functions (minute volume, mean expiratory flow rate) and effective renal plasma flow are all shown to scale to body mass using this equation. The scaling exponent 'b' in the above equation is close to 0.75 for all these parameters (Singer, 2001). Singer (2001) explains that animals of varying sizes adapt their metabolism to become more energy efficient in such a way that the energetic cost of overall metabolic activity is relatively lower in larger animals. Although there are inter-species variations in the scaling exponent, it remains constant in the same sized mammal.

GFR increases with increase in body size due to increase in both the number of nephrons in each kidney and the surface area of the glomeruli. Although there is an absolute increase in GFR with increase in body size, GFR per unit of body mass

decreases. Similar effect is seen with regards to BMR. As body size increases, the oxygen consumption per unit body mass decreases although there is an absolute increase in BMR (KREBS, 1950).

Allometric scaling shows that both GFR and BMR increase with body size with a scaling exponent of approximately 0.75 as shown by Singer (2001) using previously published data (Figure 2-6).

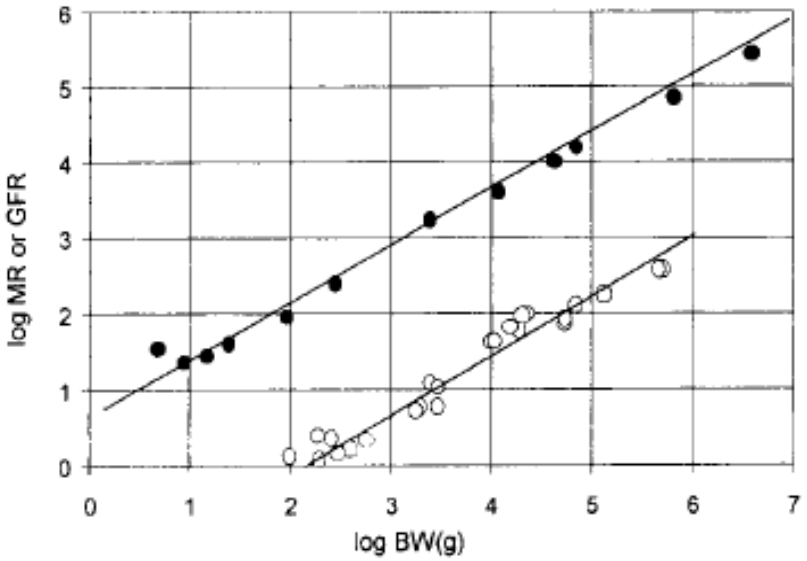


Figure 2-6: Relationship between Body weight (BW) and BMR (•) or GFR (o)

Reprinted from Singer MA, Am J Kidney Dis 37(1): 164-178; Copyright 2001, with permission from Elsevier.

Singer (2001) hypothesised that this is because the metabolic rate is the driving process for renal blood flow and hence, GFR responds to changes in metabolic rate. He also argued that the converse is not true i.e. changes in GFR should not result in corresponding changes in metabolic rate. There is circumstantial evidence to suggest that changes in metabolic rate drive corresponding changes in GFR. In patients with hypothyroidism, reduction in BMR is accompanied by reduction in GFR (Davies, Mackinnon, & Platts, 1952; Mackay & Sherrill, 1943) and opposite relationship prevails in patients with hyperthyroidism (Aizawa et al., 1986; Ford, Owens, Curd, Moyer, & Spurr, 1961; Mackay & Sherrill, 1943). This hypothesis is also supported by some clinical studies in CKD patients demonstrating no change in

metabolic rate with the reduced GFR in this patient population (Schneeweiss et al., 1990; Monteon, Laidlaw, Shaib, & Kopple, 1986).

There are survival differences among dialysis patients of different ethnicity which may be related to metabolic rate. BMR estimated by resting energy expenditure (REE) has been shown to be lower in Asians and Blacks compared to Whites (Wouters-Adriaens & Westerterp, 2008; Gallagher et al., 2006). This means that these ethnic group patients may receive sufficient dialysis even at lower levels of Kt/V. This difference in metabolic rate may be the underlying cause for the increased survival observed among Asian and Black dialysis patients compared to Whites (Jain et al., 2009; Roderick et al., 2009).

These findings definitely present a case in favour of using metabolic rate as a scaling parameter for dialysis dose. However, the counter arguments to using metabolic rate as the scaling parameter need to be considered. Although some studies showed normal REE in CKD patients, there are studies that demonstrate raised (Neyra et al., 2003; Kuhlmann, Schwickardi, Trebst, & Lange, 2001; Ikizler et al., 1996) or lowered (Avesani et al., 2004; Panesar & Agarwal, 2003) metabolic rate in patients with kidney disease. This argues against Morton and Singer's above mentioned hypothesis. Moreover, if metabolic rate is the driving process for GFR, one would expect GFR to rise with large amounts of physical activity. There is little evidence to strongly support this. Although there is some evidence that physical activity may be related to GFR (Finkelstein, Joshi, & Hise, 2006), this relationship may vary depending on the intensity of exercise and there may even be a reduction in GFR at higher levels of physical activity (Kachadorian & Johnson, 1970). Daugirdas et al (2008) used HEMO study data to quantify dialysis dose using REE-normalised Standard Kt/V and analysed the potential dose change that will be needed if REE is used as scaling parameter. The researchers showed that this approach will result in only trivial changes in recommended dialysis dose for both men and women compared to current dosing practice (Daugirdas, Levin, et al., 2008). The researchers argued that the lack of evidence demonstrating significantly higher GFR in athletes and labourers compared to office workers undermines the importance of BMR as a principal determinant of GFR.

There are some practical difficulties that could be expected in studies related to metabolic rate. The main barrier is the availability of resources – direct measurement of metabolic rate requires indirect calorimetry apparatus which is relatively expensive. Metabolic rate measurement with this device could take up to 30 minutes for each patient. Hence, it is not practical to use this in routine clinical practice and as such, it remains only as a research tool. As a result, studies related to metabolic rate in renal failure are limited leading to poor understanding of the effect of renal failure on metabolic rate and the determinants of metabolic rate in renal failure. Studies focusing on exploring these aspects of metabolic rate may shed more light on the reliability and applicability of metabolic rate as a normalising factor for dialysis dosing.

The following sections discuss two important determinants of metabolic rate – body composition and protein turnover – with regards to their role in management of patients with renal disease.

2.3.1.1 Body Composition and Metabolic Rate in Renal Failure

Body composition is a major determinant of resting energy expenditure in general population. Body composition can be broadly classified into fat mass (FM) and fat-free mass (FFM). FFM is a heterogeneous compartment and includes the masses of various organs, skeletal muscle, bone and other extracellular tissues excluding adipose tissue. With regards to metabolic rate, FFM comprises of two distinct tissue categories – high and low metabolic rate compartments. High metabolic rate (HMRO) compartment is principally made up of various organs such as brain, heart, liver, spleen and kidneys whereas the low metabolic rate compartment comprises of bone and other extracellular tissues. The metabolic activity of skeletal muscle (MM) is intermediate and has significantly higher metabolic activity compared to bone and other extracellular tissues. The different compartments of FFM and their relative energy expenditure are shown in Figure 2-7 (Müller, Bosy-Westphal, Kutzner, & Heller, 2002).

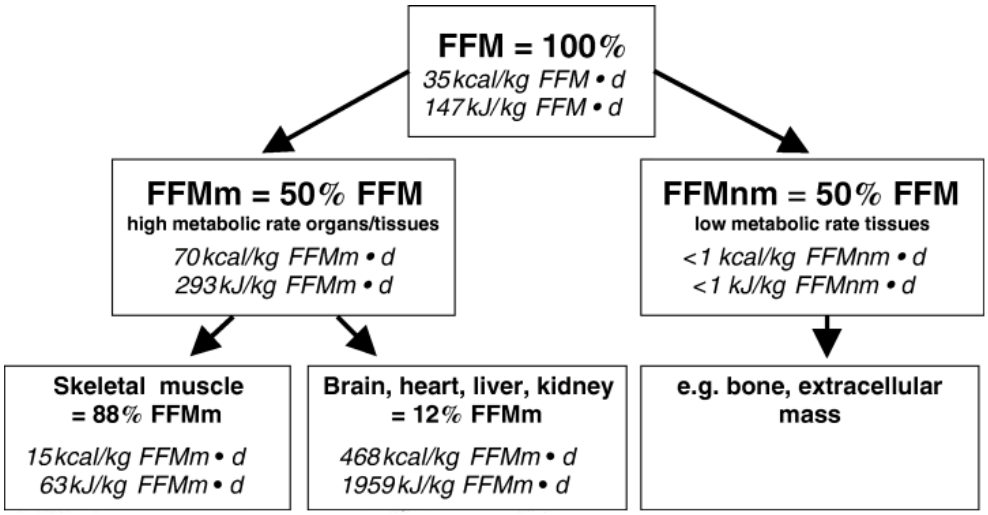


Figure 2-7: Components of Fat-free mass and their Energy Expenditure at Rest

Reproduced with permission from Muller et al, Obes Rev 3(2): 113-122; Copyright 2002, John Wiley and Sons.

The concentration of uraemic toxins in the blood is influenced by the rate of production of toxins, their distribution volume and their clearance through dialysis and/or residual renal function. It is vital to understand the relative roles of HMRO and MM in the metabolism of uraemic toxins in individuals of varying body sizes. HMRO is a highly metabolically active compartment and is responsible for the major bulk of nitrogenous metabolic waste production. Although comprising only about 5% of body weight, they contribute approximately 70-75% to the basal metabolic rate. In contrast, MM forms a greater proportion of body weight but contributes far less to the metabolic rate. Approximately 70% of muscle tissue is comprised of water. Hence, MM compartment may be considered a site of uraemic toxin sequestration.

If it is assumed that the proportion of HMRO and MM as FFM remains constant irrespective of body size, one would expect to see a constant REE/kg of FFM across adults of all sizes. However, this is not the case as shown by some clinical studies (Wang et al., 2000; Weinsier, Schutz, & Bracco, 1992; Ravussin & Bogardus, 1989). Indeed, REE/FFM decreases with greater FFM. This may be due to changes in relative proportions of HMRO mass and MM in individuals with higher body weights. Heymsfield et al (2002) measured various tissue compartments using MRI and showed whilst the proportion of HMRO mass as FFM decreases, the MM and low metabolic rate compartment mass increases with increase in FFM mass (Heymsfield

et al., 2002). This means that in larger individuals, the metabolic rate, and thereby the metabolic waste production, per unit mass of metabolically active tissue is less compared to a smaller individual.

If the above changes in body composition with body size holds true for patients with kidney disease, then this may have important implications for dosing of dialysis. However, studies exploring body composition differences in haemodialysis patients are very limited. Kotanko et al (2007) hypothesised that the concentration of uraemic toxins is lower in larger in dialysis patients for two reasons (Kotanko & Levin, 2007). Firstly, larger dialysis patients have higher muscle and fat mass providing a larger distribution volume and secondly, the relative mass of HMRO in relation to body weight is smaller in these patients (Figure 2-8). As dialysis dose is scaled to urea distribution volume, smaller patients may be receiving lower dialysis doses relative to their metabolic needs. They estimated HMRO mass using published equation and showed that small patients had significantly higher HMRO mass per kg of body weight (Kotanko & Levin, 2007). This means that the uraemic toxin concentration was relatively higher in smaller patients compared to larger patients. If this is true, does it impact on survival? The same group examined short-term survival in haemodialysis patients in relation to HMRO mass/body weight and found that patients in the lowest HMRO mass/body weight tertile group had 10% higher survival time compared to those in middle tertile and nearly 17% compared to those in the lowest tertile.

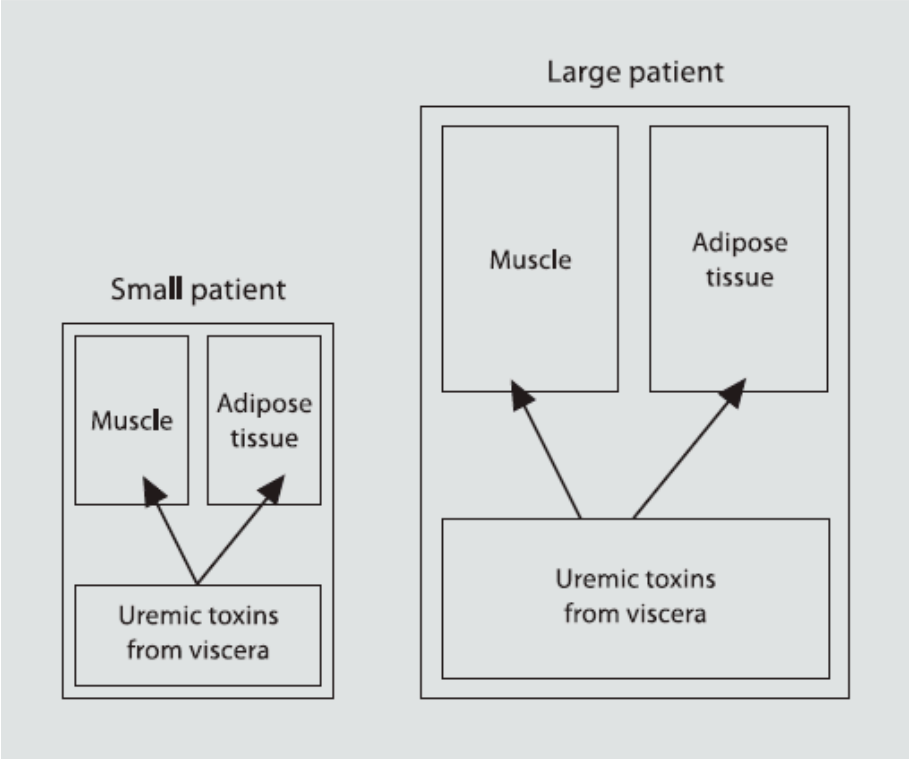


Figure 2-8: Relative sizes of HMRO, skeletal muscle and adipose tissue

Larger patients have relatively larger muscle and fat mass and lower HMRO mass/body weight compared to smaller patients. Reproduced with permission from Kotanko et al; Blood Purification 25(1): 27-30, Copyright © 2007 Karger Publishers, Basel, Switzerland.

Whilst this study was suggestive of body composition differences as a possible mechanism underlying the survival difference observed in women and small men on haemodialysis, the HMRO mass was estimated using predictive equation and was not measured directly. Moreover, this equation had not been previously validated in renal failure population.

In order to examine the relationship between body size and body composition, Sarkar et al (2006) measured various tissue components using MRI and explored their relationship with body size and estimated REE (Sarkar et al., 2006). The authors demonstrated that there was a clear inverse relationship between body size and HMRO mass per kg of body weight and hence, smaller patients had relatively higher HMRO mass compared to their larger counterparts (Figure 2-9). The authors also showed that the relative contribution of HMRO-derived REE expressed as percentage of total REE was highest in the small patients (Figure 2-10). In addition,

there was also a strong correlation between HMRO mass and estimated protein catabolic rate (PCR) ($r = 0.616$, $p < 0.001$).

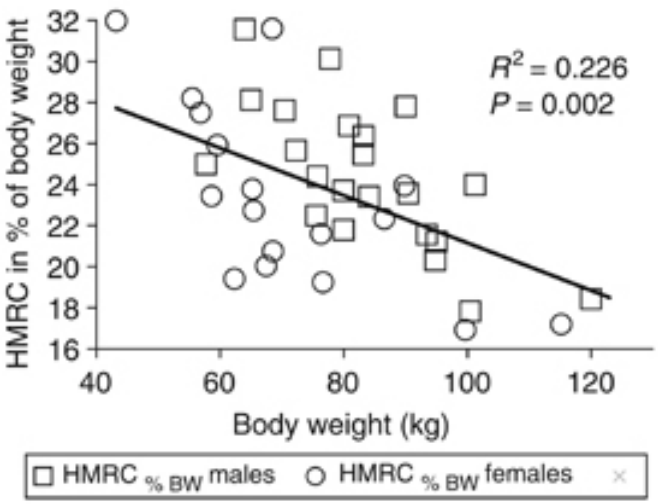


Figure 2-9: Relationship of body weight to HMRO mass (as percentage of body weight)

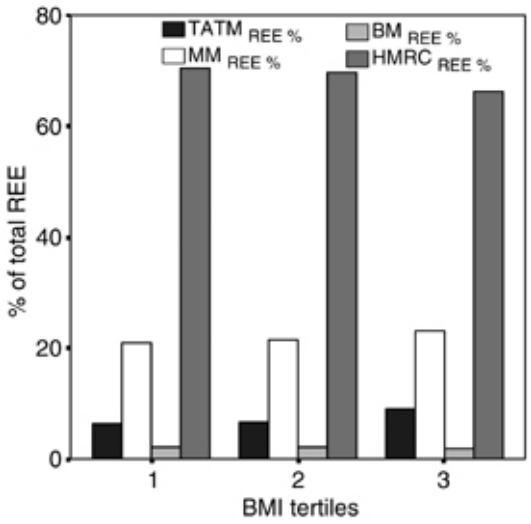


Figure 2-10: Relative contribution of various tissue components to metabolic rate in various BMI-group patients

BMI tertile 1 is the lowest and tertile 3 is the highest tertile. Reprinted by permission from Macmillan Publishers Ltd: Kidney International, Sarkar et al, 2006, 70: 1832-1839, copyright 2006

The main strength of the study was the direct measurement of body composition in haemodialysis patients, the first such study to do so. This study clearly demonstrated the importance of body composition differences in haemodialysis patients. Moreover, the observed relationship between HMRO mass and REE in smaller patients implies relatively higher metabolic waste production in these patients. PCR is a derivative of urea generation rate. Although this is an area of ongoing research (Vanholder, Glorieux, & Lameire, 2002), urea generation rate could be considered a surrogate marker for other uraemic toxin generation. The strong correlation between HMRO mass and PCR further strengthens the hypothesis that HMRO mass is the major determinant of uraemic toxin generation.

These body composition differences may not be limited to body size alone. There are significant body composition differences in individuals from different ethnic population. African American men and women have lower REE compared to Whites (Weyer, Snitker, Bogardus, & Ravussin, 1999; Albu et al., 1997; Foster, Wadden, & Vogt, 1997; Kushner, Racette, Neil, & Schoeller, 1995). This may be due to significantly lower HMRO mass and relatively higher muscle mass in African Americans (Gallagher et al., 2006). Similarly, Asians have lower REE compared to Whites (Wouters-Adriaens & Westerterp, 2008; de Boer, van Es, Voorrips, Blokstra, & Vogt, 1988). This is likely due to differences in body composition as Asians have significantly lower FFM. Indeed, many studies have shown higher fat percentage in Asians compared to Whites with similar BMI (Deurenberg, Deurenberg-Yap, & Guricci, 2002; Wang et al., 1996; Wang et al., 1994) and that the differences in REE were not significant if adjusted to FFM (Wouters-Adriaens & Westerterp, 2008; Lawrence, Thongprasert, & Durnin, 1988). As Whites may have relatively higher HMRO mass as percentage of body weight compared to Asians and Blacks, their dialysis dose requirements may be higher compared to other ethnic populations. This may partly explain the higher mortality risk noted in Caucasian haemodialysis patients.

Most of the above-mentioned studies have been carried out in healthy adults and there is dearth of information on relationship between body composition differences and metabolic rate in patients with kidney disease. This may be due to some major barriers in conducting studies to explore body composition in renal failure. Firstly, whole body MRI scans are time-consuming, cumbersome and expensive. A whole

body MRI scan takes up to an hour to complete and patients will be expected to lie in the scanner for this length of time. Patients with renal failure are likely to be elderly and frail and are not likely to tolerate such lengthy MRI scans. Secondly, if these studies are to be conducted in clinical environment in secondary and tertiary care centres, the availability of time on scanner and MRI personnel is a major limitation. A dedicated MRI facility for research is needed to facilitate these studies and it is not feasible to establish this on a wider scale. Thirdly, the time and costs associated with performing the scans and analysis of MRI images means that it cannot be carried out in large numbers.

In view of the above limitations of using MRI scan, alternate methods need to be explored. Bioimpedance devices can be used to analyse body composition. Although not considered a gold standard method for body composition analysis, studies carried out in patients with ESRD have shown these devices to be reliable and accurate compared to DEXA scan (Fürstenberg & Davenport, 2011a; Fürstenberg & Davenport, 2011b). These devices are non-invasive and provide results in a short span of time, typically within a few minutes, and hence, are reliable alternatives to MRI scan.

There is a need for clinical studies focusing on elucidating the complex relationship between body composition, REE and dialysis requirements. Such studies will improve our understanding of the inter-play of these various factors determining uraemic toxin generation and in turn, their impact on survival outcomes in renal failure patients.

2.3.1.2 Protein Turnover and Metabolic Rate in Renal Failure

Kidneys play a major role in protein and amino acid metabolism. This is achieved through the synthesis, degradation, filtration, reabsorption and urinary excretion of various amino acids. Approximately 50-70g/day of amino acids is filtered through the glomerulus and are almost completely reabsorbed by the tubules (Garibotto, Pastorino, & Dertenois, 2003). In healthy human kidneys, there is net uptake or release of several amino acids in the post-absorptive state (Brundin & Wahren, 1994; Tizianello et al., 1982; Tizianello, De Ferrari, Garibotto, Gurreri, & Robaudo, 1980). Hence, in renal failure, disturbed protein and amino acid metabolism may be expected. Indeed, many studies have shown reduced concentrations of several

amino acids and impairment of specific amino acid metabolism pathways in patients with kidney disease (Jones, Kopple, & Swendseid, 1978; Kopple, 1978; Gulyassy, Aviram, & Peters, 1970).

Malnutrition is common in patients with renal failure with approximately 20-50% of dialysis patients affected (Ikizler, 2007). These patients suffer from a specific form of protein and energy malnutrition (Ikizler & Hakim, 1996; Bergström, 1995; Kopple, 1994; Hakim & Levin, 1993), commonly termed as 'uraemic wasting' which is associated with increased mortality and hospitalisations in this patient population (Ikizler, Wingard, Harvell, Shyr, & Hakim, 1999; Goldwasser et al., 1993; Iseki, Kawazoe, & Fukiyama, 1993; Owen, Lew, Liu, Lowrie, & Lazarus, 1993). It was initially thought the uraemia per se was a catabolic state and the protein energy malnutrition was a result of this. But many studies have shown that this is not the case. Studies using nitrogen balance and whole-body amino acid kinetics in stable, non-acidotic pre-dialysis CKD patients have showed no significant increase in protein catabolism and neutral nitrogen balance albeit at a low protein turnover rate (Adey, Kumar, McCarthy, & Nair, 2000; Castellino et al., 1992; Goodship et al., 1990). Novel mechanisms such as stimulation of ubiquitin-proteosome system have been suggested as possible underlying mechanism for uraemic wasting rather than malnutrition (Lecker & Mitch, 2011; Workeneh & Mitch, 2010).

All these studies point to a derangement of protein metabolism in renal failure. Abnormal protein metabolism will have important consequences in the management of renal failure patients. In addition to the higher mortality risk associated with protein energy wasting, deranged protein metabolism may also influence uraemic toxin generation. Many of the uraemic toxins are products of cell protein metabolism and hence, excess protein intake may potentially lead to increased uraemic toxin generation (Workeneh & Mitch, 2010; Ikizler, 2007). Any increase in uraemic toxin generation is bound to influence morbidity and mortality of these patients and will impact on dialysis requirements. Protein turnover is a major contributor to basal metabolic rate and thereby, influences energy requirements of patients with kidney disease. Moreover, protein metabolism has a direct relationship with body composition, in particular fat-free mass and hence, may impact on dialysis requirements.

A good understanding of the whole body protein turnover in renal failure patients is required to tackle the above potential problems. While many protein turnover studies in non-dialysis CKD patients have demonstrated neutral protein balance, there is no such consensus in patients on haemodialysis. Most of these studies in haemodialysis patients have used isotope labelled leucine (^{13}C-leucine) to examine the whole body protein turnover. In a study of five haemodialysis patients, the nitrogen balance was found to be negative on dialysis days irrespective of the level of protein intake (Borah et al., 1978). Berkelhammer et al (1987) showed that the leucine oxidation rate was increased in HD patients compared to healthy controls (Berkelhammer et al., 1987) but this is likely due to the HD patients in the study being significantly acidotic (mean serum bicarbonate level of 18 mmol/L).

Three studies undertaken to explore the effects of haemodialysis on protein turnover showed conflicting results. The study by Lim et al (1993) showed no increase in protein degradation during HD although there was significant amino acid loss in the dialysate with no concomitant increase in protein synthesis (Lim, Bier, Flanigan, & Sum-Ping, 1993). Ikizler et al (2002) studied whole body protein kinetics and forearm protein kinetics in 11 HD patients 2 hours before, during and 2 hours after dialysis (Ikizler et al., 2002). The average whole body proteolysis rate was 3.38mg/FFM/min pre-dialysis, 3.70mg/FFM/min during dialysis session and 3.77mg/FFM/min after dialysis. There was approximately 9% increase in proteolysis during dialysis and nearly 11% increase 2 hours after dialysis. In a study conducted by Raj et al (2004) in 6 HD patients, an average increase of 28% in protein synthesis and 58% in protein degradation was noted during HD but the increase in degradation rate was higher than synthesis rate (Raj et al., 2004). However, all 3 studies concluded that the dialysis is a catabolic process although there were variations in the abnormalities noted with individual components of the protein metabolism.

Given that there are no definitive alterations observed in protein turnover during HD, is there conclusive evidence on protein metabolism in HD patients in steady-state conditions when they are not undergoing dialysis? There are only a limited number of studies which has explored this question but the answer again is not definitive. Whilst one study demonstrated neutral balance in HD patients (Raj et al., 2004), another study demonstrated negative protein balance with higher protein degradation rate in relation to the synthesis rate (Ikizler et al., 2002). In order to

minimise the inter-individual variations in protein metabolism, Lim et al (1998) conducted a longitudinal study in which whole body protein turnover was measured in the same group of CKD patients before initiation of dialysis and a few months after being established on dialysis therapy along with measurements in healthy adults as controls (Lim, Yarasheski, & Flanigan, 1998). Contrary to the above studies, they demonstrated that the whole body protein turnover and protein synthesis increased after initiation of dialysis compared to pre-dialysis CKD stage and was comparable to protein turnover rates in healthy controls. They did not find any evidence of higher protein degradation rate during pre-dialysis or dialysis stage compared to controls.

What could be the reason behind such differences in protein turnover in renal failure patients across various studies? There does not seem to be a clear answer for this. The current recommended dietary protein intake for haemodialysis patients is 1.1g/kg/day (Naylor et al., 2013). Protein intake in the study participants across these studies have been similar in the range of 0.9 – 1.2 g/kg/day and hence, this is unlikely to be a confounding factor. Lim et al (1998) showed increase in protein synthesis after dialysis initiation in spite of similar dietary protein intake in subjects before and after initiation of dialysis. Although no definitive explanation for this increased protein synthesis could be derived from the study, the dialysis patients had significantly higher serum pH (mean 7.43 ± 0.05) compared to the other pre-dialysis groups in the study and this may have contributed, at least in part, to the increased synthesis. Age is unlikely to be the confounding variable in these studies as there is no large age difference between the study cohorts. The variations in the results may be due to the small sample sizes of these studies. Whether the ethnic origin of the study subjects plays a major role in protein turnover is as yet unclear and this may be a confounding factor in these study results. There are body composition differences across various ethnic groups which will influence protein metabolism. As most studies did not report information on participants' ethnicity, this issue cannot be explored from published literature.

There has been no published literature exploring the relationship between protein turnover and metabolic rate in renal failure setting. It has been shown in healthy adults that whole body protein turnover contributes approximately 20% to the basal metabolic rate in both young and the elderly (Boirie, Beaufrère, & Ritz, 2001; Welle & Nair, 1990) but this relationship has not been studied in renal failure as yet. Although

it is not likely that the process of protein turnover is more efficient in renal failure, whether the energetic cost of protein turnover is higher in renal failure remains to be examined.

It is worth considering the practical difficulties in conducting these studies in the renal failure population. The methods to study protein turnover are cumbersome and requires study subjects to be admitted for a considerable number of hours to a research facility. Most HD patients attend the hospital facility for routine dialysis at least 3 times a week and an additional day of hospital visit is not welcome amongst this patient population. Hence, recruitment to these studies is difficult. Protein turnover studies demand appropriate laboratory equipment to measure isotope enrichments of amino acids and its availability may pose a barrier to these studies being conducted. Lastly, a close collaboration between personnel from various disciplines (e.g., dieticians, pharmacist, laboratory staff etc.) is vital for successful conduct of the study. This may be cumbersome and lack of availability of all required personnel at a single site may hinder these studies from being carried out in routine clinical practice.

Nevertheless, whole body protein turnover and its influence on metabolic rate in renal failure are poorly understood. Studies focusing on exploring these areas will help improve understanding of the protein and energy requirements of patients with renal disease as well as providing valuable information about the factors influencing metabolic rate in this patient population.

2.3.2 Physical Activity and Dialysis Dose Requirements

Physical activity is increasingly being studied at present in patients with kidney disease. Physical activity levels have been shown to be low in patients with kidney disease. In CKD patients not receiving dialysis, total physical activity levels progressively decrease with worsening renal function (Hawkins et al., 2011). Many studies have shown markedly reduced physical activity levels in haemodialysis patients even when compared to healthy sedentary individuals (Avesani et al., 2012; Zamojska, Szklarek, Niewodniczy, & Nowicki, 2006; Johansen et al., 2000). This reduction in physical activity is even more pronounced on dialysis days compared to non-dialysis days (Majchrzak et al., 2005). Dialysis patients are exposed to a number of factors that may predispose to low physical activity. Firstly, these patients are

likely to have significant comorbidities such as diabetes (and its associated vascular complications), protein-energy wasting, anaemia etc. which may restrict their activities. Uraemia per se may lead to sarcopenia thereby reducing the ability to perform regular exercise. Indeed, a study carried out on dialysis patients showed 30% of incident and 39% of prevalent dialysis patients to have signs of muscle atrophy (Carrero et al., 2008). Moreover, renal failure is predominantly a disease of the elderly and factors related to aging may also contribute to reduced physical activity (Brown, Yore, Ham, & Macera, 2005; Johansen et al., 2000). Finally, studies have shown markedly diminished physical functioning capacity in CKD patients even in earlier stages of kidney disease (Leikis et al., 2006; Castaneda et al., 2001; Boyce et al., 1997).

There are clear physical and mental health benefits associated with higher physical activity levels in patients with kidney disease. Physically active dialysis patients have reduced mortality risk compared to their sedentary counterparts (Tentori et al., 2010; Stack, Molony, Rives, Tyson, & Murthy, 2005; O'Hare, Tawney, Bacchetti, & Johansen, 2003). These studies have shown nearly 26-30% reduction in mortality risk in people who exercise regularly. Exercise training in CKD and dialysis patients has been shown to improve objective measures of physical functioning capacity and muscle strength in addition to a number of other anabolic effects (Mustata et al., 2011; Cheema & Fiatarone Singh, 2005; Castaneda et al., 2004). Patients who perform regular exercise also report higher physical functioning scores, better quality of life and are less likely to suffer from anxiety and depression (Painter, 2005; Suh, Jung, Kim, Park, & Yang, 2002).

However, there are some aspects of physical activity and its potential impact on various nutritional and metabolic factors in patients with kidney disease that have not been studied as yet. Individuals performing regular exercise and moderate to vigorous physical activity have higher rates of protein turnover and have higher dietary protein requirements compared to sedentary individuals. Indeed, it has been shown that physically active adults will be in negative nitrogen balance if their protein intake was similar to sedentary ones (Lemon, Dolny, & Yarasheski, 1997; Tarnopolsky et al., 1992). It is conceivable that a similar relationship between physical activity levels and protein turnover exists in patients with kidney disease

which may influence the nutritional management of this patient population but this has not been explored in any studies to date.

Physical activity has been shown to be a significant predictor of fat-free mass in dialysis patients (Baria et al., 2011; Majchrzak, Pupim, Sundell, & Ikizler, 2007) and will induce changes in body composition in individuals who exercise regularly. If minimum dialysis requirements ought to consider body composition differences, then physical activity levels will have significant influence on dialysis dosing practices. Moreover, the increased muscle mass may provide a survival advantage as discussed in earlier sections. The mechanisms underlying reduced mortality risk in physically active haemodialysis patients have not been studied and may be multi-factorial. However, it is likely that changes in body composition may play an important role and this relationship needs to be explored further.

It is difficult to quantify the influence of increased physical activity on concentrations of individual metabolic waste products in the tissues. Physical activity is likely to increase uraemic toxin generation in patients with renal failure and may also increase mobilisation of these toxins from sequestrated tissues. In a study conducted by Kong et al (1999), intra-dialytic exercise increased transfer of uraemic solutes from peripheral tissues leading to higher dialysis clearance of these solutes with a resultant 14% increase in sessional Kt/V (Kong, Tattersall, Greenwood, & Farrington, 1999). If the hypothesis that physical activity increases uraemic toxin generation is true, this would mean that minimum dialysis requirements will be higher in physically active patients. There is a knowledge gap in this area in that the influence of physical activity levels on dialysis dose requirements has not been explored as yet in clinical studies.

2.3.2.1 Physical Activity and Total Energy Expenditure

Physical activity is the variable component of total energy expenditure (TEE) and can be measured. If the goal is to normalise dialysis dose by a parameter that closely reflects the rate of uraemic toxin generation, it is imperative that TEE be examined as a scaling parameter amongst others. TEE, with its physical activity component, is related to body composition and uraemic toxin generation. Intuitively, TEE may seem a better scaling parameter as it encompasses both metabolic rate and physical activity, the two principal factors related to metabolic waste production. But, the

applicability and reliability of TEE as a scaling parameter for dialysis dosing has not been tested in clinical studies to date.

The gold standard method of measuring daily energy expenditure is through the use of doubly labelled water technique which is time-consuming, expensive and cumbersome. The development of various accelerometer devices has enabled easier measurement of physical activity related energy expenditure and total energy expenditure. Although, these devices could not be used in routine clinical practice at present, use of these devices in large-scale physical activity studies could lead to generation of predictive equations to estimate TEE in clinical practice. These devices are now readily available and facilitate measurement of physical activity in clinical studies.

Despite the myriad benefits demonstrated by many studies associated with exercise, the effects of physical activity on uraemic toxin production and its potential impact on dialysis dose requirements has not been explored. As discussed above, physical activity can potentially influence uraemic toxin generation in many ways and thereby, impact on dialysis requirements. Studies focusing on these aspects of physical activity will help bridge the knowledge gap and will support the development of holistic exercise programs for haemodialysis patients.

2.4 Summary

In summary, the current practise of using total body water for scaling dialysis dose may carry the risk of under-dialysis in women and small men. Factors that are closely related to uraemic toxin generation may provide more appropriate dialysis dose relative to individual metabolic needs. Many aspects of the proposed alternative scaling parameters – body surface area, resting energy expenditure and total energy expenditure – remain largely unexplored. Studies focusing on the relationship of energy expenditure measures to renal function and its impact on uraemic toxin generation are needed to examine the benefits of using these parameters in clinical practice.

2.5 Research Aims

The overarching aim of the thesis is of the form – “optimal management of advanced kidney failure should take into account metabolic factors influencing the rate of

uraemic toxin generation". To this end, studies reported in this thesis have been designed with the following objectives.

- To explore the relationship between energy expenditure and uraemic toxin generation
- To measure basal metabolic rate and total energy expenditure and examine the impact of advanced kidney disease on these energy expenditure measures
- To explore ways to quantify habitual physical activity levels in patients with advanced kidney disease
- To study the impact of physical activity on uraemic toxin generation in patients with end-stage renal disease
- To measure protein turnover rate in end-stage renal disease patients and evaluate its relationship with resting energy expenditure
- To prospectively examine the influence of metabolic factors – BSA, REE and TEE – on survival outcome in patients with advanced kidney disease on dialysis

The hypotheses related to each of the studies are mentioned in the respective chapters.

Chapter 3

3. General Methodology

3.1 Introduction

This chapter explains the design rationale for all the studies in this thesis and describes the study population at each study site and the common methodologies used in the studies reported in this thesis.

3.2 Rationale for the Studies

There is paucity of data exploring the relationship between metabolic factors such as resting energy expenditure (REE), total energy expenditure (TEE) or fat-free mass (FFM) and a measure of uraemic toxin generation such as urea generation rate. Knowledge of the interaction between these factors will help inform designing further clinical studies. A retrospective analysis was carried out examining the relationship between REE, TEE and FFM to Urea generation rate to inform better the designing of further studies for this PhD.

As discussed in chapter 2, various authors have proposed a number of alternative scaling parameters. However, there are no large-scale outcome-based studies exploring the use of such parameters. A prospective epidemiological study was therefore designed to explore the reliability of REE, TEE and BSA in predicting survival when used as normalising factor for dialysis dose. Haemodialysis patients (n=1500) were recruited from 5 different centres. REE was estimated from a predictive equation developed and validated in HD patients (Vilar et al., 2014). TEE was calculated from REE in conjunction with physical activity data collected through the Recent Physical Activity Questionnaire (RPAQ).

One of the major functions of the kidneys is excretion of metabolic waste products and it is this function that is being replaced by dialysis. Hence, factors related to metabolic activity such as resting energy expenditure or total energy expenditure could potentially be a better scaling parameter for dialysis dosing. It is important to understand the determinants of these energy expenditure variables. Whole body protein turnover is an important contributor to REE and contributes as much as 20% to REE. As kidneys play a significant role in protein turnover, alterations of protein

metabolism are expected. It is not known whether the energetic cost of protein turnover in ESRD remains the same as in general population. Hence, a cross-sectional study was designed to examine the energetic cost of whole body protein turnover in a group of haemodialysis patients (n = 6) and advanced CKD patients (n = 6).

Physical activity is the variable component of Total energy expenditure and can be easily measured. In theory, increased physical activity may lead to increased generation and mobilisation of metabolic waste products and thereby, necessitating an appropriate increase in the dialysis dose. This has not been explored in studies to date. In order to examine the influence of physical activity on urea generation rate (and thereby, dialysis requirement), a cross-sectional study was conducted to measure physical activity levels in haemodialysis patients (n=120) through wrist-worn accelerometers. The study also aimed to validate the Recent Physical Activity Questionnaire (RPAQ) in this study to facilitate physical activity data collection in routine clinical practice.

Though accelerometers provide a reliable measure of physical activity and total energy expenditure, the gold-standard method of measuring total energy expenditure is by doubly labelled water technique. To examine the effect of varying levels of kidney function on resting and total energy expenditures, a study was designed to measure TEE through doubly labelled water in 40 patients with varying stages of CKD. REE was measured using indirect calorimetry. The relationship between the measured energy expenditure and urea generation rate was also explored in this study.

An overview of each individual study carried out as part of this PhD is described below. A detailed explanation of the methodologies is provided in later relevant sections in this chapter.

3.2.1 Retrospective analysis exploring the relationship between body composition, energy metabolism and urea generation rate

As there was no published data available examining the relationship between various metabolic factors and uraemic toxin generation, a retrospective analysis of data from a previous study cohort was undertaken. This study was carried out on

166 HD patients from Lister renal unit. Adult HD patients older than 18 years and dialysing for more than 3 months were included. Exclusion criteria included patients with active infection, untreated thyroid dysfunction, hospital admission in the previous month, recent respiratory infection, treated tuberculosis in the previous 12 months, a history of infection with hepatitis B, C or HIV, and those with amputated limbs. Demographic and anthropometric data such as height and weight were collected from all patients. Comorbidity data and blood test results were collected from medical records. REE was measured using indirect calorimeter. Physical activity data was collected using Stanford 7-day recall questionnaire, which was then used in conjunction with REE to calculate TEE. Body composition was measured using a bio-impedance device. HMRO mass was estimated from previous published literature (Gallagher et al., 2006). Urea Kinetic modelling was carried out using the software Solute Solver to calculate urea generation rate (Daugirdas, Depner, Greene, & Silisteanu, 2009).

3.2.2 Prospective study of survival outcomes and alternative dialysis dose scaling parameters

To determine the impact on survival of using alternative scaling parameters for dialysis dosing, a prospective, epidemiological study was designed. 1500 subjects on in-centre haemodialysis across 5 different sites were recruited for this study. Body size parameters were obtained through direct measurements. Comorbidity data was collected using a self-report comorbidity questionnaire and physical activity data from Recent Physical Activity Questionnaire. Subjects were followed up for a period of 18 months to collect survival and dialysis adequacy data. Resting energy expenditure was estimated using the novel predictive equation mentioned above. Body surface area was calculated using Haycock formula and Total energy expenditure was calculated from REE and physical activity data. Data from this study was used to determine the impact on survival of using Kt/REE, Kt/BSA and Kt/TEE as dialysis dosing measures and to determine appropriate cut-off points for minimum dialysis requirement using these parameters.

3.2.3 Cross-sectional Study of Physical Activity and its influence on Urea Generation Rate in Haemodialysis Patients

120 patients on in-centre haemodialysis at Lister and Royal Free hospitals were recruited for this study. Adult anuric HD patients older than 18 years and dialysing for more than 3 months were included. Exclusion criteria included patients with active infection or malignancy, untreated thyroid dysfunction, hospital admission in the previous month, those with cardiac pacemakers or defibrillators, those with amputated limbs and immobility due to physical ailments. Physical activity was measured using wrist-worn accelerometer and from Recent Physical Activity Questionnaire. Body size measures were obtained through direct measurements and body composition was measured using bio-impedance spectroscopy. All study subjects were asked to prospectively complete a food diary for 3 days in the study period. These 3 days included 1 dialysis weekday, 1 non-dialysis weekday and a non-dialysis weekend day. Pre- and post-dialysis blood tests were carried out during the study period. Urea generation rate was estimated using the software Solute Solver.

3.2.4 Cross-sectional study of Whole-body Protein turnover in advanced chronic kidney disease

Measurement of whole-body protein turnover by intravenous infusion of stable isotope of leucine is a validated and reliable method. The isotope is non-radioactive. This method was employed to study WBPT in 12 patients with advanced CKD (eGFR < 20 ml/min) – 6 of them receiving in-centre haemodialysis and 6 of them in pre-dialysis stage. Resting Energy expenditure was measured using indirect calorimetry and body composition by bio-impedance analysis. A bolus dose of stable isotopes of sodium bicarbonate and leucine were administered intravenously followed by a continuous infusion of leucine isotope for 2-3 hours. Blood tests were taken at regular intervals throughout the study period, which were then analysed for isotopic enrichment of α-ketoisocaproate (α-KIC). Breath samples were collected at the time of blood tests for estimation of isotopic enrichment of ^{13}C in the expired air. The results were then used to estimate the energetic cost of protein turnover in patients with advanced CKD.

3.2.5 Prospective analysis of total energy expenditure in chronic kidney disease

The doubly labelled water isotope-excretion technique has been used safely in general population and is the gold standard method for measuring Total Energy Expenditure. In order to explore TEE in patients with different stages of CKD, a study was conducted using the stable isotope of doubly labelled water in 40 patients with CKD. Patients were recruited from the general nephrology clinic at Lister hospital. REE was measured using indirect calorimetry. Physical activity data was also collected through two recall questionnaires – Stanford questionnaire and Recent Physical Activity Questionnaire. Body composition was measured using bio-impedance analysis. Using doubly labelled water for TEE measurement in conjunction with physical activity measurement through questionnaires enabled validation of these questionnaires in CKD patients.

3.3 Study Population

All the above studies recruited patients registered under the care of the renal service at East and North Hertfordshire NHS Trust. In addition, two of the studies included patients from other sites. The study of survival outcomes with alternative scaling parameters recruited patients registered under the care of renal services at 4 different sites – Royal Free London NHS Foundation Trust, Barts and The London NHS Trust, Southend University Hospital NHS Foundation Trust and Wirral University Teaching Hospital NHS Foundation Trust. The study of physical activity and urea generation rate recruited patients under the care of Royal Free London NHS Foundation Trust.

3.4 Research Ethics

All prospective studies were given NHS ethics committee approval and local Research & Development approval. All study participants were given a written information sheet explaining the study. They were given at least 24 hours to make a decision regarding participation in the study. Written informed consent was obtained from all subjects recruited in prospective studies as per the Good Clinical Practice guidelines. Letter of approval for the studies are shown in the appendix. The NHS

Research Ethics committee and the University Ethics reference numbers for the studies are given below.

Total Energy Expenditure in Chronic Kidney Disease – NHS ref: 13/EE/0388; UH ref: LMS/PG/NHS/00255

Physical activity and urea generation rate – NHS ref: 13/EE/0053; UH ref: LMS/PG/NHS/00254

Survival outcome using alternate scaling parameters – NHS ref: 12/WA/0060; UH ref: LMS/PG/NHS/00257

Whole body protein turnover in advanced CKD – NHS ref: 13/EE/0158; UH ref: LMS/PG/NHS/00256

3.5 Haemodialysis Programme at Study Sites

3.5.1 Lister Hospital

The haemodialysis programme at the Lister hospital renal units consists exclusively of high-flux haemodialysis and haemodiafiltration using predominantly polysulphone membranes. Nearly 60% of patients were receiving online haemodiafiltration. Bicarbonate was used exclusively as the buffer. Water quality was regularly monitored to ensure tight bacteriological standards. Dialysis was prescribed and monitored according to a target two-pool total Kt/V of 1.2 per dialysis session. The two-pool total Kt/V comprises of urea clearance from dialysis ($Kt/V_{dialysis}$) and that from residual renal function (Kt/V_{renal}). Dialysis adequacy was monitored monthly through blood tests for dialysis clearance and inter-dialytic urine collection for estimation of residual renal function.

3.5.2 Royal Free Centre for Nephrology

Patients were dialysed using high flux membranes that were predominantly polysulphone. Majority of patients received online haemodiafiltration with the rest receiving high-flux haemodialysis. Water quality was regularly monitored to ensure tight bacteriological standards. Dialysis was prescribed and monitored according to target two-pool $Kt/V_{dialysis}$ of 1.2. Patients with a urine output of more than 100ml/day had the urine volume measured on a 3-monthly basis but residual renal function was

not included in the dialysis prescription. Dialysis adequacy was monitored on a monthly basis.

3.5.3 Royal London Hospital

Patients were dialysed using high flux membranes that were predominantly polysulphone. Majority of patients received high-flux haemodialysis. Water quality was regularly monitored to ensure tight bacteriological standards. Dialysis was prescribed and monitored according to target two-pool $Kt/V_{dialysis}$ of 1.2 per session without inclusion of residual renal function. Dialysis adequacy was monitored on a monthly basis.

3.5.4 Southend University Hospital

Patients were dialysed exclusively using low flux polysulphone membranes. All patients received low-flux haemodialysis. Water quality was regularly monitored to ensure tight bacteriological standards. Dialysis was prescribed and monitored according to the Urea Reduction Ratio of 70% per session. Neither residual renal function nor $Kt/V_{dialysis}$ was used to monitor the dialysis prescription.

3.5.5 Wirral University Hospital

Patients were dialysed using high-flux polysulphone membranes with nearly half of the patients on haemodiafiltration and the other half receiving high-flux haemodialysis. Water quality was regularly monitored to ensure tight bacteriological standards. Dialysis was prescribed and monitored according to target single-pool Kt/V of 1.2 per session. Dialysis adequacy was monitored on a monthly basis.

3.6 Assessment of Metabolic Rate

Basal metabolic rate (BMR) denotes the energy expenditure at sleep. As it is difficult to measure basal metabolic rate in practice, it is usually expressed as Resting Energy Expenditure (REE). REE includes the energy spent by being awake and also from the residual metabolic activity resulting from posture, spontaneous muscle activity etc. In sedentary individuals, REE contributes to around 60% of Total Energy Expenditure (TEE). Apart from REE, TEE also comprises of energy expenditure from physical activity and thermic effect of food, which is the energy expended for

digestion and assimilation of ingested food. Thermic effect of food contributes to around 10% of TEE. REE measurement for studies in this thesis was carried out through predictive equations and direct measurement using indirect calorimetry as explained below.

3.6.1 Predictive Equations

A number of predictive equations for REE have been derived in general population. There are more than 190 published formulae to estimate REE (Foster, Knox, Dempsey, & Mullen, 1987) with the commonly used ones being Harris-Benedict equation (Harris & Benedict, 1918), Mifflin-St Jeor equation (Mifflin et al., 1990) and Schofield equation (Schofield, 1985). None of these equations were derived from patients with CKD. As explained in section 2.3.1, there is evidence to suggest metabolic rate differs in CKD patients compared to healthy individuals. A novel predictive equation for REE in HD patients was derived from previous research studies at Lister hospital through direct measurements using indirect calorimetry (Vilar et al., 2014). This equation was also validated in a separate cohort of HD patients and was found to be at least as accurate as other existing equations and was also associated with less bias. The equation is given below.

$$(1)\ \ \boldsymbol{REE} = \left[-\mathbf{2.497} \times \boldsymbol{Age}(\boldsymbol{years}) \times \boldsymbol{Factor_{age}}\right] + \left[\mathbf{0.011} \times \boldsymbol{Height}^{\mathbf{2.023}}(\boldsymbol{cm})\right] + \left[\mathbf{83.573} \times \boldsymbol{Weight}^{\mathbf{0.6291}}(\boldsymbol{kg})\right] + \left[\mathbf{68.171} \times \boldsymbol{Factor_{sex}}\right]$$

where $Factor_{age}$ is 0 if age <65 and 1 if ≥65 and $Factor_{sex}$ is 0 if female and 1 if male. REE is expressed in kcal/day.

As this is a condition-specific equation for patients on haemodialysis, this equation was used in studies involving estimation of REE.

3.6.2 Indirect Calorimetry

Indirect calorimetry was used to measure REE in some of the studies. Indirect calorimeter measures oxygen consumption rate (VO_2) and carbon dioxide production rate (VCO_2) by analysing the expired air. One litre of oxygen consumed generates 3.9 kcal and one litre of carbon dioxide produced generates 1.1 kcal. This is then converted to energy expenditure using Weir equation (Cunningham, 1990; Weir, 1949). The ratio of VCO_2 / VO_2 is called the respiratory quotient (RQ). RQ is related

to the diet and denotes which kind of substrate is predominantly used as a fuel for energy production. RQ of 1.0 denotes carbohydrate as a predominant source of energy whereas RQ of 0.7 denotes fat as the source.

Indirect calorimetry was performed using a Sensormedics (CareFusion Corporation, San Diego, California, USA) Vmax 29n metabolic cart with an overhead canopy technique. The Sensormedics system is a ventilated open circuit system. The system collects the expired air through an overhead canopy which is then diluted with room air and passed through a gas analyser at a specific flow rate to analyse the oxygen and carbon dioxide concentrations. The specific flow rate is set as 5-6 times the subject's minute volume which is calculated as tidal volume multiplied by the respiratory rate per minute. The high flow rate ensures that almost all of the expired air is collected for analysis. The Sensormedics system has been shown to have good validity with a coefficient of variation of approximately 8% compared to the Deltatrac Metabolic Monitor and high reliability with no significant difference between REE measured from these two instruments (Cooper et al., 2009).

The Sensormedics system is illustrated in Figure 3-1. The expired air, diluted with room air as per the set flow rate, is analysed in the mixing chamber of the gas analyser. The system also samples the room air to measure oxygen and carbon dioxide concentrations which are used in the analysis of the diluted expired air. The analyser is calibrated using two calibration gas mixtures containing different concentrations of oxygen, carbon dioxide and nitrogen. The mass flow sensor, shown in the illustration, is also calibrated manually using a 2.5 litre syringe which generates flow-volume loops of varying intensity.

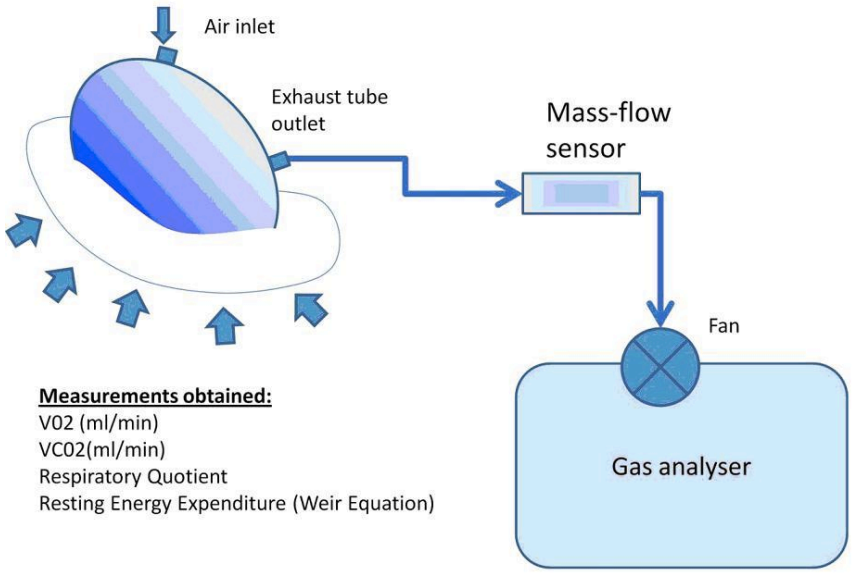


Figure 3-1: Sensormedics Indirect Calorimetry System

Reproduced with permission from Dr Enric Vilar.

Indirect calorimetry was performed in a designated thermo-neutral room with a temperature range of 21-25°C. No measurements were made if the room temperature fell outside this range. Subjects were requested in writing to refrain from eating for 2 hours prior to the test and also from intense physical activity prior to the study. Prior to testing each subject, mass flow sensor and gas analyser calibration was performed. Subjects were requested to lie down on a couch for at least 15 minutes before the REE measurement was initiated. Patients were requested to lie still for the duration of the test and not to speak unless necessary. The procedure room was kept closed during the study to prevent changes in room pressure and temperature. Overhead canopies were cleaned after each patient use as per the local Trust infection control policy for medical devices.

Indirect calorimetry was performed until a steady-state period was achieved. The steady-state was defined as a 5-minute period of <5% variation in VO_2 and VCO_2. In patients whom the steady-state was not achieved by 20 minutes from the start of the test due to high variability of VO_2 and VCO_2, a threshold of <10% variation in these parameters were used.

The oxygen consumption rate, carbon dioxide production rate and respiratory quotient were measured after the steady-state period has been achieved. REE was calculated using the Weir equation given below.

$$(2)\ \boldsymbol{REE\ (kcal/day)\ = 1.44 \times [(3.9 \times VO_2)\ +\ (1.1 \times VCO_2)]}$$

3.7 Assessment of Physical Activity

Physical activity is the most variable part of the Total Energy Expenditure (TEE) and can be measured through various ways. For studies in this thesis, a number of methods have been used for physical activity measurement. While accelerometer was used in one of the studies, questionnaire-based methods were employed in all the studies. These methods are described below.

3.7.1 Accelerometer-based Measurement

Accelerometer-based physical activity measurement was employed in one of the studies in this thesis. Accelerometers measure movement of the body parts in different axes along with the speed, duration and the intensity of the movement. This data is recorded as vectors in the respective axes which are then downloaded onto a computer using specific software. The output obtained from the accelerometers varies with the type of accelerometer. While some accelerometers give the output as energy expenditure, some others express activity in terms of time spent at various activities depending on the intensity of the activity.

Physical activity was measured using GENEActiv accelerometer (Activinsights Limited, Kimbolton, UK). GENEActiv is a wrist-worn, tri-axial accelerometer and measures hand movement in three axes. It is lightweight and worn just like a wrist watch. The splash-proof design ensures that it can be worn even during exposure to water such as swimming, shower/bath etc. GENEActiv has an internal memory capacity of 500MB. The data sampling frequency was set at 100 Hertz. With this sampling frequency, the accelerometer was capable of storing up to 8 days worth of physical activity data.

GENEActiv has been validated as a physical activity measurement tool in adults. Esliger et al (2011) showed that GENEActiv had high technical reliability (intra-instrument and inter-instrument coefficient of variation of 1.8% and 2.4% respectively) and validity (r = 0.97) compared with Multi-Axis Shaking Table (MAST) acceleration (Esliger et al., 2011). It also showed excellent concurrent validity with existing, widely used accelerometers such as ActiGraph (r = 0.92) and RT3 (r = 0.97). In addition, specific cut off points for different intensities of activities were

defined. Welch et al used these cut off points in a different cohort of subjects and found them to be as accurate as the existing wrist-worn accelerometers in identifying different intensities of activities (Welch et al., 2013). Algorithms have also been developed and published to classify daily activities using GENEActiv with high accuracy (Zhang, Rowlands, Murray, & Hurst, 2012). Although the waist-worn GENEActiv units had higher classification accuracy (98.2% - 99.1%) compared to the wrist-worn units (95.3% - 97%), algorithms based on wrist-worn units were found to be robust enough for daily physical activity monitoring and these units were also more convenient for patients to use.

Study participants were consented prior to the study. The accelerometers were pre-programmed with the demographic (age and gender) and anthropometric data (height and weight) pertaining to each individual participant prior to the study date. The device was activated using an activation button in the device on the study day. At the start of the study, the accelerometer was strapped on the non-fistula hand. Subjects were asked to wear it continuously for a period of 7 days. They were specifically informed about the waterproof nature of the device and were advised not to take it off until the end of the study. After a week, the accelerometers were collected back. All accelerometers were thoroughly cleaned as per local Trust infection control policy for medical devices. The accelerometer was then connected to a computer and the raw data was downloaded. The devices were then recharged and re-programmed with the data for the next set of study subjects.

3.7.2 Questionnaire-based methods

It is practically difficult to design large scale studies of physical activity measurement using accelerometers due to its cost and time limitations. Self-report questionnaires are a useful alternative. The primary requirements for the questionnaires chosen for the studies were that it is validated against other methods of activity measurement and it would give a measure of Metabolic Equivalent of Task (MET) which will enable estimation of Total Energy Expenditure.

Two physical activity questionnaires are used for studies in this thesis, namely, Recent Physical Activity Questionnaire (RPAQ) and Stanford 7-day recall questionnaire. RPAQ was used in all the studies while the other questionnaire was used only in the doubly labelled water study to measure TEE.

3.7.2.1 Recent Physical Activity Questionnaire

Recent Physical Activity Questionnaire (RPAQ) was developed as a tool to measure physical activity in different domains of daily life. RPAQ (shown in Appendix B) enquires about the time spent on various activities at home, work and recreational activities over the preceding 4 weeks. It also enquires about time spent on commuting to and from work. The main advantage of the questionnaire is that the questions are divided into various sections such as at home, work and leisure time which aids recollection of the activities performed. The recreational activity section has a long list of commonly performed individual activities and some blank rows were also included for the study subjects to mention any performed activity that was not mentioned in the list.

RPAQ has been validated using accelerometers in adults as a reliable tool to measure and categorise activity levels into different intensities (Besson, Brage, Jakes, Ekelund, & Wareham, 2010). In this study by Besson et al (Besson et al., 2010), total and physical activity related energy expenditure derived from RPAQ was compared with the gold standard method using Doubly labelled water. The correlation between the questionnaire-derived and measured TEE was good (r = 0.67) and the correlation for physical activity related energy expenditure (PAEE) was weak (r = 0.39). PAEE was calculated using subject's body weight and sum total of activity expressed as MET-hours/day. However, a different method of estimating TEE was used in the studies here as explained below. This method of TEE calculation was validated using the doubly labelled water technique, the results of which are described in one of the studies (chapter 7).

Study subjects completed RPAQ by themselves at the end of the study period. Subjects mentioned whether they had performed a particular activity as mentioned in the questionnaire over the preceding 4 weeks and if so, they were required to mark the frequency and the time spent on the activity. Where the time spent on the activity was expressed as a range, the average of the range was considered for analysis. The frequency of the activity over the preceding 4 weeks was converted to a daily frequency to enable calculation of daily physical activity time. If the total duration of reported activities was more than 18 hours per day, then each activity time was normalised to total activity duration of 18 hours/day as below.

$$(3)\ \boldsymbol{Adjusted\ Activity\ Time} = \frac{\boldsymbol{Reported\ Activity\ Time}}{\boldsymbol{Total\ Activity\ Time}} \times \mathbf{18}$$

The remaining time (RT) was calculated as the time not accounted for from the questionnaire assuming the Sleep time to be 6-8 hours/day.

If total activity time was ≤16, then RT = 16 – Total activity time.

If total activity time was >16, then RT = 0.

Each reported activity was given a Metabolic Equivalent of Task (MET) score as per the Compendium of Physical Activities chart (Ainsworth et al., 2011; Ainsworth, Haskell, Whitt, Irwin, & et al., 2000). Subjects were assumed to be performing light activities during the unreported RT which was given a MET value of 1.3. A MET value of 1 was given for the sleep time. Total daily MET and TEE was calculated for each subject as below.

$$(4)\ \boldsymbol{Total\ Daily\ MET} = (\boldsymbol{A_1} \times \boldsymbol{T_1}) + (\boldsymbol{A_2} \times \boldsymbol{T_2}) + \cdots\cdots + (\boldsymbol{A_n} \times \boldsymbol{T_n})$$

where A_1 to A_n represent each activity and T_1 to T_n represent the reported duration of the corresponding activity per day including sleep and RT.

$$(5)\ \boldsymbol{TEE\ (kcal/day)} = \boldsymbol{Resting\ Energy\ Expenditure\ (kcal/day)} \times \left(\frac{\boldsymbol{Total\ Daily\ MET}}{\mathbf{24}}\right)$$

3.7.2.2 Stanford 7-day Recall Questionnaire

The Stanford 7-day recall questionnaire (shown in Appendix C) was developed for the Stanford Five City Project (Blair et al., 1985). The questionnaire is based on the principle that recall of vigorous physical activity is more accurate than lighter activities. The questionnaire enquires about time spent in sleep and at moderate, hard and very hard activities over the preceding 7 days. Any time not accounted for was considered to be spent in light activity.

The questionnaire has been validated and used in many studies for physical activity measurement. Taylor et al (1984) showed the 7-day recall questionnaire had

excellent correlation (r = 0.81) compared with a prospectively recorded physical activity log (Taylor et al., 1984). The questionnaire has also been shown to have high test-retest reliability and convergent validity compared with a daily activity diary (Williams, Klesges, Hanson, & Eck, 1989) which was better than that observed between Caltrac accelerometer and daily log. A study comparing the energy expenditure measured using doubly labelled water and that estimated using the 7-day recall questionnaire showed poor correlation (r^2 = 0.14) between them (Conway, Seale, Jacobs, Irwin, & Ainsworth, 2002). Moreover, the questionnaire data overestimated physical activity in a significant proportion of study subjects. However, this level of correlation is similar to that obtained for other activity questionnaires. Although there are few studies examining the validity of the questionnaire in CKD population, a small study by Johansen et al showed the 7-day recall questionnaire to have a significant correlation with accelerometer measured physical activity (r^2 = 0.34) (Johansen et al., 2001).

Stanford 7-day recall questionnaire was used alongside RPAQ in the study measuring TEE using doubly labelled water. Subjects were asked to complete the questionnaire at the end of the study period. Using the questionnaire along with other activity questionnaires allowed comparison of the usefulness of these questionnaires in predicting total energy expenditure. A MET value was assigned to each activity depending on its intensity as per Table 3-1.

Table 3-1: Assigned MET value for activity categories in the Stanford 7-day recall questionnaire

Physical Activity Intensity	MET value assigned to the activity level
Sleep	1
Light activity	1.5
Moderate intensity activity	4
Hard activity	6
Very hard activity	10

Mean daily MET was calculated using the following formula.

$$(6)\ \boldsymbol{Mean\ Daily\ MET} = \frac{(\boldsymbol{S}\times\mathbf{1}) + (\boldsymbol{L}\times\mathbf{1.5}) + (\boldsymbol{M}\times\mathbf{4}) + (\boldsymbol{H}\times\mathbf{6}) + (\boldsymbol{VH}\times\mathbf{10})}{\boldsymbol{24\ hours}}$$

where S, L, M, H and VH are the time spent in sleep, light, moderate, hard and very hard activities respectively

TEE was calculated using the above equation (5).

3.8 Assessment of Total Energy Expenditure

3.8.1 Doubly Labelled Water Isotope Excretion Technique

Doubly labelled water technique was used for measurement of Total Energy Expenditure (TEE). The technique involves use of a stable, non-radioactive isotope of oxygen (^{18}O) and hydrogen (^{2}H) to estimate the production rates of water and carbon dioxide from which TEE is calculated. These isotopes are normally present in tap water but at a lower concentration. A measured dose of these isotope-enriched samples are given orally. The isotopes will equilibrate in the body water pool within a few hours which will raise the enrichment level of these molecules in body water. Over a period of 7-14 days, the excretion rates of these molecules are measured from urine samples. ^{2}H (also known as deuterium) leaves the body primarily as water ($^{2}H_2O$) in urine and sweat. ^{18}O, on the other hand, leaves the body as both water

($H_2{}^{18}O$) and carbon dioxide ($C^{18}O_2$). By measuring the difference between the elimination rates of both these isotopes, production rate of carbon dioxide can be calculated as explained in the section below. If the respiratory quotient (RQ) of the individual in the steady state is known, then TEE can be calculated with the above measurements. RQ can either be measured directly using indirect calorimetry or a population based estimate can be used instead.

Two different methods can be employed to measure TEE using these isotopes. One approach is the 'Two-point' method where only two samples are collected – one at the start and at the end of the study and the rate of elimination of isotopes between these two time points is used to calculate TEE. Whilst this may be convenient for the conduct of the study, the main disadvantage is that a single erroneous result will lead to major errors in energy expenditure calculation. The other approach is the 'Multipoint' method where multiple samples are collected during the study period for analysis. The principal disadvantage in this method is the cost associated with analysis of the samples. Although the multipoint method confers some advantage over the two-point method (Speakman, 1997), a study comparing them did not show significant difference in measured energy expenditure between the methods (Welle, 1990). As the excretion of water may be altered in CKD and also, since there was previous experience of using multipoint method at Lister Hospital, this method was chosen for this study. The assumptions and general theory of the doubly labelled water technique are discussed further in the relevant chapter.

3.8.1.1 Isotope administration and sampling

The isotopes – 99% deuterium oxide (^2H_2O) and 10% oxygen-18 ($H_2{}^{18}O$) – were sourced from Iso-Analytical Limited (Crewe, UK). The samples were analysed for water quality and safety at the Quality Control laboratory at Lister hospital prior to study use. The multipoint method of sample analysis was followed with the study conducted over a 14-day period.

On the study day (day zero), subjects' body weight was measured and the dose of the isotopes was calculated accordingly. Isotope samples were weighed using a Sartorius scale with an accuracy of >0.0001g. Calibration of the scale was carried out before every subject. The administered doses were 1.375g/kg body weight of 10% oxygen-18 and 0.083g/kg body weight of 99% deuterium oxide. 1ml of this

sample was retained for analysis and the remaining was ingested. The actual administered dose was calculated from the ingested volume. The flask was washed out with 2-3 chasers of tap water to ensure that the entire isotope sample was ingested.

Participants were asked to provide a baseline urine sample before the ingestion of the isotope. They were provided verbal and written instructions on urine sample collection during the 14-day period. They were asked to collect daily urine samples, beginning 24 hours after the sample administration, in a sterile universal sample pot and were asked to write the date and time of urine collection on the sample bottle. Subjects were asked to store the samples in the fridge or freezer for the duration of the study period. At the end of the 14-day period, all the samples were collected and frozen at -20°C in the research facility at Lister hospital until analysis.

3.8.1.2 Sample Analysis

The urine samples were analysed by Iso-Analytical Limited using a continuous flow isotope ratio mass spectrometry. Samples from days 1, 8 and 14 were used for analysis. If there was no sample collected on these days, then the closest sample to the missed sample was used. Standard laboratory water – calibrated against Vienna Standard Mean Oxygen Water and Standard Light Atlantic Precipitation – was used as the reference for analysis. Table 3-2 shows the list of standard notation as recommended by the International Atomic Energy Agency (Prentice, 1990). This notation has been used in the analysis of the samples.

Table 3-2: Standard Nomenclature recommended by IAEA

Group	Name	Standard Notation
Isotopes	Oxygen	O or ^{16}O and ^{18}O
	Hydrogen	H or ^{1}H
	Deuterium	^{2}H
Dosing Variables	Dose of ^{18}O administered	A_O
	Dose of ^{2}H administered	A_D
	Dose diluted for analysis (^{18}O)	a_O
	Dose diluted for analysis (^{2}H)	a_D
Pool sizes	Pool size measured using ^{18}O	N_O
	Pool size measured using ^{2}H	N_D
Removal rate constants	Rate constant for ^{18}O	K_O
	Rate constant for ^{2}H	K_D
Production rate	Uncorrected production rate	r
	Corrected production rate	r’
	Production rate of CO_2	rCO_2
Enrichment	Parts per million	ppm
	Atom percent excess	‰
	Isotope enrichment relative to a standard	δ
International standard isotope reference waters	Vienna-Standard Mean Oxygen Water	V-SMOW
	Standard Light Antarctic Precipitation	SLAP

3.8.1.3 Calculation of Total Energy Expenditure

TEE calculation was carried out as set out in the report by the International Atomic Energy Agency (Prentice, 1990) and published literature by Speakman (Speakman, 1997). The isotopic enrichment in the sample was expressed as a fraction of the initial dose facilitating further calculation for TEE. As the administered dose was diluted in 100ml of water, the following formula was used to calculate fractional enrichment.

$$(7)\ \boldsymbol{Fractional\ Enrichment} = \frac{\boldsymbol{\delta_{post} - \delta_{pre}}}{\boldsymbol{\delta_{dose} - \delta_{tap}}} \times \frac{\boldsymbol{18.02 \times a}}{\boldsymbol{W \times A}}$$

where W is the amount of water used to dilute the dose (in grams), A is the dose administered to the participant (grams) and a is the mass of the dose diluted for analysis (grams).

For each isotope, a linear regression plot of the natural logarithm of fractional enrichment versus time was generated. The intercept and the slope of the regression line was calculated which was used to estimate the pool size and rate constant as below.

$$(8)\ \boldsymbol{Pool\ Size}\ [\boldsymbol{N}\ (\boldsymbol{moles})] = \frac{\boldsymbol{1}}{\boldsymbol{Intercept}}$$

$$(9)\ \boldsymbol{Rate\ constant}\ [\boldsymbol{K}\ (\boldsymbol{days^{-1}})] = \boldsymbol{slope}$$

where N denotes N_O or N_D and K denotes K_O and K_D for the respective isotopes.

Both oxygen and hydrogen distribute to a varying extent in pools other than body water with hydrogen distributing more than oxygen in other pools. To adjust for this, a correction factor was applied to the calculated pool sizes as below.

$$(10)\ \boldsymbol{Corrected\ N_D} = \frac{\boldsymbol{N_D}}{\boldsymbol{1.0427}}$$

$$(11)\ \boldsymbol{Corrected\ pool\ size}\ (\boldsymbol{N}) = \frac{\boldsymbol{Corrected\ N_D + N_O}}{\boldsymbol{2}}$$

Total body water was then calculated from the following equation.

$$(12)\ \boldsymbol{Total\ Body\ Water = N \times 18.01528\ (g/mol)}$$

The corrected production rate of carbon dioxide is then calculated from the following equation published by Speakman(Speakman, Nair, & Goran, 1993).

$$(13)\ \boldsymbol{r'CO_2 = \frac{N}{2.196} \times [K_O - (1.0427 \times K_D)]}$$

Total energy expenditure was calculated using the following Weir equation.

$$(14)\ \boldsymbol{Total\ Energy\ Expenditure = r'CO_2 \times 22.4 \times \left[1.1 + \left(\frac{3.9}{RQ}\right)\right]}$$

where RQ is the respiratory quotient measured by indirect calorimetry

3.9 Assessment of Whole Body Protein Turnover

3.9.1 Leucine Isotope method

There are broadly two different methods to measure whole body protein turnover - precursor method and the end-product method. Both methods involve administering a non-radioactive, stable isotope of an amino acid either intravenously or orally and sampling body fluids (plasma or urine) to measure isotopic enrichment which is then used to calculate protein turnover. The major difference between the methods is the site of sampling and the product of protein metabolism that is sampled. In the precursor method, the isotope is administered intravenously and timed plasma samples are obtained to measure the isotopic enrichment of the administered amino acid itself or its immediate transaminated product. The end-product method involves administering amino acid isotope intravenously or orally. However, what is measured is the isotopic enrichment of the ingested amino acid in the end-product of the protein metabolism – urea or ammonia – and not the amino acid itself as in the precursor method. Both these methods have different set of assumptions in their methodologies.

In order to study whole body protein turnover (WBPT), the various methods and amino acid isotopes available were examined and the method that would be appropriate for subjects with advanced kidney disease was chosen. The

disadvantages of the other methods are explained in the protein turnover study chapter. In our study, WBPT was measured with the use of a carbon-labelled stable isotope of leucine (^{13}C – leucine). There are a number of advantages in using leucine for WBPT study. Firstly, leucine is an essential amino acid and hence, is not synthesised de novo in the body. This means that apart from that produced as a result of protein breakdown, the only source of leucine would be that present in the sample administered the dose of which can be measured. Secondly, the fate of ingested leucine is well known and fairly straight-forward. Leucine in the precursor pool (plasma and intracellular stores) is either taken up for protein synthesis or oxidised resulting in the release of carbon dioxide. As the isotope is carbon labelled (^{13}C), the amount of oxidation in steady state can be measured through the isotopic enrichment of carbon dioxide ($^{13}CO_2$) in the expired air. Thirdly, with an appropriate priming of the bicarbonate pool and a priming dose of leucine, the study can be completed in a relatively short time of 2-3 hours. The schema of leucine turnover is illustrated in Figure 3-2.

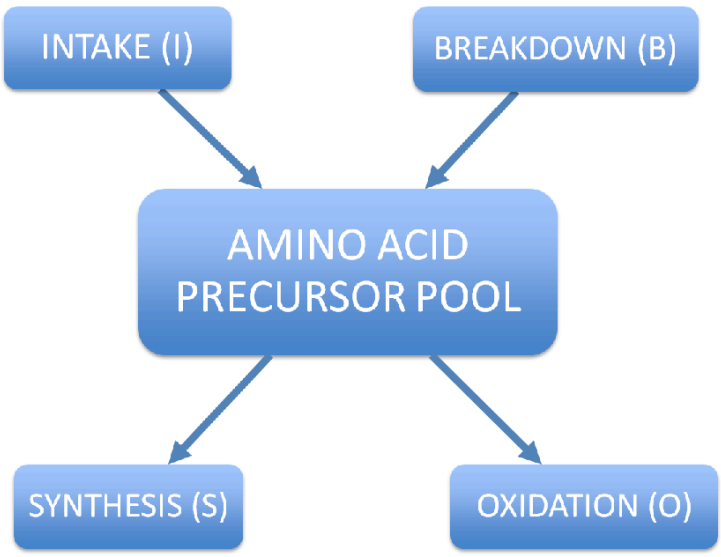


Figure 3-2: Model of Leucine Turnover

In the steady state, the rate of appearance of leucine (R_a) into plasma from protein breakdown and intake is equal to the rate of disappearance (R_d) of leucine from

plasma through protein synthesis and oxidation. This rate is denoted as the leucine flux (Q). Calculation of the flux is explained in a later section.

3.9.1.1 Isotope administration and sampling

The isotope samples – l-^{13}C-leucine (99% atom excess) and ^{13}C-sodium bicarbonate – were sourced from Sigma-Aldrich Company Limited (Dorset, UK). The providers conduct sterility and pyrogen testing of the samples. A copy of the test certificate is shown in Appendix F. The samples were provided in sterile, screw top ampoules in a powder form which is reconstituted with 0.9% sodium chloride to the desired volume.

Subjects were given verbal and written instructions for the study. They were asked to avoid strenuous physical activity for the three days preceding the study day. They were also asked to maintain a food diary and were advised to avoid protein supplements and excessive protein intake for those three days. They were also asked to attend the research facility in empty stomach after overnight fasting on the study day. Participants' body weight was measured to determine the dose to be administered. A cannula was inserted into the antecubital fossa for the isotope infusion. For haemodialysis patients, their existing vascular access (arteriovenous fistula or tunnelled dialysis catheter) was used for obtaining blood samples for analysis. For blood sampling in CKD patients, an intravenous cannula was inserted in the hand on the other side to that used for infusion as recommended by published literature (Waterlow, 2006). The required dose of the sodium bicarbonate isotope was reconstituted in 10ml of 0.9% sodium chloride solution and the leucine isotope in 50ml of 0.9% sodium chloride solution. 0.5ml of the leucine isotope infusate was removed and stored for analysis of isotopic enrichment. A bolus dose of 0.11mg/kg body weight of ^{13}C-sodium bicarbonate and 1mg/kg body weight of l-^{13}C-leucine were administered. Following the bolus dose, leucine isotope infusion was started at a dose of 1mg/kg/hr and continued for 3 hours. At the end of the study period, the cannulae were taken out and subjects were given a meal to eat.

A baseline blood sample (2.5ml) was collected before the injection of the bolus dose. A breath sample was also obtained at the same time by asking the subjects to blow into Exetainer® tube designed for expired air collection. Thereafter, both blood and breath samples were collected every 30 minutes for the first 2 hours of the infusion

and every 15 minutes between 2 and 3 hours. Subjects were not allowed to eat or drink during this period.

Indirect calorimetry was also performed as explained in section 3.6.2 at the start of the study and around 2 hours after the start of the infusion. This was carried out to obtain direct measurements of REE and VCO_2 (carbon dioxide consumption rate) which was used for calculation of protein turnover as explained below.

3.9.1.2 Sample Analysis

Breath samples obtained were stored in the airtight Exetainer® bottles at room temperature. Blood samples were collected in Lithium-Heparin coated (with gel separator) bottles. After collection, the blood samples were centrifuged at a speed of 3000 revolutions per minute for 10 minutes. Plasma obtained from centrifugation was stored in Microtainer® bottles at -20°C.

Plasma and breath samples were analysed at the Department of Metabolic and Molecular Physiology, University of Nottingham, Royal Derby Hospital Campus using isotope ratio mass spectrometry. The plasma samples were transported in the frozen state from Lister hospital.

3.9.1.3 Calculation of Whole Body Protein Turnover

WBPT was calculated as per previous published literature (Lim et al., 1998). Leucine taken up for oxidation intracellularly is transaminated into α-ketoisocaproate (α-KIC). Measurement of α-KIC enrichment in plasma has been shown to be a better marker of intracellular leucine enrichment compared to direct measurement of leucine in plasma (Matthews et al., 1982). Hence, plasma enrichment of α-KIC was measured in the samples. As explained in the earlier section 3.9.1, leucine and α-KIC flux (Q) was calculated using the following formula.

$$(\mathbf{15})\ \boldsymbol{Q} = \boldsymbol{I_i} \times \left[\left(\frac{\boldsymbol{\varepsilon_i}}{\boldsymbol{\varepsilon_p}}\right) - \mathbf{1}\right]$$

where I_i is the infusion rate of the isotope, ε_i is the isotopic enrichment of leucine in the infusate and ε_p is the enrichment of leucine or α-KIC in the plasma.

The rate of carbon dioxide release ($F^{13}CO_2$) from tracer leucine oxidation will be calculated as follows:

$$(16)\ \boldsymbol{F^{13}CO_2} = \left(\frac{\boldsymbol{VCO_2 \times \varepsilon_{CO_2}}}{\boldsymbol{BW}}\right) \times \left(\frac{\boldsymbol{60 \times 41.6}}{\boldsymbol{100 \times 0.77}}\right)$$

where VCO_2 is CO_2 production rate (ml/min, as measured by indirect calorimetry), * ε_{CO2} is $^{13}CO_2$ enrichment in the expired breath at isotopic steady-state and BW is body weight. The constants 60 (min/hr) and 41.6 (μmol/ml) convert VCO_2 from ml/min to μmol/hr. The factor 100 changes moles% enrichment from a percent to a fraction and the factor 0.77 accounts for the fraction of $^{13}CO_2$ produced by [I -^{13}C] leucine oxidation released from the bicarbonate pool into the expired gas.

The rate of leucine oxidation (O) is calculated as:

$$(17)\ \boldsymbol{O} = \boldsymbol{F^{13}CO_2} \times \left[\left(\frac{\boldsymbol{1}}{\boldsymbol{\varepsilon_p}}\right) - \left(\frac{\boldsymbol{1}}{\boldsymbol{\varepsilon_i}}\right)\right] \times \boldsymbol{100}$$

In a steady state condition, the rate of appearance of leucine in plasma should be the same that of leucine disappearance. This is expressed as the mass balance relationship below.

$$(18)\ \boldsymbol{Q = S + O = B + I}$$

where Q is the flux, S is the synthesis rate, O is the oxidation rate, B is the breakdown rate and I is the rate of intake. The whole body protein synthesis and breakdown rate can be calculated using the following formulae.

$$(19)\ \boldsymbol{S = Q - O}$$

$$(20)\ \boldsymbol{B = Q - I}$$

3.10 Assessment of body composition and body water

3.10.1 Watson formula

Total body water (TBW) estimation was carried out using the below formula published by Watson et al(Watson et al., 1980).

For males:

$$\boldsymbol{TBW} = \mathbf{2.447} - (\mathbf{0.09516} \times \boldsymbol{Age}) + (\mathbf{0.1074} \times \boldsymbol{Height}) + (\mathbf{0.3362} \times \boldsymbol{Weight})$$

For females:

$$\boldsymbol{TBW} = -\mathbf{2.097} + (\mathbf{0.1069} \times \boldsymbol{Height}) + (\mathbf{0.2466} \times \boldsymbol{Weight})$$

where TBW is expressed in Litres, age in years, height in cm and weight in kg

3.10.2 Bio-impedance analysis

Whole-body Bioimpedance analysis was carried out in many of the studies in this thesis using Inbody 720® (Biospace Company Limited, Seoul, Korea). Participants were asked to stand on the base platform of the analyser with a hand-held tactile electrode on each side for 2 minutes. The base platform also had tactile electrodes for heels and toes. The device measures body composition using multi-frequency currents. The outputs obtained from the device are intracellular-, extracellular- and total body water, skeletal muscle mass, fat-free and fat mass besides many other variables.

3.11 Demographic and clinical data

Demographic and clinical data were collected using respective renal electronic databases at the study sites. All data collected were anonymised using respective study ID numbers. The data collected included demographic and body size parameters (age, gender, ethnicity, height, weight) and clinical parameters (blood pressure, pulse rate). Height was measured using a wall-mounted stadiometer. Weight was measured using calibrated weighing scales used routinely in clinical practice. Besides these, parameters pertaining to dialysis adequacy were also obtained from the renal databases where appropriate.

3.12 Assessment of extra-renal comorbidities

Assessment of extra-renal comorbidity was carried out in two ways – information collected through a novel self-report comorbidity questionnaire and that from renal databases. Information obtained from medical records was used to calculate the Charlson Comorbidity Index as previously described (Charlson, Pompei, Ales, & MacKenzie, 1987).

3.12.1 Self-report Comorbidity Questionnaire

In order to facilitate comorbidity data collection in a large scale prospective study, a self-report comorbidity questionnaire was designed. The development and reliability of this questionnaire in predicting short-term survival is discussed in detail in a subsequent chapter. Seven medical conditions that are commonly prevalent in patients with ESRD were chosen to be included in the questionnaire. The medical conditions were expressed in a plain language that could be understood by individuals without any prior medical knowledge. Also, participants had the option of adding 3 additional medical conditions other than the ones that were listed. For each of the seven listed conditions and the additional ones the participants could add, they were asked 3 questions – whether they had the disease and if they did, whether they were receiving any treatment and whether the disease was limiting their activities. Participants were asked to tick the respective box if the answer to the corresponding question was in the affirmative. The questionnaire took about 2-3 minutes for participants to complete. A Composite Self-report Comorbidity Score (CSCS) was calculated from the questionnaire (see chapter 5 for scoring methods) which was used in subsequent survival analysis.

3.13 Analysis of the accelerometer data

In the study involving direct measurement of physical activity, the accelerometer (GENEActiv) data was downloaded onto a computer for further analysis. Individual raw activity readings were aggregated to 1-minute epochs and provided in the form of the signal magnitude vector (SVM) as per previous published literature (Esliger et al., 2011). The resulting SI unit for the outcome variable was g-minutes (g-min). Validated algorithms were used to calculate time spent in different activity states (sedentary, light, moderate and vigorous - intensity PA).

3.14 Blood analyses

In the cross-sectional study of physical activity measurement, the following parameters were measured in the serum – urea and electrolytes, full blood count and C-reactive protein.

For the protein turnover study, plasma samples were analysed for isotopic enrichment of α-KIC. Blood samples were collected in Lithium-Heparin coated (with gel separator) bottles. After collection, the blood samples were centrifuged at a speed of 3000 revolutions per minute for 10 minutes. Plasma obtained from centrifugation was stored in Microtainer® bottles at -20°C. Plasma samples were analysed by Dr Ken Smith at the Department of Metabolic and Molecular Physiology, University of Nottingham, Royal Derby Hospital Campus using isotope ratio mass spectrometry.

3.15 Statistical methods

An explanation of specific statistical methods used is included in the relevant chapters. Statistical analysis was performed using SPSS v19. A general overview of the methods employed is mentioned below. A p-value of < 0.05 was considered significant in all the analyses.

3.15.1 Regression techniques

Linear, non-linear and logistic regressions were used when appropriate to examine the relationship between different variables as explained in relevant chapters.

3.15.2 Univariate Survival Analyses

Univariate survival analysis was carried out using Kaplan-Meier method in the prospective survival outcomes study and also in examining the predictive value of the self-report comorbidity questionnaire with regards to short-term survival.

3.15.3 Multivariate Survival Analyses

Multivariate survival analysis was carried out using Cox proportional hazard model for the two studies mentioned in the above section. Some variables such as age, residual renal function and comorbidity score were dichotomised in certain analyses and these are explained in relevant sections as are the other variables that were included in the models.

Chapter 4

4. Body Composition, Energy Metabolism and Uraemic Toxin Generation

4.1 Introduction

Uraemic toxins accumulate in the blood and tissues in kidney failure and exert adverse effects on various organ systems (Au-Yeung et al., 2006; Glorieux, Helling, et al., 2004; D'Hooge et al., 2003). To maintain life in advanced kidney failure, these toxins need to be removed by dialysis. Patients with end-stage kidney disease have a wide range of clinical problems resulting from loss of kidney function and management of this need to focus on all the clinical manifestations of kidney failure. Hence, judging the minimum adequate dose of dialysis based on clearance of uraemic toxin alone is difficult. As the primary function of dialysis is to clear metabolic wastes, it might be expected that the minimum requirements for adequate dialysis would be related to the rate of metabolic waste production i.e. metabolic rate. But, this is not the basis of current clinical practice.

Haemodialysis adequacy is currently measured by a dimensionless parameter Kt/V, where K is the dialyser urea clearance, t is the duration of a single dialysis session and V is the Urea distribution volume (or Watson Volume) which is equal to the total body water. As discussed in Chapter 2, this practice is based on the assumption that uraemic toxin production is a function of its distribution volume and hence, it is not normalised based on the generation rate of the toxin (urea in this instance). This may have a number of unintended consequences.

The HEMO study showed survival benefit for women when given higher dialysis doses compared to men (Depner et al., 2004). A number of other studies have also shown an inverse relationship between body size and mortality in haemodialysis patients (Johansen et al., 2004; Port et al., 2002; Leavey et al., 2001; Leavey et al., 1998). This phenomenon of “reverse epidemiology” is discussed in detail in section 2.2.3. The underlying reason for the survival difference may be related to differences in body composition and metabolic activity (Huang et al., 2010; Spalding et al., 2008; Beddhu et al., 2003). As a result, some authors have suggested alternative

parameters that could be used for normalising the dialysis dose as discussed in chapter 2 (Daugirdas, Levin, et al., 2008; Spalding et al., 2008).

Metabolic rate, expressed as resting energy expenditure (REE), is the sum total of all the metabolic activities at rest and as such, reflects the rate of metabolic waste production. In addition, any physical activity is expected to contribute to metabolic waste generation and for this reason, total energy expenditure (TEE) – comprised of REE, physical activity related energy expenditure and the thermic effect of food – may be a better indicator of total uraemic toxin generation. Body composition can be broadly grouped into two compartments – Fat-free mass (FFM) and adipose tissue (fat) mass. Adipose tissue contributes only a minor fraction to the metabolic activity at rest. An important component of FFM is the High Metabolic Rate Organ (HMRO) compartment. HMRO compartment comprises of liver, kidneys, spleen, heart and brain and has significantly higher metabolic rate compared with other compartments. The combined mass of these organs (HMRO mass) and FFM has been shown to correlate closely with REE (Gallagher et al., 1998). In order to replace the current dialysis scaling parameter (Watson Volume) with any of these parameters, it is important to understand how these parameters relate to metabolic waste production. No studies to date have examined the relationship between these parameters and a specific marker of uraemic toxin generation.

There is considerable debate as to the choice of an ideal uraemic toxin marker. Urea was chosen as the marker for small solute toxin generation in this study for the following reasons. The current adequacy target for dialysis is based on urea clearance. Urea clearance by dialysis has been shown to correlate with patient survival in HD patients (Bloembergen et al., 1996; Owen et al., 1993). Urea generation rate (G) is an accepted surrogate marker of small solute uraemic toxin generation and can be calculated through formal urea kinetic modelling.

The primary aim of the study was

- To examine the relationship between body composition and energy expenditure and uraemic toxin generation
- To identify predictors of G in haemodialysis patients

The experimental hypotheses for this study were that these metabolic parameters – REE, TEE, FFM and HMRO mass – have a closer relationship with uraemic toxin generation compared to Watson Volume and that one or more of these factors may determine uraemic toxin generation better than Watson Volume.

This was a retrospective study conducted from an existing dataset. Patient recruitment and the study assessments were carried out for another study as part of Dr Vilar's PhD (Dr Enric Vilar, PhD, University of Hertfordshire – degree awarded Feb 2012) at Lister Hospital. The modelling of urea generation rate and the study results shown here are carried out as part of this study and have not been reported in Dr Vilar's PhD previously.

4.2 Methods

4.2.1 Ethical Review

The study was approved by the Essex Research Ethics Committee (Ref: 08/H0301/86). All study participants gave written informed consent at the start of the study.

4.2.2 Study Design

This was a cross sectional study of 166 haemodialysis patients. All study participants underwent a comprehensive metabolic analysis along with questionnaire-based assessment of physical activity. The data obtained was used to examine the relationship between energy expenditure variables and urea generation rate.

4.2.3 Study Population

A total of 166 chronic HD patients older than 18 years of age and have been established on HD programme for more than 3 months were recruited. Exclusion criteria included patients with active infection, untreated thyroid dysfunction, hospital admission in the previous month, recent respiratory infection, treated tuberculosis in the previous 12 months, a history of infection with hepatitis B, C or HIV, and those with amputated limbs.

4.2.4 Study Assessments

The following data was collected on all patients.

4.2.4.1 Anthropometric Measurements

Demographic and anthropometric measurements were collected as explained in section 3.11. Height and weight were measured using calibrated scales. Body Mass Index (BMI), Body surface area (BSA) using Haycock formula (Haycock, Schwartz, & Wisotsky, 1978) and Watson volume (Watson et al., 1980) were calculated using this data.

4.2.4.2 Dialysis Data

Parameters required for Urea Kinetic Modelling to estimate Urea Generation (G) and eKt/V were collected. These included pre- and post-dialysis blood urea levels, interdialytic urine collection for estimation of urea clearance, dialyser characteristics, blood and dialysate flow rates, ultrafiltration volume and dialysis session length.

4.2.4.3 Resting Energy Expenditure Measurement

Measurement of REE was carried out as explained in the General Methodology section 3.6.2. All participants were given written and verbal instructions to be followed prior to the procedure.

4.2.4.4 Physical Activity Assessment and estimation of Total Energy Expenditure

All study participants completed the Stanford 7-day recall questionnaire. Total energy expenditure was calculated from the questionnaire data in conjunction with measured REE as explained in general methodology chapter (section 3.7.2.2) using *equation (5)*.

4.2.4.5 Body Composition Analysis

Bioimpedance was performed using a Xitron Hydra 4200 in whole body continuous recording mode. Adhesive electrodes were placed on the hand and foot and the data was recorded over a period of five minutes at rest. Intracellular (ICW) and extracellular water (ECW) volume was obtained from the bioimpedance. Fat-free mass (FFM) was calculated as shown in the following section.

4.2.4.6 Urea Kinetic Modelling

Solute Solver (Daugirdas, Depner, et al., 2009) is a formal urea kinetic modelling software. It models a dialysis session based on specific dialysis parameters that are input into the system. The dialysis data collected as mentioned above was used to model the dialysis session. The software was used to calculate 2-pool urea generation rate (G) and Standard Kt/V besides many other outcome variables.

4.2.5 Calculation of Study-related Parameters

4.2.5.1 Body Size Parameters

The equations used for calculation of Body mass index (BMI) and BSA (Haycock equation) is shown below.

$$\boldsymbol{Body\ Mass\ Index\ (BMI)} = \frac{\boldsymbol{Weight\ (kg)}}{\boldsymbol{Height\ (m)^2}}$$

$$\boldsymbol{Body\ Surface\ Area\ (BSA)} = \mathbf{0.024265} \times \boldsymbol{Weight\ (kg)}^{\mathbf{0.5378}} \times \boldsymbol{Height\ (cm)}^{\mathbf{0.3964}}$$

Watson Volume was calculated using Watson equation explained in the general methodology chapter (section 3.10.1).

4.2.5.2 HMRO mass

HMRO mass is the combined mass of brain, heart, liver, kidneys and spleen. HMRO mass was estimated from a previously published model as mentioned below (Gallagher et al., 2006).

$$\boldsymbol{HMRO\ mass} = \mathbf{1.223} - (\mathbf{0.008} \times \boldsymbol{Age}) + (\mathbf{0.801} \times \boldsymbol{Height}) + (\mathbf{0.016} \times \boldsymbol{Weight}) + (\mathbf{0.305} \times \boldsymbol{Sex}) - (\mathbf{0.251} \times \boldsymbol{Race})$$

where Age is in years, Height in metres and Weight in kilograms; Sex is 0 if female and 1 if male; Race is 0 if White and 1 if Black

This equation has been used reliably in a study of haemodialysis patients exploring the relationship between body composition and survival (Kotanko & Levin, 2007).

4.2.5.3 Fat-free mass

Fat-free mass was calculated from the Intracellular (ICW) and Extracellular fluid volume (ECW) measured by bioimpedance as follows.

$$\boldsymbol{Fat-free\ mass\ (FFM)} = \boldsymbol{(dICW \times ICW) + (dECW \times ECW)}$$

where dICW and dECW are the densities of ICW and ECW – 1.521 and 1.106 – respectively.

4.2.6 Statistical Analysis

Normally distributed data are presented as mean ± standard deviation and non-normally distributed data as median (inter-quartile range). The differences between means were tested using Student's t-test and of differences between medians by Mann-Whitney U-test. Analysis of variance with Bonferroni adjustment was carried out to test the differences among multiple group means. Linear regression was used to examine the relationship between variables. Multiple regression analysis was used to determine the predictors of G. A p-value of <0.05 was assumed to indicate statistical significance.

4.3 Results

4.3.1 Baseline Demographics

A total of 166 patients were studied. The demographic, body size and dialysis-related characteristics are given in Table 4-1.

All body size parameters except BMI were significantly greater in men than women. There were no biochemical or haematological gender differences. eKt/V, but not Standard Kt/V, was slightly higher in women than men.

Table 4-1: Demographic, Body size and Dialysis characteristics of participants

Values expressed as mean ± SD for normally distributed data and median (*interquartile range) for non-normally distributed data. Kt/V – Urea clearance normalised to total body water.

	All patients (n = 166)	Males (n = 97)	Females (n = 69)	p-value
Age (years)	62.0 ± 15.5	60.8 ± 15.8	63.6 ± 15.1	NS
Diabetes (%)	27	30	23	NS
Ischaemic Heart Disease (%)	31	39	19	0.005
Weight (kg)	74.0 ± 19.1	79.4 ± 18.2	66.4 ± 17.7	<0.001
Height (m)	1.68 ± 0.10	1.74 ± 0.07	1.60 ± 0.08	<0.001
Body mass index (kg/m^2)	26.5 ± 6.0	26.7 ± 5.7	26.3 ± 6.5	NS
Body surface area (m^2)	1.84 ± 0.25	1.98 ± 0.26	1.74 ± 0.26	<0.001
Watson V (L)	38.0 ± 8.0	42.5 ± 6.6	31.7 ± 4.8	<0.001
Blood urea (mmol/L)	23.0 ± 6.3	22.4 ± 5.8	23.9 ± 6.9	NS
Serum creatinine (µmol/L)	865 ± 258	895 ± 272	825 ± 232	NS
Bicarbonate (mmol/L)	23.2 ± 2.8	23.3 ± 2.6	23.1 ± 3.0	NS
Haemoglobin (g/dL)	11.4 ± 1.2	11.4 ± 1.1	11.4 ± 1.3	NS
High CRP (>5 mg/L) (%)	50	51	48	NS
Haemodiafiltration (%)	74	84	61	0.002
Blood flow rate (Qb - ml/min)	330 ± 65	347 ± 62	307 ± 64	<0.001
Duration of session (Td - ml/min)	191 ± 36	197 ± 38	182 ± 33	0.007
Residual urea clearance* (ml/min)	0.6 (2.0)	0.8 (2.4)	0.5 (1.4)	NS
Equilibrated Kt/V (total)	1.41 ± 0.32	1.36 ± 0.28	1.48 ± 0.36	0.029
Total Standard Kt/V	2.47 ± 0.36	2.44 ± 0.32	2.51 ± 0.39	NS

4.3.2 Energy expenditure and Urea Generation Rate

Table 4-2 shows energy expenditure and urea generation rate data for the study participants. Both REE and TEE were low in women. Mean urea generation rate (G) was 4.3 ± 1.6 mg/min in women and 5.5 ± 2.2 mg/min in men ($p < 0.001$). TEE, but none of the other parameters, was lower in those with ischaemic heart disease (2135 ± 381 vs. 2304 ± 647 kcal/day; $p = 0.039$). REE (1651 ± 337 vs. 1523 ± 346 kcal/day; $p = 0.035$) and HMRO mass (3.59 ± 0.51 vs. 3.38 ± 0.45 kg; $p = 0.012$) were higher in diabetic patients. This was likely related to the higher body weight in diabetic patients (81.1 ± 20.9 vs. 71.4 ± 17.7 kg; $p = 0.003$).

Table 4-2: Energy Expenditure and Urea Generation Rate values

	All patients (n = 166)	**Males (n = 97)**	**Females (n = 69)**	**p-value**
Resting Energy Expenditure (kcal/day)	1588 ± 347	1679 ± 329	1387 ± 298	<0.001
Mean daily MET	1.44 ± 0.12	1.45 ± 0.13	1.42 ± 0.11	0.237
Exercise related Energy Expenditure (kcal/day)	689 ± 286	761 ± 315	588 ± 201	<0.001
Total Energy Expenditure (kcal/day)	2247 ± 583	2440 ± 590	1976 ± 452	<0.001
Fat-free mass (kg)	50.0 ± 17.2	56.4 ± 16.9	41.1 ± 13.2	<0.001
High Metabolic Rate Organ mass (kg)	3.4 ± 0.5	3.7 ± 0.3	3.0 ± 0.3	<0.001
Urea Generation Rate (mg/min)	5.03 ± 2.06	5.54 ± 2.19	4.32 ± 1.64	<0.001
Urea Generation Rate/Kg (mg/min/kg)	0.069 ± 0.023	0.070 ± 0.021	0.068 ± 0.027	NS

4.3.3 Factors related to Urea Generation Rate

We examined the relationship between G and various study parameters which is shown in Table 4-3. All parameters except age were significantly correlated. The highest correlations were noted for REE ($R^2 = 0.303$), TEE ($R^2 = 0.333$) and fat-free

mass (FFM) (R^2 = 0.366). The relationship between these three variables and G are shown in Figure 4-1 to Figure 4-3.

Table 4-3: Univariate correlations with Urea Generation Rate

	R^2	p-value
Age	0.012	NS
Body Mass Index	0.153	<0.0001
Weight	0.252	<0.0001
BSA (Haycock formula)	0.257	<0.0001
Watson Volume	0.286	<0.0001
High Metabolic Rate Organ Mass	0.256	<0.0001
Resting Energy Expenditure	0.303	<0.0001
Exercise related Energy Expenditure	0.260	<0.0001
Total Energy Expenditure	0.333	<0.0001
Fat Free Mass	0.366	<0.0001

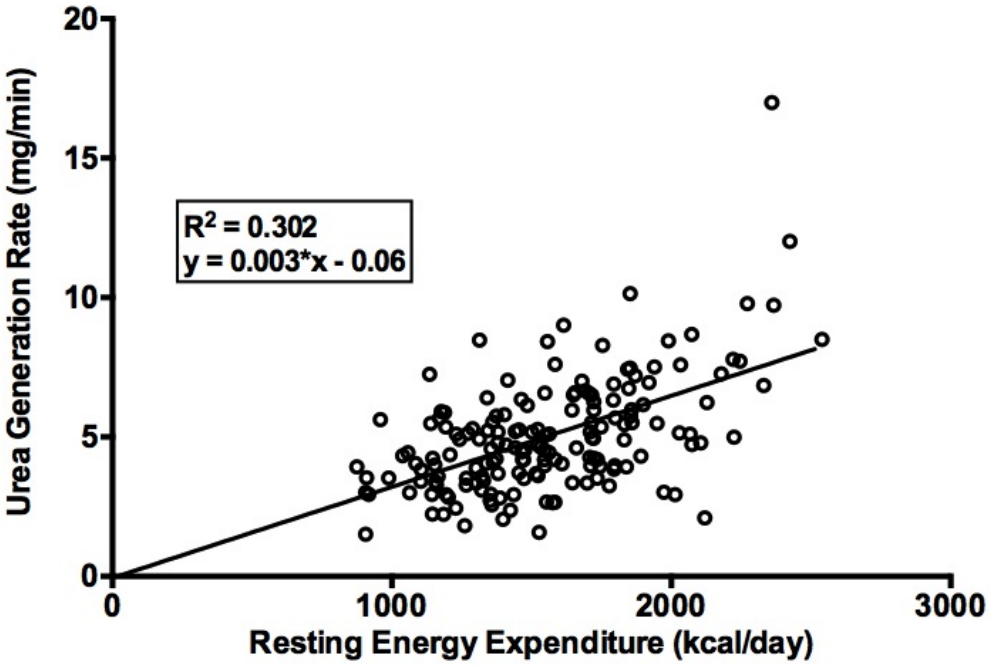


Figure 4-1: Relationship between REE and Urea generation rate

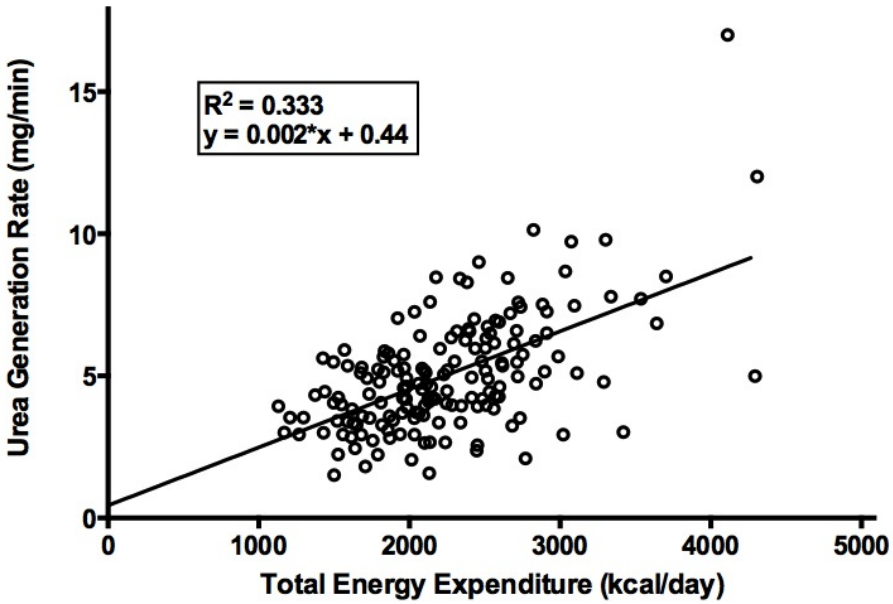


Figure 4-2: Relationship between TEE and Urea generation rate

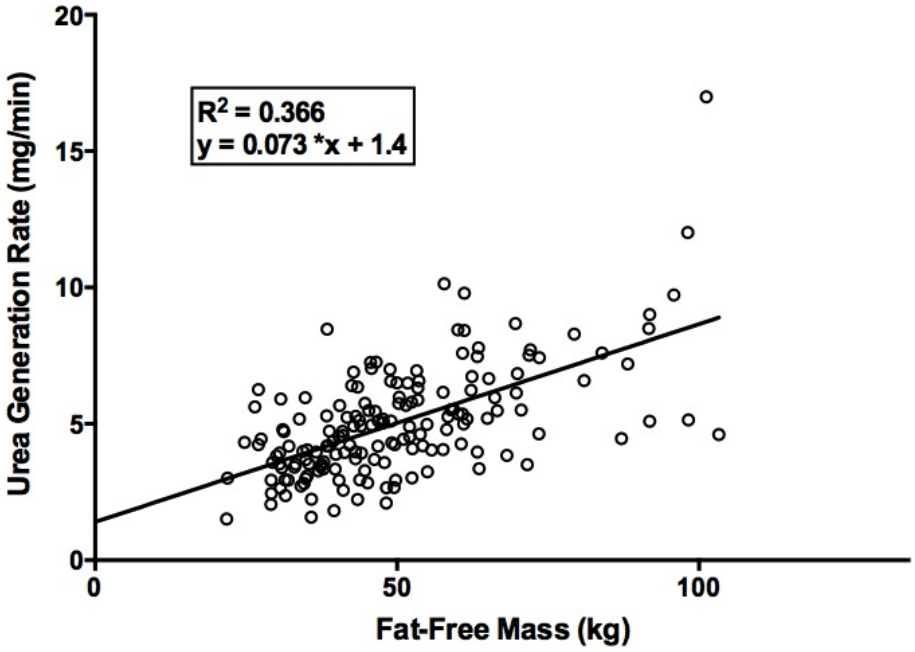


Figure 4-3: Relationship between FFM and Urea generation rate

4.3.4 Gender and Body Size Differences in Urea Generation Rate

Differences in urea generation rate based on gender and body size are shown in Figure 4-4 and Figure 4-5. The body size measure shown in the graph is Body mass index (BMI) with the cut-off being the median value. In both cases, the difference in G was significant ($p < 0.001$) with males and heavier individuals having higher levels.

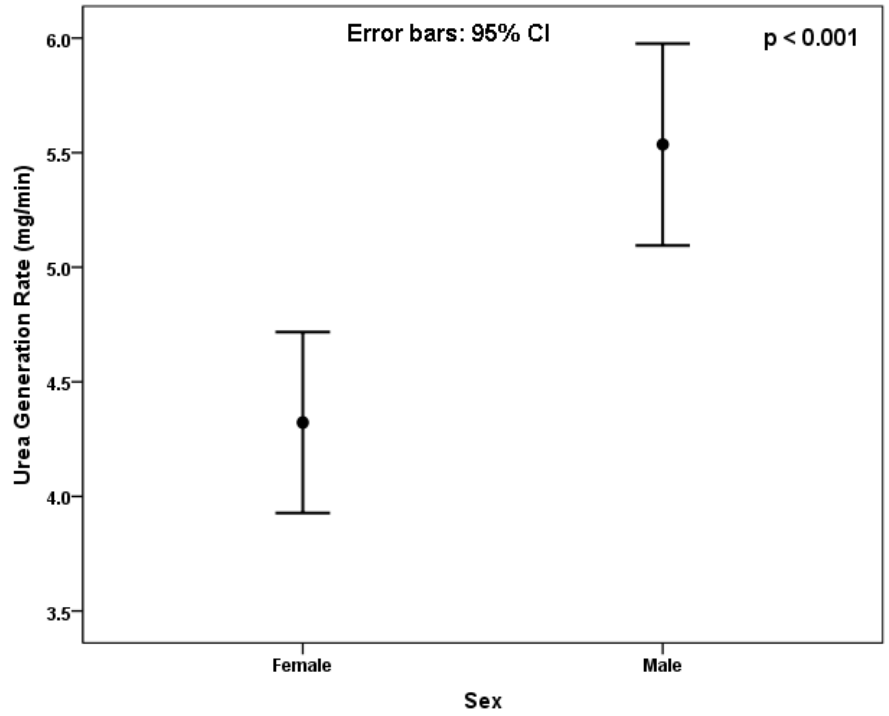


Figure 4-4: Gender Differences in Urea Generation Rate

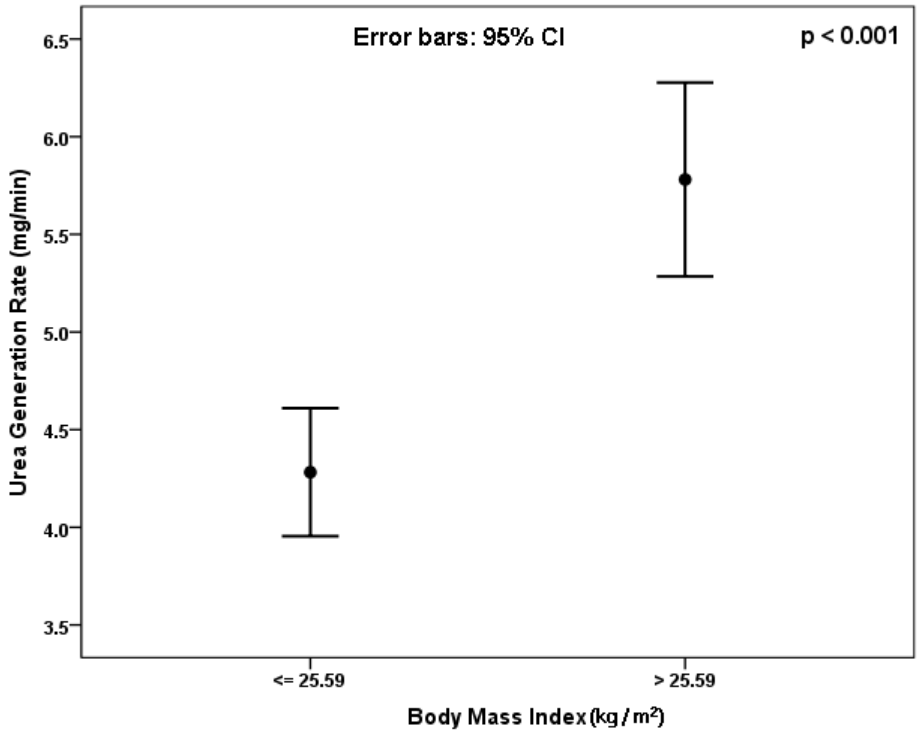


Figure 4-5: Body Size Differences in Urea Generation Rate

4.3.5 Effect of normalising G to Energy Expenditure

The effect of normalising G to energy expenditure measures, REE and TEE, was explored with regards to the gender and body size differences. Figure 4-6 and Figure 4-7 shows the gender and body size difference respectively when G was normalised to REE. There was no significant difference between the groups in both cases.

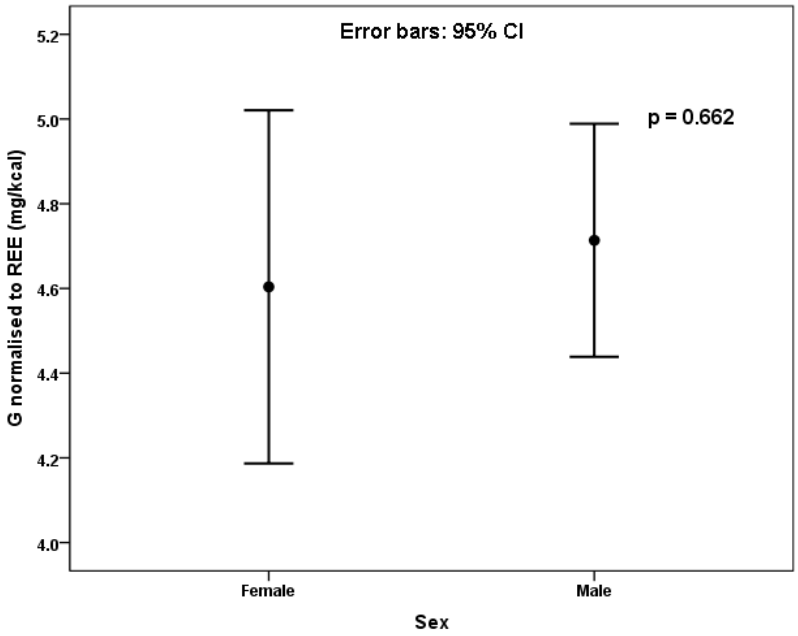


Figure 4-6: Differences in G normalised to REE based on Gender

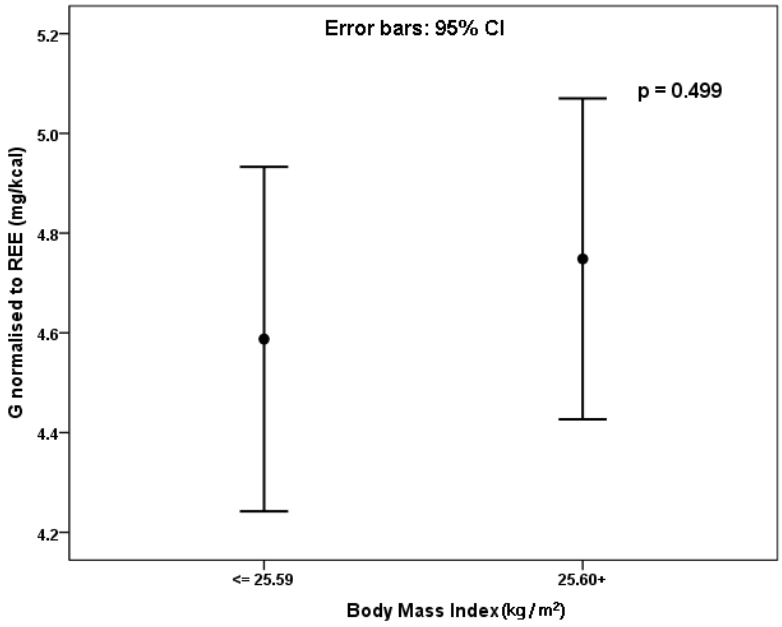


Figure 4-7: Differences in G normalised to REE based on Body Size

Similarly, the gender and body size difference when G was normalised to TEE is shown in Figure 4-8 and Figure 4-9. As with REE, there was no significant difference noted between the groups.

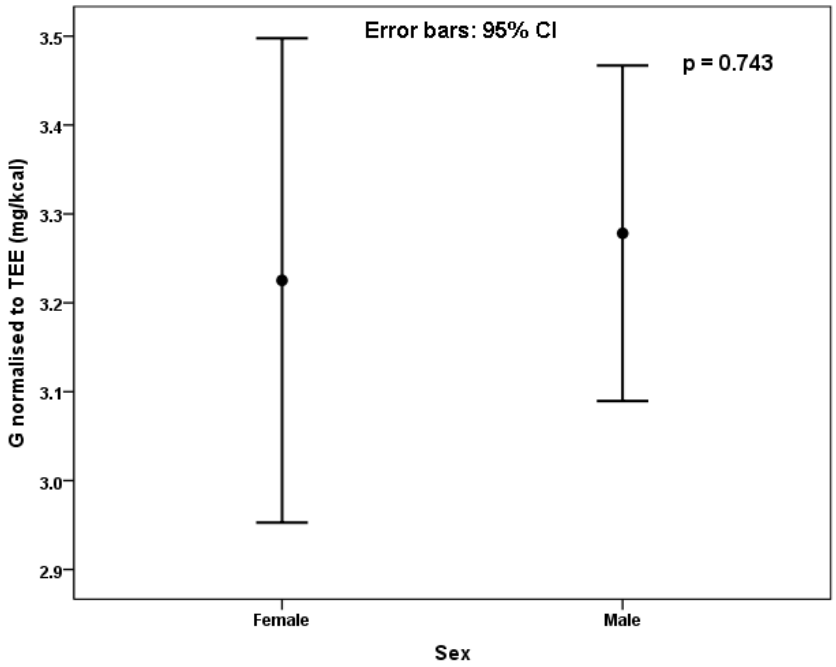


Figure 4-8: Differences in G normalised to TEE based on Gender

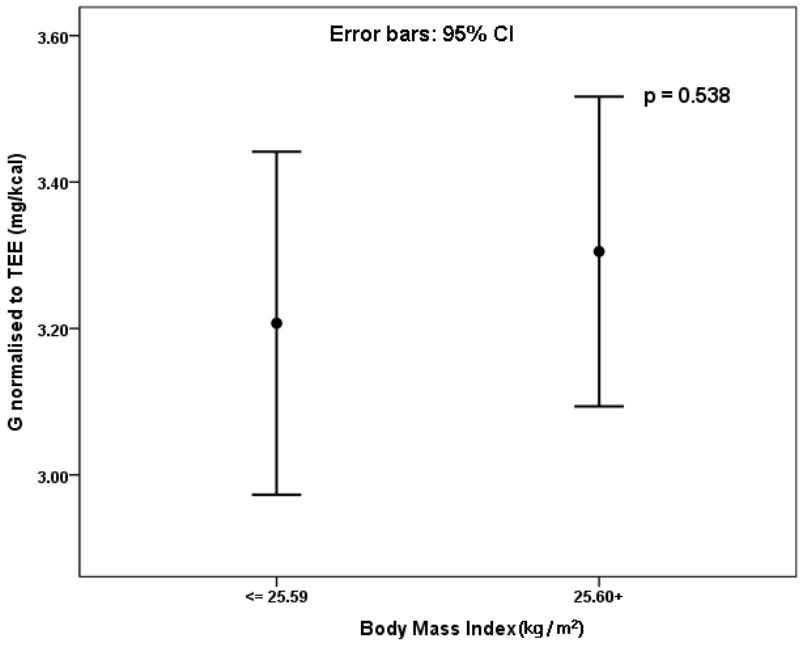


Figure 4-9: Differences in G normalised to TEE based on Body Size

4.3.6 Predictors of Urea Generation Rate

A number of multivariate regression models to identify independent predictors of G were explored. The variables considered were age, sex, weight, BMI, Watson Volume (V), HMRO mass, BSA, REE, TEE and FFM. The best fit model is shown in Table 4-4. In this model, the only independent predictors of G were TEE and FFM and the model accounted for 42% of variance in G (adjusted R^2 = 0.415).

Table 4-4: Multivariate Linear regression model of independent predictors of Urea generation rate

B is the unstandardised regression coefficient.

	B	Standard Error (B)	Beta	t	p-value
Constant	0.137	0.493		0.277	0.782
Fat-free mass (kg)	0.048	0.009	0.397	5.014	<0.001
Total energy expenditure	0.001	0.000	0.316	3.997	<0.001

4.3.7 Relationship between Urea generation rate and Watson Volume

The relationship between G normalised to body weight and Watson Volume was examined. Gender-specific V was calculated and was categorised with the mean as cut-off. There was a negative correlation between V and G/kg in women (R = -0.393; p = 0.001) but not in men. Gender differences in G/kg in relation to V are shown in Figure 4-10. Women of less than the mean gender-specific V had higher G/kg than those with higher V (p = 0.008). No such difference was noted in similarly grouped men.

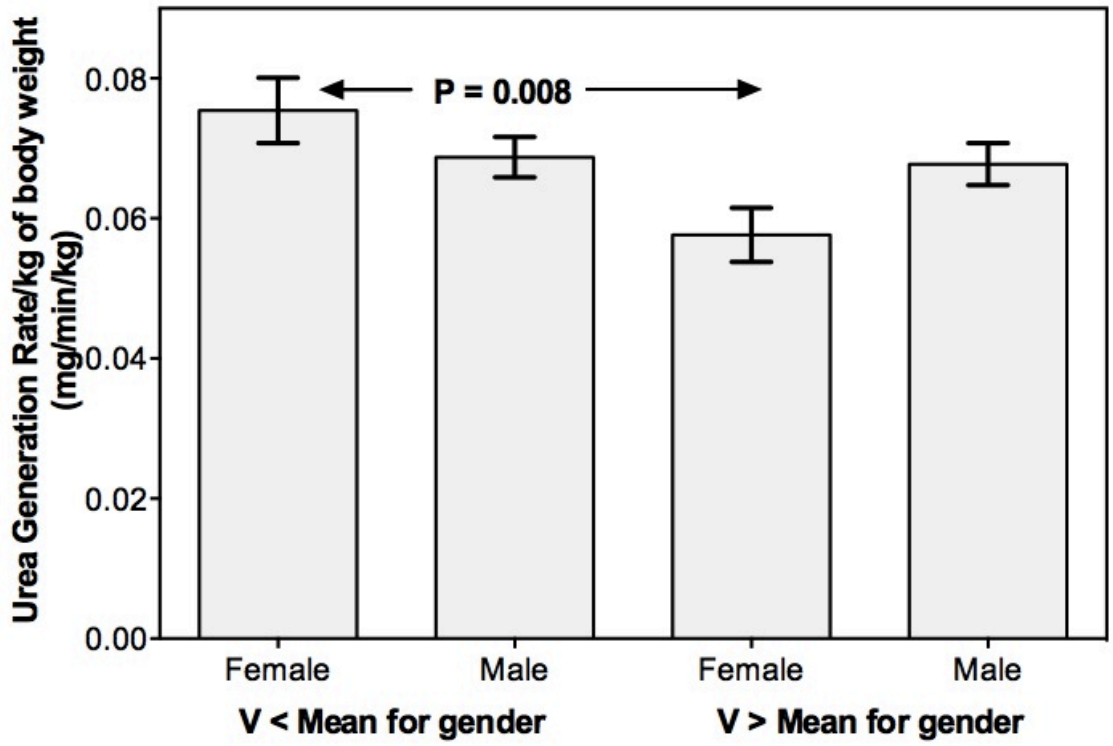


Figure 4-10: Relationship between Watson Volume and Urea generation rate normalised to body weight

The relationship between V and G normalised to FFM was also explored. G/FFM correlated with V in women (R = -0.268; p = 0.026) but not in men. Figure 4-11 shows the gender differences in G/FFM in relation to V. Again, women of less than mean gender-specific V had higher G/FFM although the difference was statistically significant only in comparison to men with higher than mean gender-specific V (p = 0026).

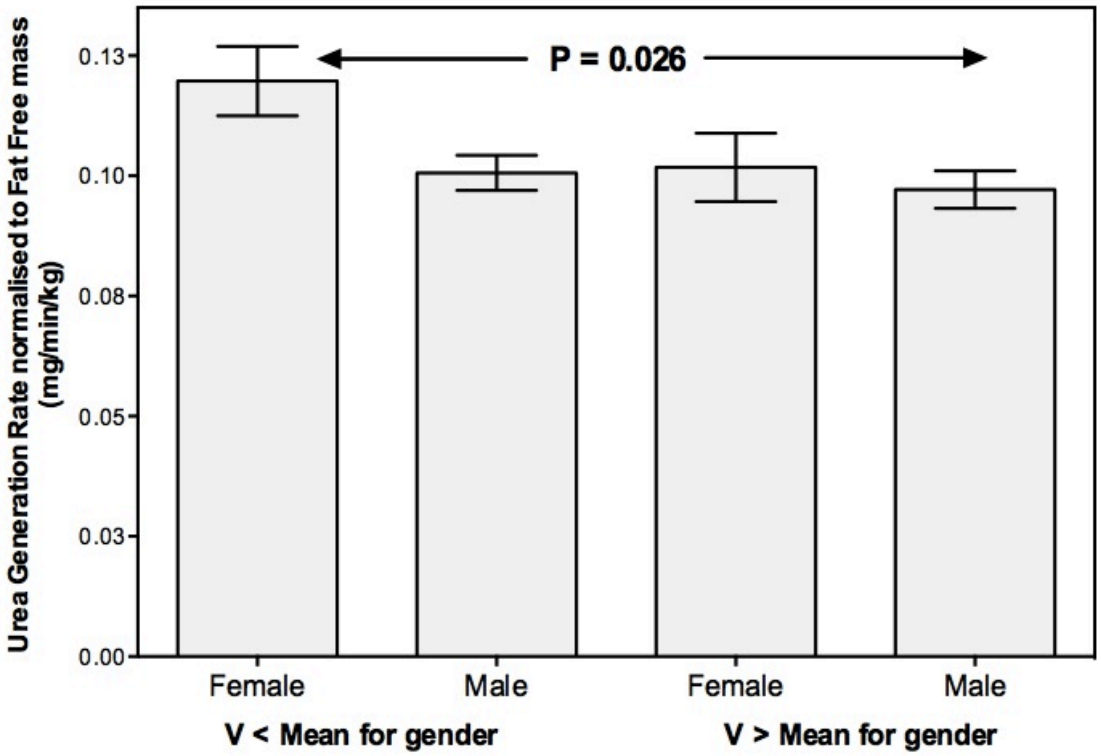


Figure 4-11: Relationship between Watson Volume and Urea Generation Rate normalised to Fat-free mass

4.4 Discussion

This study was designed to explore the relationship between various parameters related to energy metabolism and uraemic toxin generation as defined by the urea generation rate. The study results show that factors related to energy metabolism – REE, TEE and FFM – correlated better with G compared to Watson volume (V). In addition, the study explored the independent predictors of G and found that only TEE and FFM were significant variables in the model. Moreover, the study also showed that small women (with less than mean V) have significantly higher G relative to body weight compared to women with higher V. Similar results were obtained when G was normalised to FFM, although the statistical significance was only achieved in comparison to larger men. These findings are in favour of accepting the experimental hypothesis that these metabolic parameters have a closer relationship to uraemic toxin generation compared to Watson Volume.

Few studies have assessed the relationship between energy metabolism, body composition and urea generation rate. Sarkar et al (Sarkar et al., 2006) examined the relationship between FFM, HMRO mass and protein catabolic rate, a derivative of G. The study found that HMRO mass was the only independent predictor of G. It is worth noting that REE was estimated and not measured and the relationship between energy expenditure and PCR was not explored. This is the first study to date to have reported the relationship between measured REE and urea generation rate. The relationship between TEE, and thereby physical activity, and G had not been explored in any previous studies involving HD patients. Similarly, FFM has not been examined in relation to urea generation rate previously. This study has examined all these parameters in a single cohort which helps to identify the best parameter to predict uraemic toxin generation.

The current study data also showed that TEE and G were closely correlated and that Exercise related Energy Expenditure correlated significantly with G. TEE was also an independent predictor of G in multivariate analysis. TEE comprises of REE, physical activity related energy expenditure and thermic effect of food. The finding that TEE correlates better than REE to G implies that physical activity may contribute to uraemic toxin generation. However, this has not been demonstrated in clinical studies to date. It could be argued that the relationship between physical activity and G relates to the dietary habits of physically active individuals. Physically active patients may have increased dietary protein intake resulting in increased net protein catabolism thereby having an impact on G. An alternative explanation is that physical activity contributes significantly to small solute uraemic toxin generation - patients with habitually high levels of physical activity tending to have increased production and mobilisation of metabolic waste products, and increased small solute uraemic toxin generation. These possibilities are not mutually exclusive – in either case dialysis requirements would be higher.

As discussed in Chapter 2, there is an inverse relationship between body size and mortality risk in haemodialysis patients in that obese patients have better survival which is in contrast to the general population. It has been suggested that the likely reason for this phenomenon is that uremic toxin generation per unit of body mass is greater in patients with low body mass who also tend to have reduced body water volumes. As a result, the concentration of uremic toxins in body fluids is greater in

small subjects implying higher requirements for dialysis (Kotanko et al., 2007). This implies that a metabolic parameter which inherently corrects for gender and body size differences may provide equivalent dialysis doses to all patients. In this context, the study data shows that no gender and body size differences were apparent when G was normalised to REE or TEE. This implies that differences in energy metabolism may be implicated in the gender and body size differences in relation to G.

The study also explored whether there is a need for higher dialysis doses to specific subgroups based on gender and body size. It demonstrates that small women have relatively higher urea generation rate irrespective of whether G is normalised to body weight or FFM. Though previous studies have shown that low-weight patients have relatively higher HMRO mass (Kotanko & Levin, 2007; Sarkar et al., 2006), this is the first study demonstrating the link between gender, body size and uraemic toxin generation.

4.5 Limitations

The study is limited by its cross-sectional nature and its relatively small sample size. In addition, the study only considers the factors influencing urea generation. The generation rate of other uraemic toxins may be influenced by a different set of variables. The study did not collect information regarding dietary protein intake of study subjects which would have enabled the investigation of the influence of physical activity on G independent of protein catabolism. Finally, the physical activity data was collected through a recall questionnaire, which enquires about physical activity in the preceding 7 days. Recall bias is therefore a potential confounder of the physical activity data.

4.6 Conclusion

In conclusion, the study data has demonstrated that REE, TEE and FFM are closely related to urea generation in HD patients and that FFM and TEE are independent predictors of urea generation rate. These associations may imply that smaller women have relatively higher small solute uraemic toxin production and hence may require relatively higher dialysis doses, as has been previously suggested (Ramirez et al., 2012; Spalding et al., 2008). Patients with high physical activity levels may also have higher requirements for dialysis. It is worth exploring the applicability of

these metabolic factors in deciding minimum dialysis adequacy targets. The relationship of physical activity to uraemic toxin generation also requires further exploration in prospective clinical studies.

Elements of this chapter are reproduced with permission from International Society for Hemodialysis (Sridharan et al, Hemodial Int 2013; 17(4): 502-509).

Chapter 5

5. A Self-report Comorbidity Questionnaire for Haemodialysis Patients

5.1 Introduction

Comorbidity is an important outcome measure in patients with ESRD and has been shown to be a significant predictor of mortality in this patient population (van Manen et al., 2002; Chandna, Schulz, Lawrence, Greenwood, & Farrington, 1999; Khan, 1998; Khan et al., 1993). A reliable means of obtaining comorbidity data is vital for clinical and research purposes. Charlson Comorbidity Index (CCI) was developed to predict patient survival using comorbidity data (Charlson et al., 1987) and has been traditionally used as a marker of comorbidity burden. CCI has been shown to be a significant predictor of clinical outcomes in ESRD patients (Rattanasompattikul et al., 2012; Goodkin et al., 2003). As CCI is collected through medical records, it is time-consuming and is limited by the availability of medical records and the accuracy of the documentation of specific medical conditions.

A simple, self-report questionnaire would be a useful alternative tool for obtaining comorbidity data. After all, the patient is the source of the comorbidity data documented in the medical records. Moreover, patient-derived comorbidity data is reliable and valid compared to that from medical records (Katz, Chang, Sangha, Fossel, & Bates, 1996; Linet, Harlow, McLaughlin, & McCaffrey, 1989). Self-report questionnaires derived from general population may not be suitable for patient population with chronic illness such as end-stage renal disease. It is important to establish the role of self-report questionnaires in disease-specific patient population.

In order to facilitate comorbidity data collection from patients with ESRD in one of the studies in this thesis and also for use in clinical practice, a self-report comorbidity questionnaire was developed. This study was conducted to examine the level of agreement between the questionnaire and medical records and also to explore the relationship between the questionnaire-derived comorbidity score and short-term survival in haemodialysis patient population.

5.2 Materials and Methods

5.2.1 Self-report Questionnaire Design

The self-report questionnaire, shown in Appendix D, was based on that developed by Sangha et al (Sangha, Stucki, Liang, Fossel, & Katz, 2003). This questionnaire has been validated in a cohort of acute medical and surgical patients and correlates well with subsequent health status and resource utilisation. Eight comorbid conditions commonly prevalent in patients with ESRD were chosen to be included in the questionnaire. Participants also had the option of adding three additional comorbid conditions that were not listed. As can be seen from the questionnaire, the first 2 items enquire about cardiac disease, one relating to current and the other past history to enable more complete data capture about cardiovascular status. The conditions were expressed in plain language that could be understood by patients without prior medical knowledge. The questionnaire was translated into Bengali and Urdu to include patients from different ethnic backgrounds who did not have sufficient knowledge of English to complete the questionnaire. The translation was carried out by Straker Translations (London, UK) and was reviewed by two independent reviewers, who were native speakers of these languages, to confirm the accuracy of the translation.

For each of the listed conditions and the ones the participants could add, subjects were asked 3 questions – whether they had the condition, and if they did, whether they were receiving any treatment and whether the condition was limiting their activities. If the answer to a particular question was in the affirmative, then they were asked to tick the respective box. All participants completed the questionnaire on their own without any help from healthcare staff.

5.2.2 Scoring of the Questionnaire

Each positive response had a score of 1. Hence, the maximum score was 3 for each medical condition – 1 for the presence of the disease, 1 for being on treatment and 1 if the disease was limiting their activities. Of the listed conditions, the first 2 referred to cardiac disease and were considered as one item for purposes of scoring. A judgement was made on the admissibility of the optional items as significant comorbidities. In the event of the additional items listed by patients, only

cerebrovascular disease ("stroke") was considered an additional significant comorbidity and included in the scoring. In addition since the level of agreement for depression between the questionnaire and the medical records was poorest of all listed conditions and since depression did not contribute to any of the models tested, this parameter was omitted from the scoring scheme. Hence the Composite Self-report Comorbidity Score (CSCS) was derived from 7 conditions – giving a potential maximum of 21. Age was not included in this score.

5.2.3 Ethical Review

The study was approved by the North Wales Ethics Proportionate Review Committee (ref: 12/WA/0060 and UH Ethics ref: LMS/PG/NHS/00257). All participants provided informed written consent.

5.2.4 Subjects

Patients on maintenance, in-centre HD were recruited from the renal units of East and North Hertfordshire NHS Trust. The study included patients older than 18 years, dialysing thrice weekly and those able to understand English, Bengali or Urdu sufficiently to complete the questionnaire. Exclusion criteria included patients with no capacity to consent, those dialysing other than thrice weekly and those with limb amputations.

5.2.5 Study Protocol

The questionnaire was administered to each participant when they attended their regular HD session and were asked to complete it by the end of the session. The demographic information and their documented comorbidities were obtained from the local renal database. CCI was calculated as previously described (Charlson et al., 1987). All participants were followed up for 18 months to obtain survival data.

5.2.6 Statistical Analysis

Statistical analysis was carried out using SPSS ® version 19 (SPSS Software, IBM Corporation, Armonk, New York, USA). Normally distributed data are presented as mean ± SD, and non-normally distributed as median (interquartile range [IQR]). Correlations between scores were determined using the Spearman coefficient.

The agreement for individual items between the questionnaire and medical-record derived CCI was assessed using the inter-rater kappa (κ) statistic (Cohen, 1960). The overall agreement, defined as the number of cases in which both the patient responses and medical records agreed (both “yes” and “no” responses) divided by the total number of cases, was also calculated.

Logistic regression models were used to identify independent predictors of survival in the study population. All models included age, sex, and ethnicity as variables. Individual patient-reported comorbid conditions that were significantly associated with survival were identified, as well as the contribution of the Composite Self-report Score (CSCS) and the CCI.

Receiver Operator Curves (ROC) were constructed to compare the utility of the CSCS and the CCI in predicting mortality as well as defining the optimal cut-off points for this prediction for both these scales. The levels of agreement for the cut-off point in the scales were compared using the inter-rater κ statistic. In addition, the predictive power of these cut-off points in predicting mortality was compared in Cox Regression models. A p-value of < 0.05 was considered significant in all the analyses.

5.3 Results

A total of 282 patients were recruited out of 350 haemodialysis patients in our unit over a period of 1 month. There were 177 males (62.8%). The mean age was 64.1 ± 15.3 years. The ethnic make-up was 201 white (71.3%), 51 South Asian (18.1%) and 30 black (10.6%). For the open-ended questions, 46 patients (16.3%) indicated 1 additional disease, 10 patients (3.5%) indicated 2 diseases and 1 patient indicated 3 diseases. Frequently mentioned additional diseases included hypertension (11), stroke (10), hypothyroidism (5), visual impairment (5), and peripheral vascular disease (2). Of these only stroke was considered as significant additional comorbidity as discussed earlier. Hypertension was not included since this is a feature of chronic kidney disease and present in a very high proportion of patients on haemodialysis and Peripheral vascular disease since it was listed only twice. The other conditions mentioned by patients were hearing loss, back pain, inguinal hernia, insomnia, lymphoedema, gout, glaucoma and diverticulitis.

5.3.1 Prevalence of Comorbid Conditions

Table 5-1 shows the prevalence of each item as determined from the medical records and the comorbidity questionnaire. Prevalence of heart disease, diabetes mellitus and cancer was similar between the two instruments. The prevalence of both lung and liver disease as obtained from medical records was higher than the self-report. For the prevalence of arthritis, cerebrovascular disease and depression the opposite prevailed.

Table 5-1: Prevalence of comorbid conditions according to Self-report comorbidity questionnaire and medical records

	Prevalence (%)		
	Medical Records	**Comorbidity Questionnaire**	**Limiting Disease**
Diabetes	84 (29.8)	82 (29.1)	22 (7.8)
Heart disease	98 (34.8)	92 (32.6)	37 (13.1)
Cancer	20 (7.1)	18 (6.4)	3 (1.1)
Liver disease	12 (4.3)	7 (2.5)	3 (1.1)
Lung disease	29 (10.3)	13 (4.6)	8 (2.8)
Arthritis	22 (7.8)	72 (25.5)	47 (16.7)
Cerebrovascular disease	10 (3.5)	22 (7.8)	2 (0.7)
Depression	17 (6.0)	35 (12.4)	15 (5.3)

5.3.2 Level of Agreement

The level of agreement between the questionnaire data and medical records is shown in Table 5-2. Overall agreement exceeded 80% for all items with the highest agreement for diabetes mellitus (99%) and the lowest for arthritis (81%). Table 5-2 also shows the κ statistic and the interpretation of the κ for each item. There was

almost perfect agreement between the two instruments for diabetes, substantial agreement for heart disease and cancer, and moderate agreement for liver disease.

Table 5-2: Level of agreement between the questionnaire and medical records

	Level of Agreement (%)	Kappa (95% CI)	Interpretation of Kappa
Diabetes	99	0.97 (0.93, 1.00)	Almost Perfect
Heart disease	83	0.62 (0.52, 0.72)	Substantial
Cancer	96	0.72 (0.55, 0.89)	Substantial
Liver disease	97	0.51 (0.20, 0.83)	Moderate
Arthritis	81	0.35 (0.09, 0.50)	Fair
Lung disease	91	0.34 (0.10, 0.58)	Fair
Cerebrovascular disease	93	0.34 (0.09, 0.56)	Fair
Depression	88	0.29 (0.06, 0.51)	Fair

There was only fair agreement for lung disease, arthritis, depression and cerebrovascular disease. There was no association observed between gender and agreement of patient self-reports with medical records for any of the items listed. Using age as a dichotomous variable (age less than 65 vs. 65 or more) in this analysis, there were significant differences in level of agreement for arthritis and depression. κ was significantly higher in older patients for arthritis and in younger patients for depression ($p = 0.03$ for both).

5.3.3 Composite Comorbidity Score

The distributions of the CSCS and CCI are shown in Figure 5-1. The median CSCS was 2 (IQR3). The median CCI was 6 (IQR3). The CSCS correlated with the CCI (rho = 0.531: $p<0.001$).

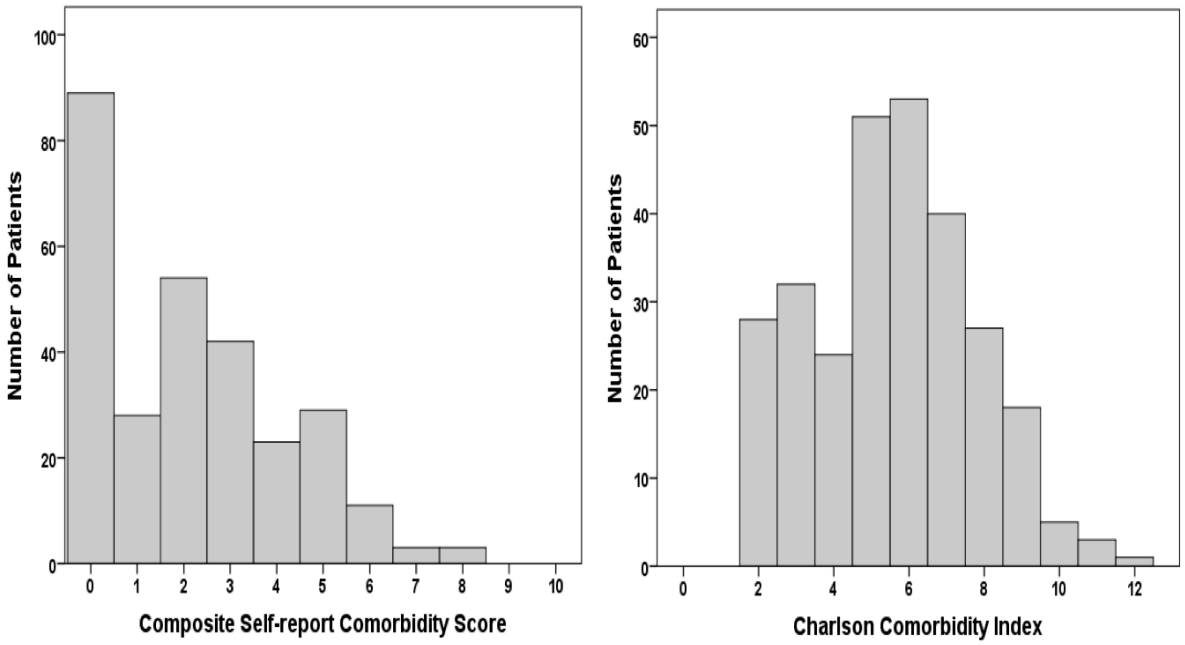


Figure 5-1: Histogram showing distribution of CSCS and CCI

5.3.4 Survival Prediction and Logistic Regression

Of the 282 participants, 58 (20.6%) died in the 18 months following recruitment. Table 5-3 shows the best Logistic Regression Model for predictors of survival at 18 months based on individual self-report comorbid conditions (Hosmer and Lemeshow Chi-square 11.115; p = 0.195, Nagelkerke R-square value 0.197). Variables considered were age, sex, ethnicity, presence of self-report heart disease, cerebrovascular disease, cancer, diabetes, liver disease, lung disease, and arthritis. Heart disease, liver disease and arthritis were, along with age, the significant predictors of survival.

Table 5-3: The best Logistic Regression Model for predictors of survival at 18 months based on individual self-report comorbid conditions

	B	S.E.	Wald	p-value	Exp(B)
Constant	-4.169	.902	21.374	.000	.015
Age	.030	.013	5.287	.021	1.030
Heart Disease	1.242	.319	15.124	.000	3.462
Liver Disease	1.794	.856	4.392	.036	6.013
Arthritis	.858	.334	6.582	.010	2.358

The logistic regression models for the CSCS and the CCI are shown in Table 5-4. The models including these individual parameters showed similar goodness of fit (Hosmer and Lemeshow Chi-square 4.151 [p = 0.843] and 1.878 [p = 0.985]) and predictive power (Nagelkerke R-square values 0.202 and 0.211 respectively). Each parameter had a highly statistically significant relationship to survival within the models (p <0.001 in both cases). Interestingly the model was improved by inclusion of both parameters (Nagelkerke R-square 0.250) and in this model (Table 5-4 – lower panel) both parameters retained a high degree of statistical significance.

Table 5-4: Logistic regression models for survival at 18 months

MODEL 1: Nagelkerke R^2 = 0.202	**B**	**S.E.**	**Wald**	**p-value**	**Exp(B)**
Age (Years)	.030	.013	5.540	.019	1.031
Sex (Male v Female)	.440	.344	1.635	.201	1.553
Ethnicity (Non-white v White)	-.222	.381	.340	.560	.801
CSCS	.392	.081	23.386	.000	1.480
Constant	-4.616	.962	23.026	.000	.010
MODEL 2: Nagelkerke R^2 = 0.211					
Age (Years)	-.015	.016	.894	.344	.985
Sex (Male v Female)	.211	.348	.367	.545	1.235
Ethnicity (Non-white v White)	-.213	.376	.322	.570	.808
CCI	.521	.105	24.445	.000	1.683
Constant	-3.641	.909	16.035	.000	.026
MODEL 3: Nagelkerke R^2 = 0.250					
Age (Years)	-.002	.016	.021	.886	.998
Sex (Male v Female)	.298	.355	.702	.402	1.347
Ethnicity (Non-white v White)	-.315	.387	.663	.416	.730
CCI	.365	.117	9.715	.002	1.440
CSCS	.262	.092	8.180	.004	1.300
Constant	-4.251	.972	19.125	.000	.014

5.3.5 Receiver Operator Characteristics (ROC) Analysis

ROC curves were constructed to compare the performance of CSCS and CCI in predicting death within the follow-up period, and to determine the best cut-off values of both these parameters for this prediction (Figure 5-2). The Area under the Curve in ROC analysis was similar for these parameters with overlapping 95% confidence intervals: 0.724 (0.651 – 0.797) and 0.754 (0.684 – 0.823) respectively. The best cut-off points for predicting mortality during the follow-up period were determined as CSCS > 3 and CCI > 6. Patients with CSCS > 3 are subsequently referred to as having high CSCS. Likewise high CCI values refer to CCI > 6.

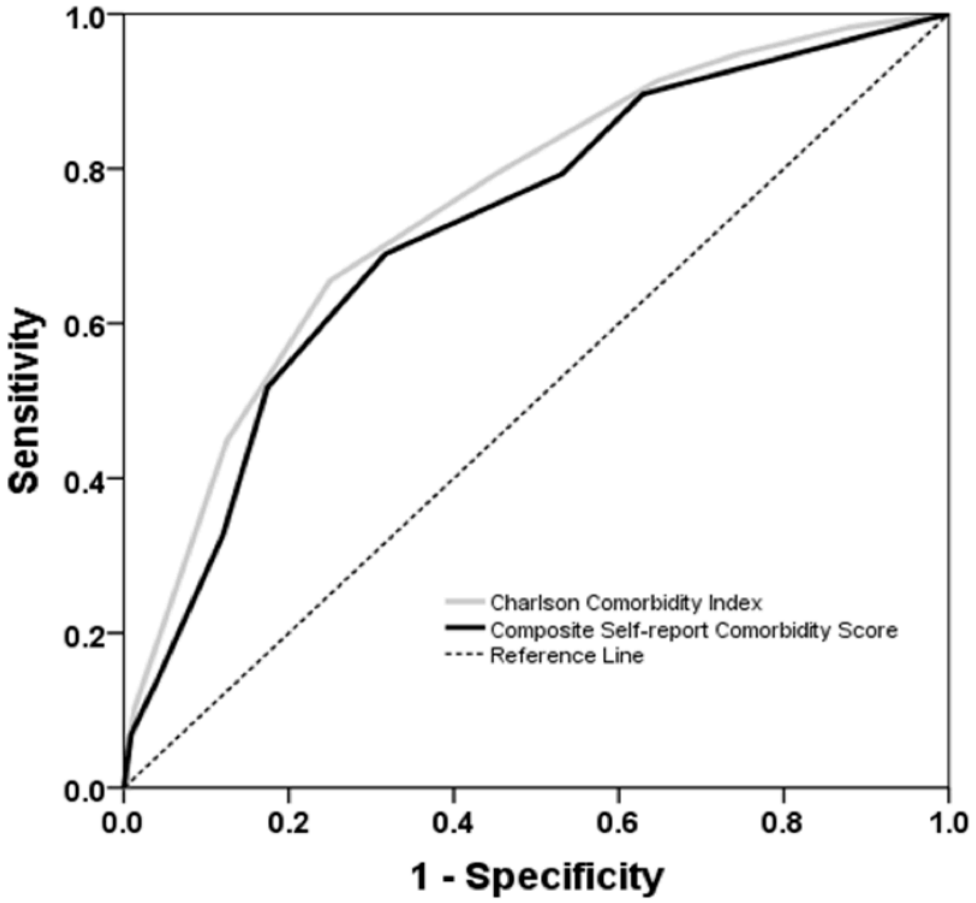


Figure 5-2: Receiver Operator Characteristic curves comparing the performance of CSCS and CCI in predicting death

5.3.6 Comparison of cut-off points in mortality prediction

Figure 5-3 compares the adjusted survival of patients with high CSCS (69 patients) and those with high CCI (94 patients). In both cases survival is adjusted for age, sex and ethnicity in Cox Regression models. Both high CSCS and high CCI were highly predictive of mortality within their separate models ($p < 0.001$ in both cases).

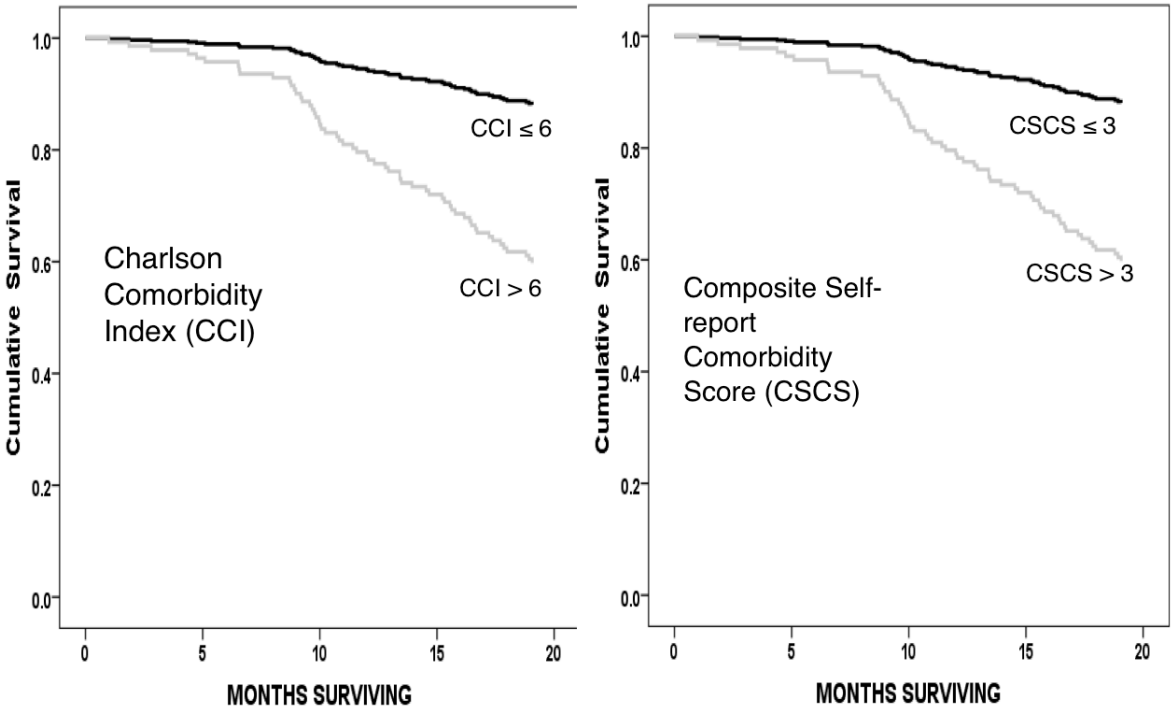


Figure 5-3: Adjusted Survival for patients with high comorbidity scores

Hazard Ratios were over 4 for both parameters with overlapping 95% Confidence Intervals (4.050 [3.362 – 6.947] and 4.139 [2.202 – 7.774] respectively). In spite of this, when these parameters were included together in the same Cox model, both retained their significance, and Hazard Ratios of each were similar, approaching 3 (Table 5-5).

The level of agreement between patient with high CSCS and high CCI was only fair ($\kappa = 0.325$; $p < 0.001$). The main difference between these high comorbidity groups relates to mean age which, unsurprisingly, was significantly higher in the high CCI group than in the high CSCS group (73.6 ± 8.7 vs. 67.4 ± 11.7; $p < 0.001$). There were other differences. The best levels of agreement between CCI (high and low) and the presence or absence of individual components of the CCI, were for heart

disease (κ 0.447), diabetes (κ 0.443), cerebrovascular disease (κ 0.301) and cancer (κ 0.205). On the other hand, the best agreements between CSCS (high v low) and the presence or absence of individual components of the CSCS, were with heart disease (κ 0.491), arthritis (κ 0.442) and diabetes (κ 0.396).

Table 5-5: Cox model of predictors of short-term survival in haemodialysis patients

	B	S.E.	Wald	p-value	Exp(B)
Age (Years)	.011	.012	.840	.359	1.011
Sex (Male v Female)	.405	.299	1.831	.176	1.500
Ethnicity (Non-white v White)	-.411	.315	1.700	.192	.663
CCI > 6	1.020	.334	9.348	.002	2.773
CSCS > 3	1.067	.292	13.309	.000	2.907

5.4 Discussion

Information on comorbid conditions is essential in routine clinical practice and also for research purposes. This study describes a simple, self-report questionnaire to obtain comorbidity data in patients with advanced kidney failure, receiving treatment by dialysis. The study data shows that the questionnaire has very good overall level of agreement with the medical records data. The questionnaire-derived comorbidity score – the CSCS – was significantly predictive of short-term survival in this patient group and may have clinical utility.

There was almost perfect or substantial levels of agreement between the prevalence of self-reported diabetes, heart disease and cancer and the prevalence of these conditions derived from the detailed examination of the patients' medical records. The level of agreement for liver disease was moderate. For arthritis, lung disease, cerebrovascular disease and depression the levels of agreement were only fair. Whilst lung disease and cerebrovascular disease were under-reported by patients,

arthritis and depression were reported more frequently compared to the medical records.

There are a number of factors which may contribute to these discrepancies. Results from study data indicates that even though lung disease was under-reported in the questionnaire, a high proportion of those patients who did report having this condition indicated that their disease limited their activities. This suggests that patients with milder forms of lung disease may not attribute much significance to related symptoms (e.g. “smoker’s cough”) or may not be aware of the diagnosis at all. The under-reporting of cerebrovascular disease was almost certainly caused by the fact that this was not a condition specified on the questionnaire and patients wishing to report this condition had to write this in under “other medical conditions”. The conditions which were over-reported by patients, arthritis and depression, are predominantly diagnosed and treated in primary care. This may sometimes lead to their not being documented in hospital records unless they are receiving medication for that condition or if its severity warrants secondary care referral. The poorest level of agreement was found with depression and this, and its failure to contribute to any of the survival models that were considered, led to the exclusion of this parameter from contributing to the CSCS.

The study data shows that CSCS was a significant predictor of survival in haemodialysis patients. The main individual comorbid conditions contributing to this predictive power in this patient group were the presence of heart disease, liver disease and arthritis. The power of the CSCS and the CCI in predicting mortality over the 18-month follow-up period was compared. In logistic regression models which allowed adjustment for age, sex and ethnicity, both these parameters were found to have similar predictive power (Table 5-4). Since the CCI includes a term for age, such a modelling approach is required to allow for this in the comparison. The ROC analysis also showed that the parameters performed similarly in predicting death within the follow-up period. The area under the ROC curve was greater for CCI than for CSCS (0.754 vs. 0.724). This difference was not significant and is probably accounted for by the fact that age is not controlled for in this method of comparison.

ROC analysis was also used to help determine the best cut-off points for the CSCS and CCI for predicting mortality during follow-up. The high comorbidity groups were

determined as CSCS >3 and CCI >6. The performances of these parameters in predicting death were similar in Cox Models, again controlling for age, sex and ethnicity.

Interestingly the use of CSCS together with CCI in a logistic regression model improved the model with both terms retaining significant predictive power. The findings were similar with the use of both high CSCS and high CCI in a Cox Model. These findings, together with the only fair level of agreement between high CSCS and high CCI suggest that these parameters make different contributions to the assessment of comorbidity. The obvious difference relates to the inclusion of a contribution of patient age in the calculation of CCI. Indeed it was found that high CCI group was significantly older than the high CSCS group. There were other differences between these two groups. Both were similarly influenced by their components relating to heart disease, and diabetes but the high CCI group was more influenced by components relating to cerebrovascular disease and cancer, and the high CSCS group by the component related to arthritis.

Instruments such as the CCI rely on availability and accuracy of medical records and as such, may be limited in its utility for clinical and research purposes. A self-report comorbidity questionnaire can help collect this information reliably and with relative ease. Various self-report health measures have been previously studied in patients with renal failure (Cavanaugh et al., 2008; Thong et al., 2008). This self-report questionnaire has the advantage of being brief, easily understandable by patients and at the same time being comprehensive enough to include commonly prevalent comorbid conditions in ESRD patient population. Also, the questionnaire enquires about the treatment and limitations imposed by specific diseases which can be used as a surrogate marker of the severity of the disease. The high level of agreement between the self-report and clinical records with respect to diabetes, heart disease and cancer suggests that the utility of the self-report approach could embrace the collection of such condition-specific data for inclusion in survival models.

5.5 Limitations

It has been shown previously that medical records may in themselves have substantial errors (Merkus et al., 2000; Peabody, Luck, Glassman, Dresselhaus, & Lee, 2000; Luck, Peabody, Dresselhaus, Lee, & Glassman, 2000) and hence, using

this method as a “gold standard” may be less than ideal. Also, with any questionnaire-based technique there is a potential for recall bias. Though patients had the option of adding any additional diseases that were not listed, it is possible that patients may not recall milder forms of existing comorbid diseases and this may exclude some important comorbid conditions such as cerebrovascular disease and peripheral vascular disease. Further development of this questionnaire should include specific enquiry about the presence and severity of these conditions. Finally this study only examined the predictive capacity of this self-report questionnaire with regards to short-term survival in haemodialysis patients and as such, the results should not be extrapolated to other groups of patients with kidney disease, or to the assessment of long-term survival.

5.6 Conclusion

In summary, this self-report comorbidity questionnaire is a simple and reliable tool for obtaining comorbidity data in clinical practice and research studies involving patients with end-stage renal disease on haemodialysis. There is strong agreement between this self-report instrument and data derived from medical records on important comorbid conditions that have an influence on patient outcome. The instrument also provides information on severity of the comorbid diseases. In addition, the comorbidity score generated (CSCS) has comparable predictive power for short-term survival in haemodialysis patients to the CCI. Further work is needed to adapt the questionnaire and to examine its applicability in studies assessing long-term survival and other clinical outcomes in patients with kidney disease.

Elements of this chapter are reproduced from a published article - Sridharan et al, BMC Nephrol 2014; 15(1): 134.

Chapter 6

6. Impact of Renal Function on Energy Expenditure

6.1 Introduction

The relationship between metabolic rate and Glomerular filtration rate (GFR) is discussed in section 2.3.1. Singer (2001) showed, through studies on different animal species of varying body size, that metabolic rate and GFR scale to body weight by approximately the same exponent (Singer, 2001). He hypothesised that the reason behind this observation is that the metabolic rate is the driving process for renal blood flow. He also argued that the converse is not true i.e. a reduction in GFR as happens in Chronic Kidney Disease (CKD) should not result in a reduction of metabolic rate. There is circumstantial evidence to support this hypothesis. Studies carried out in patients with hypothyroidism have shown reduction of GFR (Davies et al., 1952; Mackay & Sherrill, 1943) and an increase in GFR in those with hyperthyroidism (Aizawa et al., 1986; Ford et al., 1961; Mackay & Sherrill, 1943). It was suggested that this effect on GFR was likely driven by corresponding changes in the metabolic rate in these thyroid disorders. However, there have been no clinical studies carried out in patients with different stages of CKD to explore the relationship between metabolic rate and CKD.

Studies undertaken in this thesis have focused on basal metabolic rate (i.e. resting energy expenditure) and physical activity assessment methods in haemodialysis patients. It is vital to focus on these two elements together in the form of total metabolic rate i.e. Total Energy Expenditure (TEE) in the context of CKD. TEE consists of REE, physical activity-related energy expenditure (PAEE) and thermic effect of food, which comprises about 10% of TEE.

Understanding TEE in CKD patients could be beneficial in many ways. As metabolic waste generation is dependant on total metabolic activity, it is plausible that TEE could be a better marker of uraemic toxin load compared to REE as it includes metabolic waste production from physical activity as well. A better knowledge of TEE will enable better dietary and nutritional management of patients with chronic kidney

disease. Moreover, studying TEE across the spectrum of CKD will help to understand the relationship between metabolic rate and GFR and test the hypothesis proposed by Singer.

The gold standard technique of studying TEE in free-living conditions is by the doubly labelled water method (DLW). The alternative method is to obtain physical activity data through questionnaires and in conjunction with a measure of REE, TEE can be estimated. However, this is prone to measurement errors and will not provide accurate measurements of TEE as would be needed in studies examining the relationship with GFR. In addition, measuring TEE accurately will enable to explore its relationship with body size parameters such as total body water. As discussed in previous sections of this thesis, the practice of using total body water to normalise dialysis dose may lead to under-dialysis in some group of patients. Therefore, exploring the relationship between TEE and total body water may further our understanding of the gender differences in survival that have been reported in haemodialysis patients.

Total body water calculation (Watson Volume) was derived from a cohort of normal individuals but is extensively used in dialysis patients for scaling dialysis dose. TEE measurement by the doubly labelled water technique will help to examine the validity of using Watson volume in patients with CKD. In addition, the accuracy of other tools of body water measurement such as bioimpedance can also be validated using this method.

The doubly labelled water method of measuring TEE is associated with considerable costs and is time-consuming as measurements are typically carried out over a period of 10-14 days. These factors make this prohibitive for use in routine clinical practice. A useful alternative is to validate physical activity questionnaires using this technique which can then be employed in clinical practice for estimation of TEE. There have been no clinical studies published so far in CKD employing the doubly labelled water technique and therefore, no physical activity questionnaires validated for use in patients with CKD using this gold standard method.

As little is known about total energy requirements specifically in renal disease, this study was designed to explore certain aspects of TEE in CKD with the following objectives:

1. To measure TEE in free-living conditions in patients with CKD
2. To examine the influence, if any, of declining kidney function on TEE
3. To examine the relationship between TEE and body water measured by bioimpedance and also that estimated from Watson equation
4. To explore the relationship between measured TEE and urea generation rate which is a surrogate marker of uraemic toxin production
5. To examine the validity of physical activity questionnaires for TEE estimation in patients with CKD

The null hypothesis for this study was that a reduction in renal function will lead to reduction in metabolic rate and the experimental hypothesis was that declining renal function does not have a corresponding effect on metabolic rate.

There are two separate aspects of this study – the first four objectives exploring the clinical aspects and the last one focusing on validating activity questionnaires. Hence, for clarity and ease of reading, the results are presented in two chapters. The methodology discussed below applies to both the chapters. This chapter discusses the effect of renal function of TEE and the relationship between TEE and body water. The next chapter discusses the validation of two physical activity questionnaires against the doubly labelled water method.

The results presented in this chapter are from a total of 80 patients with CKD. 40 of these patients were recruited as part of this study. The other 40 patients were recruited for another similar study conducted as part of Dr Vilar's PhD (Dr Enric Vilar, PhD, University of Hertfordshire – degree awarded Feb 2012) at Lister hospital where the current study was conducted too. The protocol for that study was essentially the same as the current one. There were two additional assessments carried out in the recent cohort of 40 patients. Firstly, the Recent Physical Activity Questionnaire (RPAQ) was used in addition to the Stanford questionnaire in the current study and secondly, subjects were asked to perform a 24-hour urine collection prior to the study day to enable estimation of urea generation rate. In addition, the bioimpedance device used for assessment in the current study is different to the one used in the previous cohort. However, the core methodology of

measuring REE and TEE were exactly the same for the two studies. In view of this, results from these two studies are presented as a single cohort in both these chapters.

6.2 Considerations on use of doubly labelled water in kidney disease

6.2.1 General Considerations

The methodology of the doubly labelled water technique is explained in section 3.8.1. A comprehensive discussion about the method and the theory behind its use can be seen in two seminal publications on this topic – a textbook by John Speakman(Speakman, 1997) and a document by the International Atomic Energy Agency which also provides guidance on the methodology and calculation of TEE(Prentice, 1990). This section discusses only the general considerations on the use of doubly labelled water in kidney disease.

The technique involves use of a stable, non-radioactive isotope of oxygen (^{18}O) and hydrogen (^{2}H) to estimate the body water pool and production rate of carbon dioxide from which TEE is calculated. These isotopes are normally present in tap water but at a lower concentration. After ingestion of a measured dose, these isotopes will equilibrate in the body water pool within a few hours which will raise the enrichment level of these molecules in body water. The administered dose needs to enrich the concentration of these isotopes in the body water sufficiently to allow for the study to be conducted over a period of 7-14 days. During the study period, the excretion rates of these molecules are measured from urine samples. ^{2}H (also known as deuterium) leaves the body primarily as water ($^{2}H_2O$) in urine and sweat. ^{18}O, on the other hand, leaves the body as both water ($H_2{}^{18}O$) and carbon dioxide ($C^{18}O_2$). By measuring the difference between the elimination rates of both these isotopes, production rate of carbon dioxide can be calculated which will enable calculation of TEE. If the respiratory quotient of the individual is known, then the oxygen consumption rate and REE can also be calculated.

Doubly labelled water method has not been used in patients with end-stage renal disease previously due to a number of potential problems. Individuals receiving haemodialysis or peritoneal dialysis will have substantial short-term changes in the amount of body water pool which will lead to errors in isotope measurement. In

addition to the above mentioned routes of excretion, the isotopes are also likely to be excreted through dialysis which necessitates collection of dialysate effluent for isotope enrichment measurement and probably a much higher dose for administration, thereby increasing the costs of the study substantially. Moreover, the elimination rates of ^{2}H and ^{18}O may not be the same on dialysis which will introduce an error in the calculation of carbon dioxide production rate. However, the technique can be used reliably in patients with CKD who are not receiving any form of dialysis therapy. For this reason, we chose to conduct the study in patients with different stages of CKD.

6.2.2 Safety of the isotope

The technique is very safe and has been used reliably in many human studies over the last few decades. Both the isotopes are non-radioactive. Potability testing of the sample water was carried out to ensure that the sample was fit for ingestion by study participants. A copy of the test report is shown in Appendix E.

6.2.3 Assumptions in the methodology

As with any physiological study, the doubly labelled water method involves several assumptions(Prentice, 1990). It is assumed that the volume of the body water pool remains constant throughout the measurement period. Both ^{2}H and ^{18}O distribute to some extent in non-water body pools but this has been quantified and an appropriate correction factor is applied in the calculations as shown in section 3.8.1.3.

There is an assumption that the isotopes leave the body only in the form of water and carbon dioxide. However, a small amount of urea is also excreted in faeces which produces an error of around 2% (Montoye, 1997). It is also assumed that the background levels of isotopes remain constant throughout the study period. Whilst theoretically, the background enrichment levels may change if the subject is to consume tap water from a different locality, in practical terms, this is unlikely to be a major problem during the 14-day study period.

It is assumed that the concentration of the isotopes in water and carbon dioxide leaving the body are the same as those in body water at that time i.e. there is no isotopic fractionation. Fractionation can be minimised by storing the samples in air-

tight containers and by freezing. Finally, it is also assumed that the water or carbon dioxide that has left the body does not re-enter the body.

6.2.4 Sampling Methods

As discussed in section 3.8.1, the samples can be collected using a 'two-point' or 'multi-point' approach. The multi-point approach was chosen for this study as this would minimise potential problems because of sampling or analysis errors. Study participants were asked to collect a urine sample every day for a period of 14 days following the dose ingestion. However, only samples from days 1, 8 and 14 were used for analysis.

6.3 Methods

6.3.1 Ethical Review

The study was reviewed and approved by Hatfield NHS Research Ethics Committee (ref: 13/EE/0388 and UH Ethics ref: LMS/PG/NHS/00255). All participants gave written informed consent.

6.3.2 Overview of Study Design

This was a prospective cross-sectional study of 80 CKD patients – 40 each in CKD stages 1-3 and CKD stages 4&5 group. Consenting subjects participated in a 14-day study. Subjects performed a 24-hour urine collection beginning the day prior to the study day. On day 0, a comprehensive metabolic analysis was performed and subjects were given a measured dose of doubly labelled water isotope. Participants were then asked to collect a urine sample each day, starting 24 hours after the isotope ingestion for a period of 14 days for measurement of isotopic enrichment and calculation of TEE. Subjects were asked to complete two physical activity questionnaires at the end of the study period for validation of these questionnaires.

6.3.3 Study Population

All subjects were recruited from the outpatient clinics of Lister Hospital. Potential participants were approached in person when they attended the renal clinic and were given the information sheet about the study. They were given the opportunity to ask

any questions about the study before consenting for the study. The inclusion and exclusion criteria are detailed below.

6.3.3.1 Inclusion Criteria

- Adult CKD patients older than 18 years of age in all stages of CKD

6.3.3.2 Exclusion Criteria

Patients with

- untreated hypo- or hyperthyroidism
- active malignancy
- ongoing sepsis
- active vasculitis or connective tissue disease
- history of hospitalisation in the last month
- current pregnancy
- cardiac pacemakers or defibrillators
- unexplained weight loss
- HIV, Hepatitis B or Hepatitis C positive status
- limb amputation
- no capacity to consent for themselves
- immobility due to any physical ailments

6.3.4 Study Assessments

6.3.4.1 Demographic and Clinical Data

Data comprising of age, sex, ethnicity and comorbid conditions were collected for all study participants through direct measurements and individual medical records. Charlson Comorbidity Index was calculated according to previously published literature (Charlson et al., 1987).

6.3.4.2 Metabolic Analysis and Blood test

All participants performed a 24-hour urine collection beginning the day prior to the study for estimation of urea generation rate. Subjects were asked to fast for at least 3 hours prior to the study on the study day. On Day 0, participants attend the research facility. Accurate height and weight was measured. Bio-impedance analysis was carried out as described in section 3.10.2. Subjects were then asked to rest on a couch and indirect calorimetry was performed to measure REE as described in the General Methodology section 3.6.2. Following REE measurement, 5 ml of blood sample was taken for measurement of renal function and urea generation rate. The blood sample was analysed for measurement of serum urea, creatinine and full blood count.

6.3.4.3 Doubly labelled water administration

The doubly labelled water isotope administration along with calculation of the required doses and subsequent calculation of TEE are explained in detail in the General Methodology section 3.8.1. After ingestion of the measured dose of the sample on Day 0, subjects were asked to collect the first urine sample after 24 hours on Day 1. From then on, they were asked to collect a urine sample each day for a period of 14 days approximately around the same time every day. However, as explained above, only the samples on Day 1, 8 and 14 were used for analysis. If any of these samples were missing, the sample closest to the missing day (±1 day) was used. Samples were collected at the end of the study period and frozen at -20°C until analysis was performed.

6.3.4.4 Physical Activity Questionnaires

At the end of the 14-day period, participants completed two physical activity questionnaires – Recent Physical Activity Questionnaire (RPAQ) and Stanford 7-day recall questionnaire. The questionnaires are described in detail in section 3.7.2.

6.3.5 Estimation of Renal Function and Urea Generation Rate

Renal function was assessed using estimated GFR (eGFR) from the MDRD equation and by creatinine clearance estimation from Cockcroft-Gault (CrCl-CG) equation. The equations are given below.

$$eGFR = 186 \times \left(\frac{creatinine}{88.4}\right)^{-1.154} \times (age)^{-0.208} \times (0.742\ if\ female) \times (1.210\ if\ black)$$

$$CrCl - CG\ (ml/min) = \frac{(140 - age) \times weight\ (kg) \times (1.23\ if\ male, 1.04\ if\ female)}{Serum\ creatinine\ (\mu mol/L)}$$

Categorisation of the renal function into one of the five CKD stages was carried out as discussed in section 1.1.2 (**Error! Reference source not found.**).

Urea generation rate (G) was calculated as follows.

$$G\ (mmol/min) = \frac{Urine\ Volume\ (L) \times Urine\ Urea\ Concentration\ (mmol/L)}{Duration\ of\ Urine\ collection\ (minutes)}$$

6.3.6 Estimation of Total Body Water

Total body water was estimated in two ways – using whole-body bioimpedance (TBW_{BI}) and Watson formula (TBW_{WF}) which is given below.

For males:

$$TBW_{WF} = 2.447 - (0.09516 \times Age) + (0.1074 \times Height) + (0.3362 \times Weight)$$

For females:

$$TBW_{WF} = -2.097 + (0.1069 \times Height) + (0.2466 \times Weight)$$

where TBW_{WF} is expressed in Litres, age in years, height in cm and weight in kg

Total body water measurement from the doubly labelled water (TBW_{DLW}) was calculated from equation 11 explained in section 3.8.1.3.

6.3.7 Estimation of Physical Activity Level from Questionnaires

In RPAQ, participants were enquired about performance of various activities and the time spent doing each of them over the preceding 4 weeks. Each activity is then assigned a MET value as per the Compendium of Physical Activities (Ainsworth et al., 2011; Ainsworth et al., 2000; Ainsworth et al., 1993). Assuming the sleep time to be 6-8 hours per day and the unreported time to be spent in low intensity activity with a MET value of 1.3, the total daily MET was calculated by summation of the MET values for all the activities. Activity related MET was then calculated as shown below.

$$\boldsymbol{Activity\ related\ MET = Total\ MET - \left((24 - Sleep\ duration) \times 1\right)}$$

Stanford 7-day recall questionnaire enquires about time spent on different intensities of activities over the preceding 7 days including average sleep duration per day. Total MET and activity related MET from Stanford questionnaire was calculated in a similar manner to RPAQ as explained above.

6.3.8 Statistical Analysis

Normally distributed data are presented as mean ± standard deviation and non-normally distributed data as median (inter-quartile range). Differences in TEE between various subgroups were compared using unpaired t-test or one-way ANOVA as appropriate. Correlations between variables were analysed using Pearson correlation coefficient for normally distributed data and Spearman's for non-normally distributed data. Multiple regression models were constructed to examine the relationship between TEE and REE with renal function. The variables included in the model were age, weight, gender and eGFR. Collinearity testing was carried out for all the variables included in the regression model and variables with only low variance inflation factor (<10) were included. A p-value of <0.05 was assumed to indicate statistical significance.

Total Body Water estimations from Watson formula (TBW_{WF}) and whole-body bioimpedance (TBW_{BI}) were compared with that measured using doubly labelled water (TBW_{DLW}) using Bland-Altman technique (Bland & Altman, 1986).

6.4 Results

6.4.1 Population Demographics

The demographics and biochemical characteristics of study participants are shown in Table 6-1.

Table 6-1: Demographic and Biochemical characteristics of Study Participants

Significance in differences tested by: *unpaired t-test **Fisher's Exact test; CCI: Charlson Comorbidity Index

	All Patients	CKD Stages 1-3	CKD Stages 4-5	p-value
Number of patients	80	40	40	
Age (years)	56.7 ± 16.2	52.7 ± 17.0	60.7 ± 14.6	0.028*
Male Gender	48 (60%)	21 (52.5%)	27 (67.5%)	0.254**
Weight (kg)	77.0 ± 13.0	77.0 ± 13.8	77.0 ± 12.3	0.990*
Height (cm)	169.0 ± 9.1	168.2 ± 8.0	169.8 ± 10.1	0.435*
CCI	4.0 ± 2.5	2.9 ± 2.5	4.6 ± 2.1	0.001*
eGFR (ml/min)	41.6 ± 32.1	66.6 ± 27.8	16.7 ± 6.1	< 0.001*
CrCl-CG (ml/min)	50.7 ± 41.2	79.7 ± 40.3	21.7 ± 9.2	< 0.001*
Haemoglobin (g/dL)	12.1 ± 1.7	12.8 ± 1.5	11.3 ± 1.5	< 0.001*

There were 40 patients in each CKD group (stages 1-3 and stages 4-5). Subjects in the advanced CKD group were older and had higher comorbidity than those in the group with lesser degrees of CKD. There were no significant differences in the body size parameters such as weight and height between the groups. As would be expected, subjects in the advanced CKD group had significantly lower level of renal function ($p < 0.001$) and lower haemoglobin level ($p < 0.001$).

6.4.2 Half-life of Isotopes and Isotope Enrichment

The mean baseline enrichment of ^{2}H and ^{18}O in the urine before administration of the isotopes was 151 (± 1.08) ppm and 1994 (± 1.82) ppm respectively. Following the ingestion of isotopes, the enrichment in the urine sample collected approximately 24 hours later was 289 (± 19.87) ppm for ^{2}H and 2242 (± 45.75) for ^{18}O. Figure 6-1 shows the relationship between half-life of ^{2}H and that of ^{18}O. As would be expected according to the principles of these isotope behaviours, the half-life of ^{18}O was shorter with a mean of 6.6 days compared to the half-life of ^{2}H with a mean of 8.5 days.

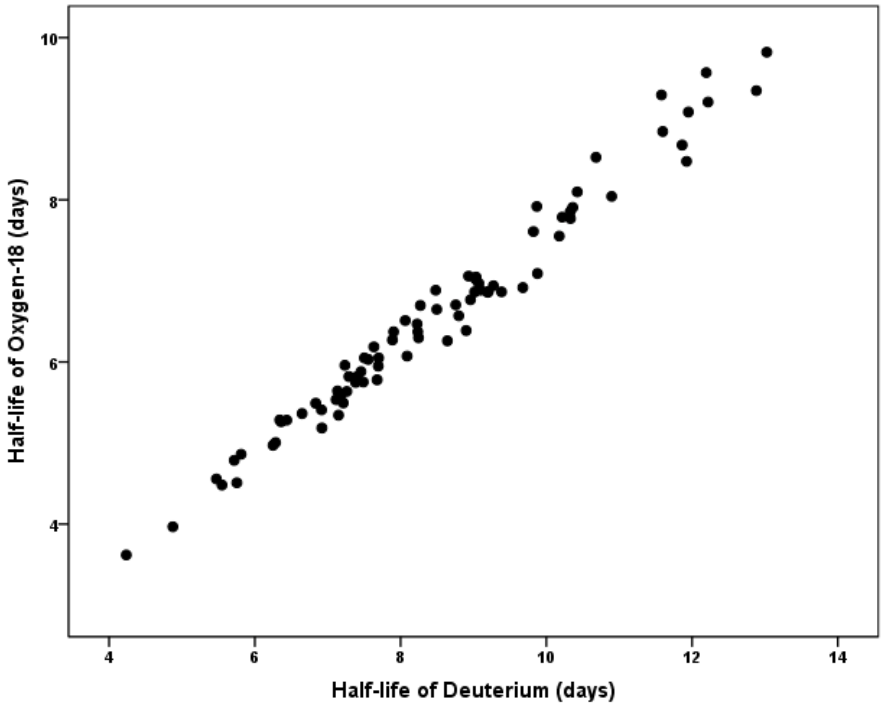


Figure 6-1: Half-lives of ^{2}H and ^{18}O

6.4.3 Total Energy Expenditure Calculation

Urine samples from day 1, 8 and 14 were analysed for isotopic enrichment of ^{2}H and ^{18}O. Fractional enrichment of both the isotopes, calculated from equation 7 (section 3.8.1.3), were plotted as a function of time. The exponential curve of each isotope was linearised using logarithmic transformation of the fractional enrichment. The

intercept and slope of the line from this plot was used to calculate the pool size and carbon dioxide production rate as explained in section 3.8.1.3 (equations 8 – 15). The corrected pool size and carbon dioxide production rate was then used to calculate TEE.

For all patients, there was a clear separation of isotope fractional enrichment levels of ^{2}H and ^{18}O by the end of the study observation period implying that the study duration was sufficiently long to ensure accurate calculation of carbon dioxide production rate.

Figure 6-2 and Figure 6-3 shows the exponential and logarithmic plot of both isotopes from two patients. There was a close linear relationship between time and the logarithm of fractional enrichment. Pearson correlation coefficient between these two variables for both ^{2}H and ^{18}O were high. For ^{2}H, the mean coefficient r was -0.99 (± 0.0007) and for ^{18}O, it was -0.99 (± 0.0006).

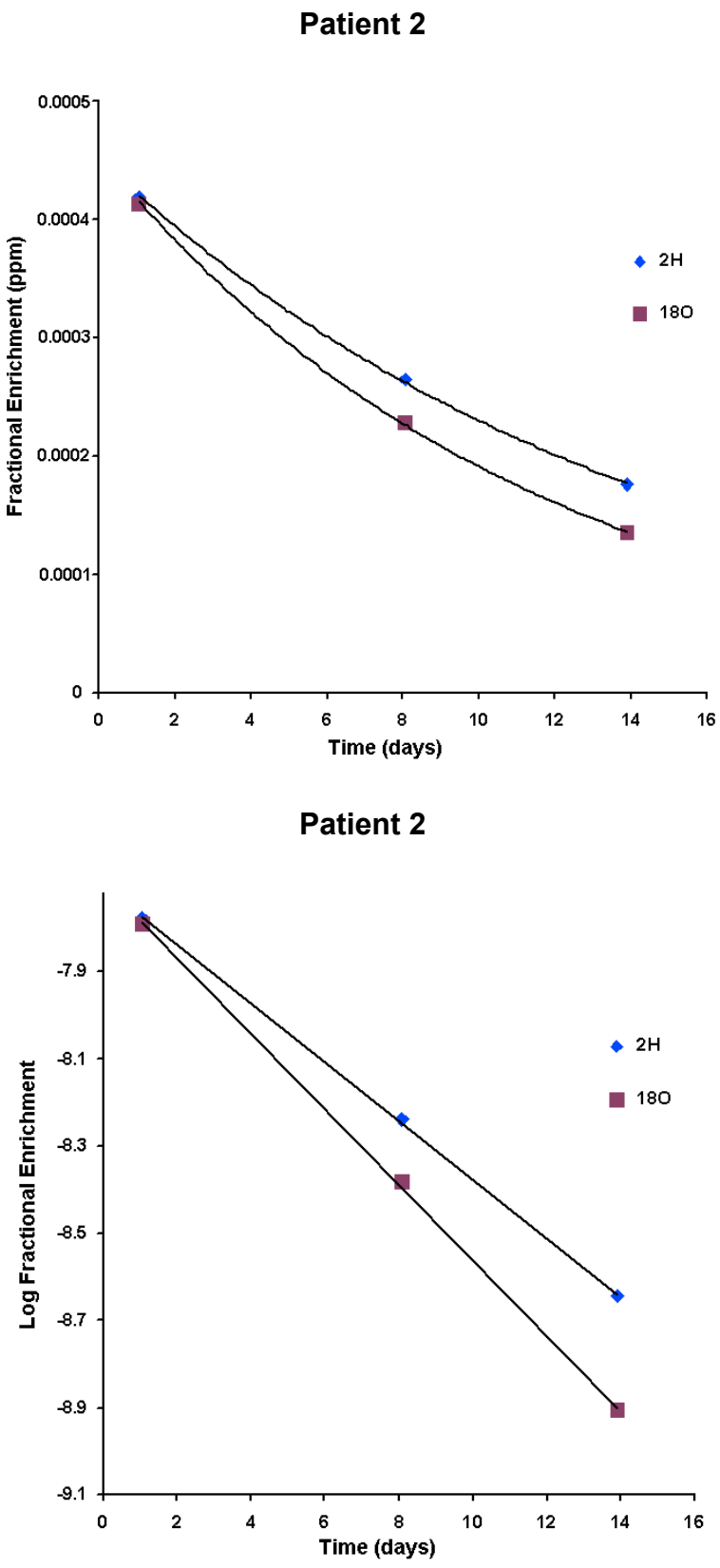


Figure 6-2: Exponential (top panel) and logarithmic (bottom panel) plots of isotope fractional enrichment over time.

The intercept and slope from the logarithmic plot was used for TEE calculation.

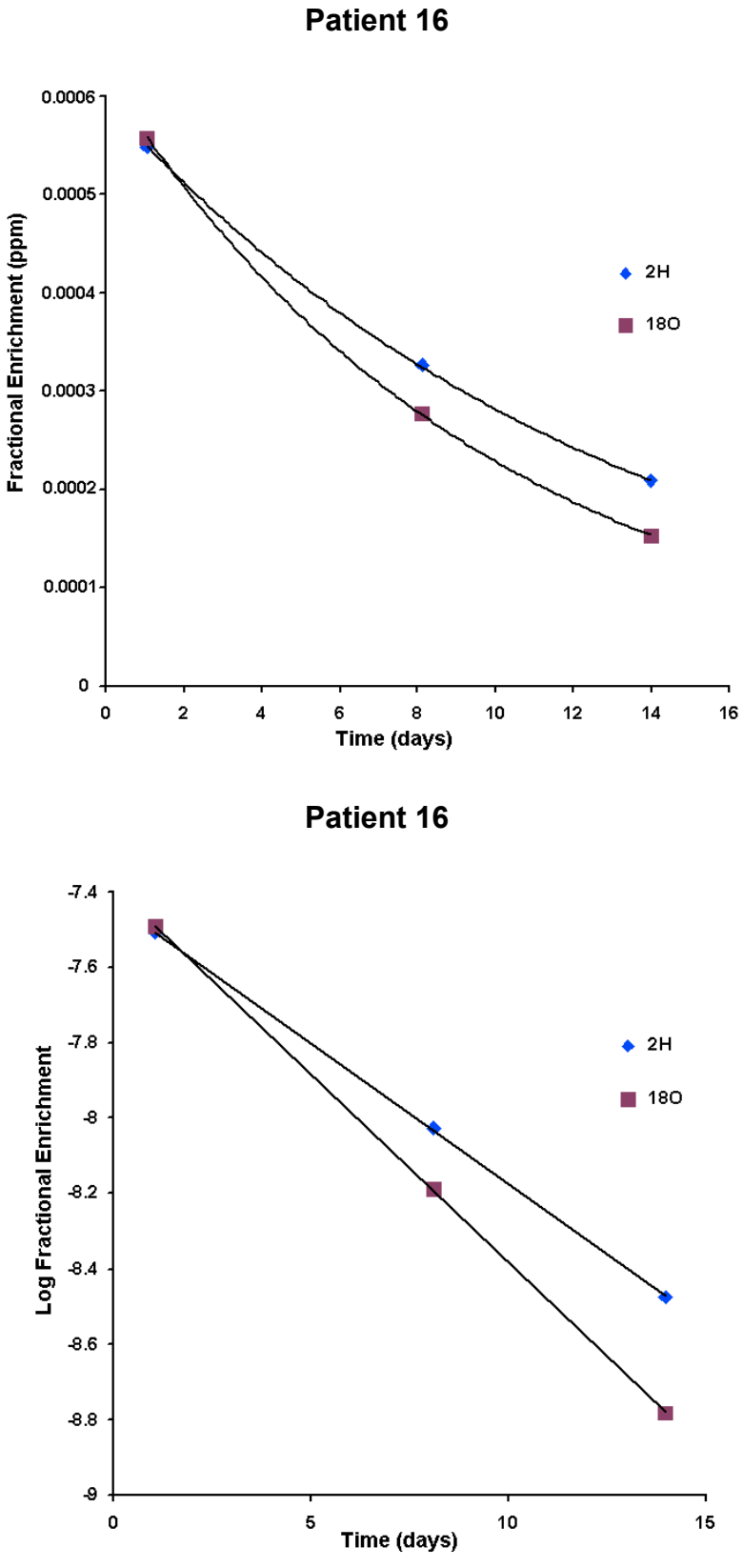


Figure 6-3: Exponential (top panel) and logarithmic (bottom panel) plots of isotope fractional enrichment over time.

The intercept and slope from the logarithmic plot was used for TEE calculation.

6.4.4 Energy Expenditure and Renal Function

Mean measured REE (from indirect calorimetry) and TEE (from doubly labelled water) for the whole cohort and for CKD groups are shown in Table 6-2. Both REE and TEE did not differ significantly between the CKD groups.

Table 6-2: Energy Expenditure of Study Participants

	All patients (n = 80)	CKD Stages 1-3 (n=40)	CKD Stages 4-5 (n=40)	p-value
REE (kcal/day)	1560 ± 301	1580 ± 305	1540 ± 300	0.555
TEE (kcal/day)	2481 ± 476	2501 ± 454	2460 ± 502	0.708
REE/kg (kcal/kg/day)	20.4 ± 3.1	20.7 ± 3.1	20.1 ± 3.1	0.379
TEE/kg (kcal/kg/day)	32.7 ± 6.3	33.0 ± 5.6	32.5 ± 7.0	0.724

The plots of eGFR against REE and REE normalised to body weight are shown in Figure 6-4 and Figure 6-5. It can be seen that there is poor relationship between the estimated GFR and either of the variables.

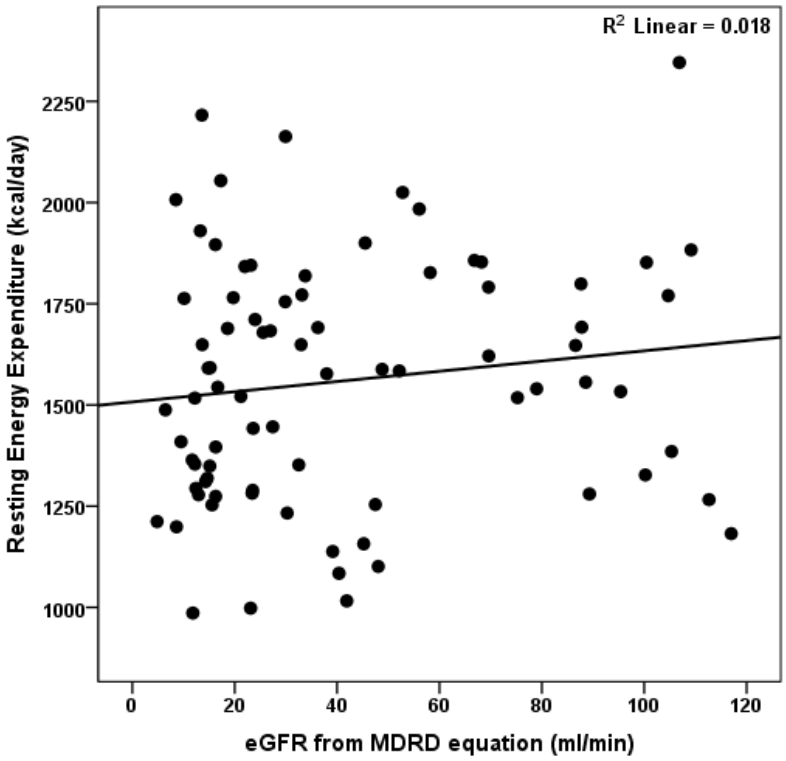


Figure 6-4: Relationship between eGFR and REE

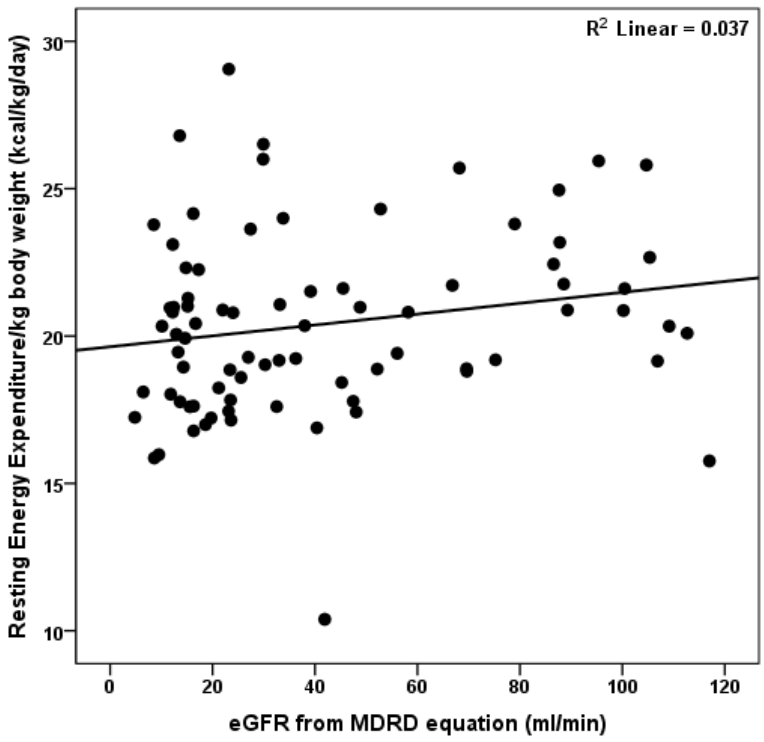


Figure 6-5: Relationship between eGFR and REE/kg body weight

The plots of eGFR against TEE and TEE/kg are shown in Figure 6-6 and Figure 6-7. As with REE, there is a poor relationship between eGFR and either of these variables.

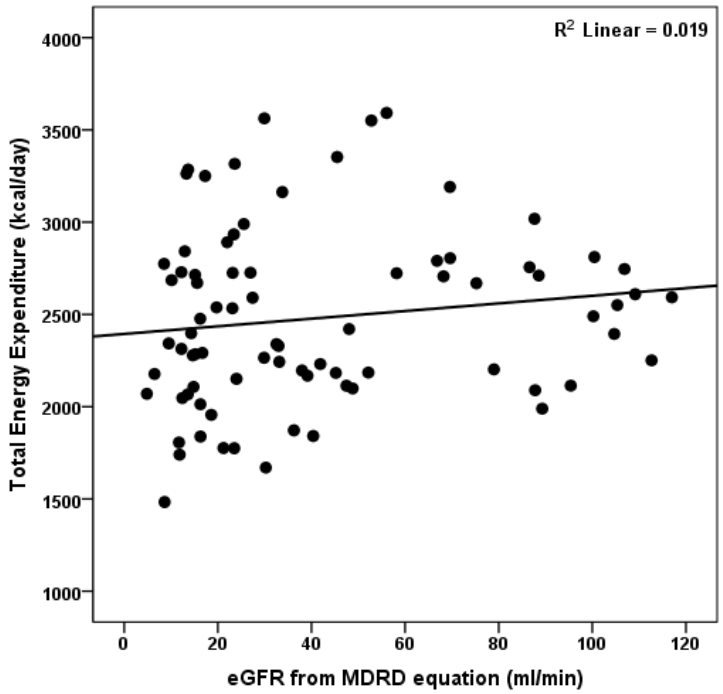


Figure 6-6: Relationship between eGFR and TEE

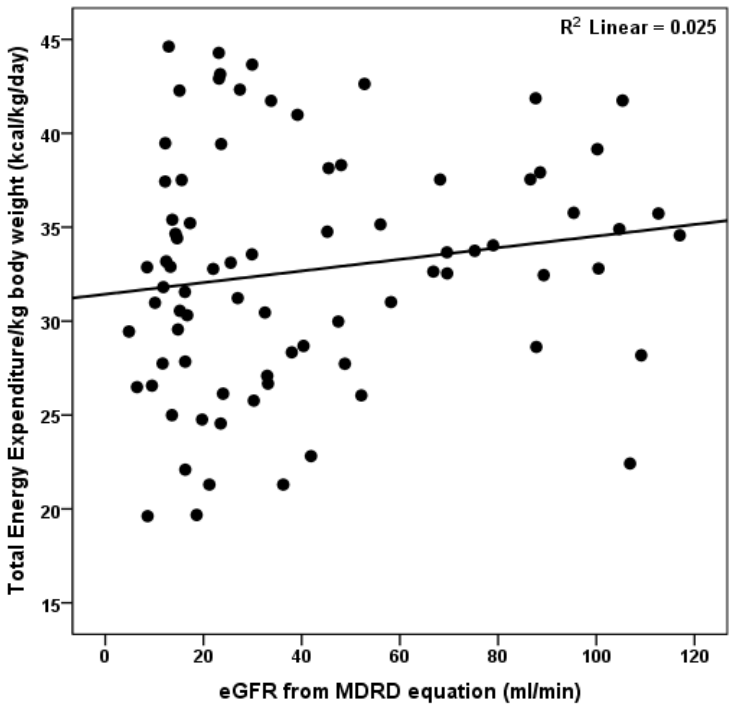


Figure 6-7: Relationship between eGFR and TEE/kg body weight

Multivariate linear regression models with REE and REE normalised to body weight as dependent variable are shown from Table 6-3 and Table 6-6. The variables considered were age, sex, weight, height, fat-free mass (from bioimpedance measurement) and renal function expressed as eGFR from MDRD equation or Creatinine clearance from Cockcroft-Gault equation. Sex and weight were found to be significant predictors in all the models. In the models including eGFR, age was also found to be a significant predictor. Renal function was not significant in any of the models irrespective of the method of estimation of renal function (eGFR or CrCl-CG).

Table 6-3: Linear regression model of determinants of REE with eGFR

Adjusted R^2 = 0.505

	Standardised coefficient (Beta)	t	p-value
Constant		3.211	0.002
Age	-0.190	-2.040	0.045
Sex	0.291	3.446	0.001
Weight	0.591	7.115	< 0.001
eGFR	0.100	1.073	0.287

Table 6-4: Linear regression model of determinants of REE normalised to body weight with eGFR

Adjusted R^2 = 0.221

	Standardised coefficient (Beta)	t	p-value
Constant		12.088	< 0.001
Age	-0.246	-2.101	0.039
Sex	0.370	3.488	0.001
Weight	-0.327	-3.134	0.002
eGFR	0.145	1.237	0.220

Table 6-5: Linear regression model of determinants of REE with CrCl-CG

CrCl-CG: Creatinine Clearance using Cockcroft-Gault equation; Adjusted R^2 = 0.505

	Standardised coefficient (Beta)	t	p-value
Constant		3.557	0.002
Age	-0.173	-1.710	0.091
Sex	0.296	3.474	0.001
Weight	0.563	6.271	< 0.001
CrCl-CG	0.115	1.097	0.276

Table 6-6: Linear regression model of determinants of REE normalised to body weight with CrCl-CG

CrCl-CG: Creatinine Clearance using Cockcroft-Gault equation; Adjusted R^2 = 0.220

	Standardised coefficient (Beta)	t	p-value
Constant		12.966	< 0.001
Age	-0.225	-1.775	0.080
Sex	0.375	3.510	0.001
Weight	-0.365	-3.240	0.002
CrCl-CG	0.159	1.206	0.232

Similar models were generated with TEE and TEE normalised to body weight as dependent variables which are shown from Table 6-7 and Table 6-10. Age, weight and height were found to be significant predictors in all these models. As with REE, renal function was not a significant predictor in any of the models.

Table 6-7: Linear regression model of determinants of TEE with eGFR

Adjusted R^2 = 0.392

	Standardised coefficient (Beta)	t	p-value
Constant		-0.419	0.676
Age	-0.327	-3.100	0.003
Sex	0.217	1.924	0.058
Height	0.276	2.467	0.016
Weight	0.299	3.193	0.002
eGFR	0.054	0.519	0.605

Table 6-8: Linear regression model of determinants of TEE normalised to body weight with eGFR

Adjusted R^2 = 0.443

	Standardised coefficient (Beta)	t	p-value
Constant		2.336	0.022
Age	-0.305	-3.020	0.003
Sex	0.248	2.297	0.024
Height	0.238	2.226	0.029
Weight	-0.555	-6.191	< 0.001
eGFR	0.083	0.833	0.408

Table 6-9: Linear regression model of determinants of TEE with CrCl-CG

CrCl-CG: Creatinine Clearance using Cockcroft-Gault equation; Adjusted R^2 = 0.392

	Standardised coefficient (Beta)	t	p-value
Constant		-0.415	0.679
Age	-0.319	-2.770	0.007
Sex	0.217	1.926	0.058
Height	0.279	2.473	0.016
Weight	0.284	2.790	0.007
CrCl-CG	0.058	0.494	0.623

Table 6-10: Linear regression model of determinants of TEE normalised to body weight with CrCl-CG

CrCl-CG: Creatinine Clearance using Cockcroft-Gault equation; Adjusted R^2 = 0.444

	Standardised coefficient (Beta)	t	p-value
Constant		2.305	0.024
Age	-0.283	-2.568	0.012
Sex	0.250	2.318	0.023
Height	0.245	2.277	0.026
Weight	-0.583	-5.984	< 0.001
CrCl-CG	0.107	0.949	0.346

6.4.5 Physical Activity-related Energy Expenditure and Renal Function

Table 6-11 shows the physical activity-related energy expenditure (PAEE) derived from doubly labelled water method and mean daily MET value estimated from the two activity questionnaires. Irrespective of the method of activity assessment, there was no difference noted between the two CKD groups.

Table 6-11: Physical Activity level of Study Participants

DLW: Doubly labelled water method; RPAQ: Recent Physical Activity Questionnaire; *Median (inter-quartile range); † Mann-Whitney U test

	All patients	CKD Stages 1-3	CKD Stages 4-5	p-value
DLW method (kcal/day)	672 ± 367	671 ± 325	674 ± 410	0.963
Activity MET – RPAQ*	9.02 (8.5)	8.51 (7.3)	10.74 (9.3)	0.569†
Activity MET – Stanford*	18.59 (4.6)	18.50 (5.2)	18.63 (4.4)	0.552†

6.4.6 Comparison of body water measurement between Watson volume and DLW

The total body water (TBW) as estimated by Watson formula (TBW_{WF}) and that measured from doubly labelled water (TBW_{DLW}) correlated significantly with a Pearson coefficient $r = 0.831$ ($p < 0.001$). The relationship between the two measurements is shown in Figure 6-8.

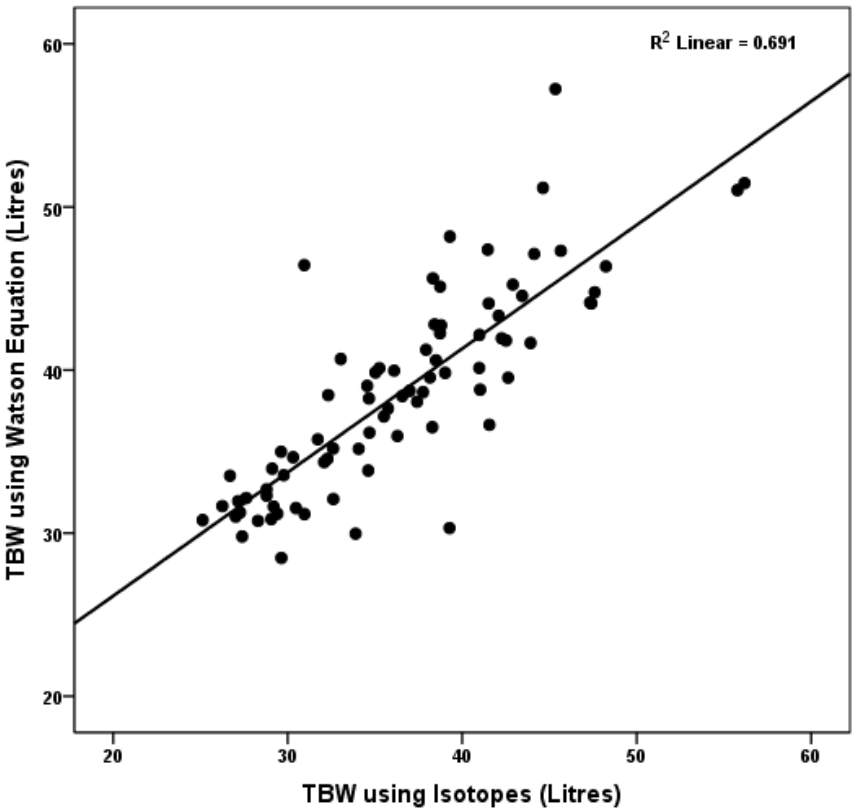


Figure 6-8: Relationship between TBW_{DLW} and TBW_{WF}

A Bland-Altman plot comparing the two measurements is shown in Figure 6-9. The Watson formula overestimated the TBW with a mean bias of -2.17 Litres. The lower and upper 95% confidence interval limits for the bias were -9.6 and 5.27 Litres respectively.

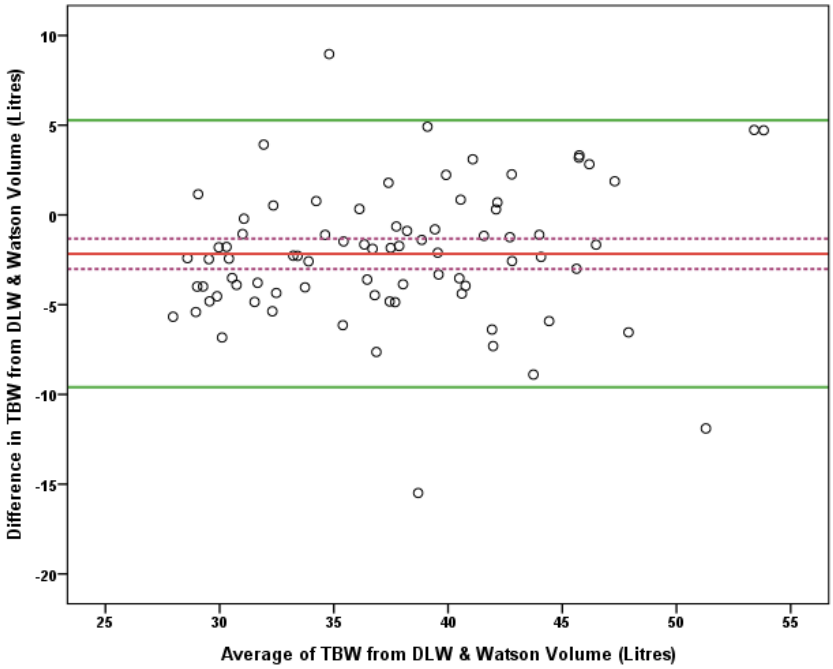


Figure 6-9: Bland-Altman Plot of difference between TBW_{DLW} and TBW_{WF}

Difference between total body water measured by DLW and Watson formula plotted against the mean of the two measurements. A negative sign indicates an overestimation and a positive sign indicates an underestimation by Watson formula.

6.4.7 Comparison of body water measurement between Bioimpedance analysis and DLW

The bioimpedance analysis for the 40 patients from the recent cohort was used for this analysis. TBW measured through bioimpedance (TBW_{BI}) and TBW_{DLW} correlated well with a Pearson coefficient (r) of 0.922 ($p < 0.001$). The relationship between the two measurements is shown in Figure 6-10.

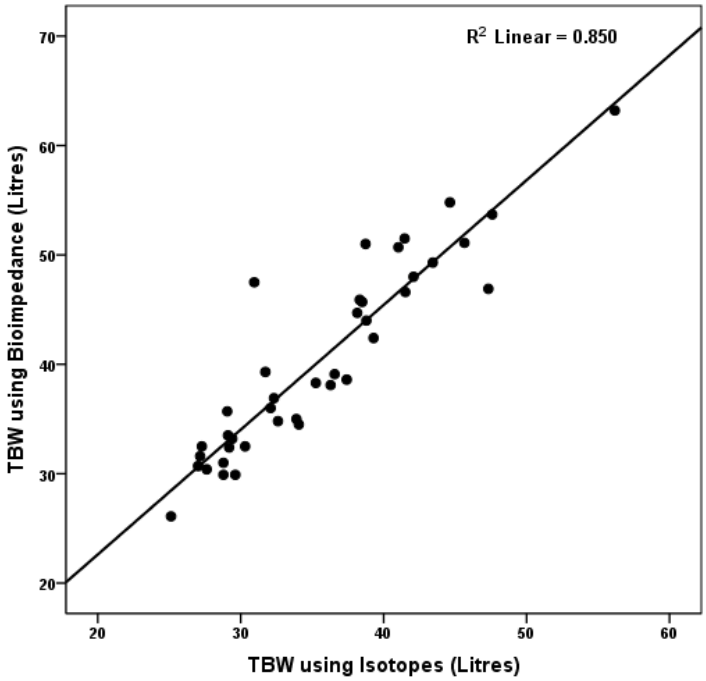


Figure 6-10: Relationship between TBW_{DLW} and TBW_{BI}

A Bland-Altman plot was constructed to compare the TBW measurements from the two methods which is shown in Figure 6-11. Bioimpedance analysis overestimated TBW with a mean bias of -4.8 Litres. The lower and upper limits of agreement were -11.7 and 2.1 Litres respectively.

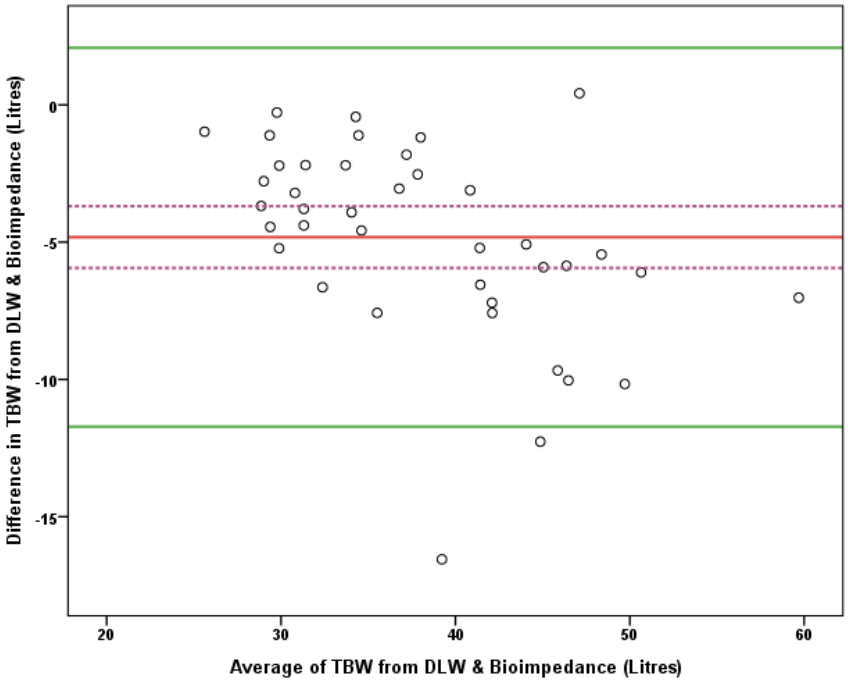


Figure 6-11: Bland-Altman Plot of difference between TBW_{DLW} and TBW_{BI}

Difference between total body water measured by DLW and Bioimpedance plotted against the mean of the two measurements. A negative sign indicates an overestimation and a positive sign indicates an underestimation by Bioimpedance.

6.4.8 Relationship between TEE and Urea Generation rate

Urea generation rate (G) was calculated for 37 patients in the recent cohort (3 patients did not provide 24-hour urine collection sample). There was significant correlation between G and TEE with a Pearson coefficient (r) of 0.646 ($p<0.001$). The relationship between TEE and G are shown in Figure 6-12. There was a linear relationship between TEE and G with TEE alone explaining 42% of variance in G.

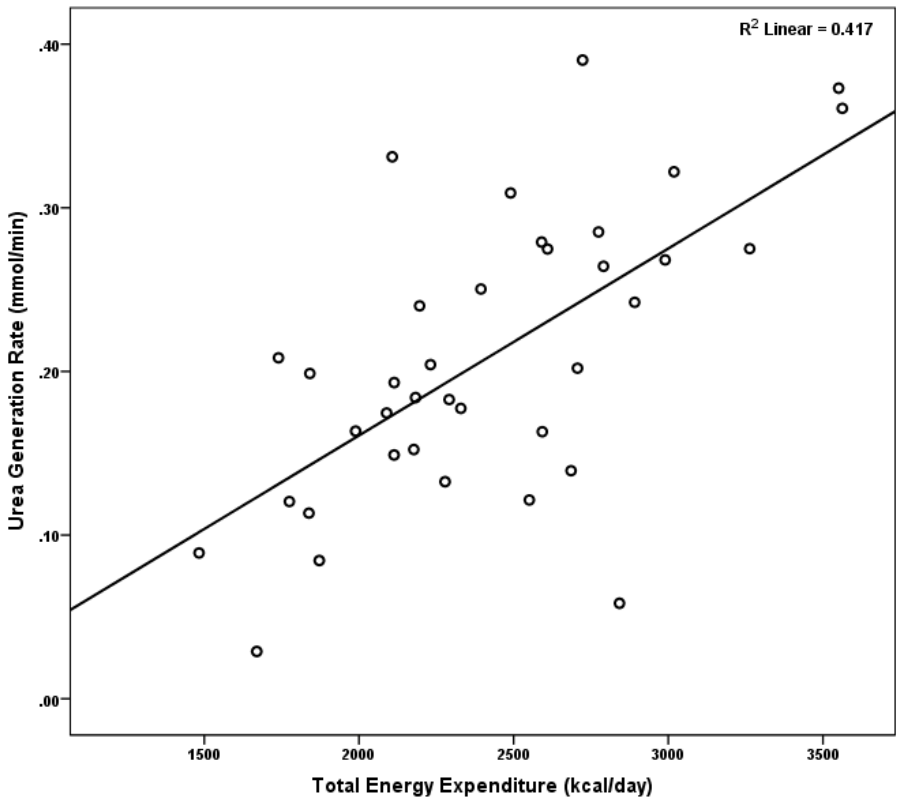


Figure 6-12: Relationship between TEE and Urea Generation Rate

6.5 Discussion

Due to the paucity of studies exploring total energy requirements in CKD, little is known about TEE in renal disease. One of the primary aims of this study was to explore the impact of varying kidney function on metabolic rate and total energy expenditure. The study data shows that there is no significant impact of worsening renal function on basal metabolic rate or TEE. Both Watson formula and Bioimpedance analysis perform reasonably well in measuring total body water when compared against that from doubly labelled water. There is also a strong relationship between TEE and urea generation rate implying that TEE may be closely related to uraemic toxin generation. The current study data is in favour of rejecting the null hypothesis and accepting the experimental hypothesis.

The difficulties associated with measuring TEE using DLW method in dialysis patients are discussed in an earlier section of this chapter. Due to this, the criteria for study inclusion focused on patients with varying level of kidney function but not yet receiving dialysis. Total energy requirements were compared between subjects with

mild-moderate (CKD stages 1-3) and advanced renal disease (CKD stages 4-5). Study participants between the groups were well matched with regards to gender proportion and body size parameters. The mean age of the advanced CKD group was higher but this would be expected as renal failure is predominantly a disease of the elderly. Similarly, the mean haemoglobin was slightly but significantly lower in this group which, again, would be expected with progression of renal disease.

The isotopes (^{2}H and ^{18}O) performed well and in a predictable manner in the study group. The mean half-life of ^{18}O (6.6 days) was lower than that of ^{2}H (8.5 days). This is because ^{18}O is excreted as both carbon dioxide and water whereas ^{2}H is excreted predominantly as water only. The log-transformed fractional enrichment of both the isotopes correlated strongly in all patients. This suggests that the limited multi-point technique used in the study is valid for the purpose of TEE measurement.

The effect of varying level of renal function on REE and TEE was examined by comparing direct measurements of REE and TEE between the mild-moderate and advanced CKD groups. In order to permit comparison of energy expenditure measures across individuals of different body sizes, both REE and TEE were also examined after normalising them to body weight. There was no significant difference in TEE, TEE/kg, REE or REE/kg between these groups when compared using unpaired t-test. In addition, in multivariate linear regression analysis which takes into account the potential confounding variables such as age and body size measures, renal function was not a predictor of REE or TEE. Renal function explained between 1-3% of variance with any of these parameters. This implies that GFR does not influence basal metabolic rate and renders support to the hypothesis proposed by Singer (Singer, 2001) that metabolic rate is the driving process for renal blood flow whilst the converse is not true.

Previous studies examining the effect of renal function on REE have shown conflicting results. In a small study of 16 CKD patients, Panesar et al (2003) found that lower levels of GFR were associated with reduced REE (Panesar & Agarwal, 2003). Monteon et al (1986) showed in 10 non-dialysed patients with CKD and 16 haemodialysis patients that there was no difference in REE across the groups (Monteon et al., 1986). On the other hand, Kuhlmann (2001) measured REE sequentially in 22 patients as their renal function worsened and found that REE per

body cell mass increased with decreasing renal function (Kuhlmann et al., 2001). The authors suggested that this could be due to relative increase in lean body mass associated with weight reduction in chronic renal disease. The current study results show that there was no significant difference in REE with reducing renal function in 80 patients.

The level of renal function can be expressed using many ways. Two different measures of renal function – eGFR by MDRD equation and Creatinine clearance by Cockcroft-Gault equation – were used in this study to examine the relationship between renal function and energy expenditure. The most commonly used method at present in clinical practice is as estimated GFR (eGFR) by MDRD equation (Levey et al., 1999). This equation has been shown to underestimate GFR in those with higher levels of kidney function (GFR > 60 ml/min) (Poggio et al., 2005; Lin, Knight, Hogan, & Singh, 2003). Creatinine clearance estimated from Cockcroft-Gault equation performs better as a measure of renal function at higher levels of renal function compared to MDRD equation (Lin et al., 2003). For this reason, both these measures were used in this study. However, irrespective of the renal function measure, there was no significant impact of renal function noted on REE and this was also the case even after normalising REE to body weight.

The impact of declining renal function on TEE was also explored in this study. As with REE, there was no significant impact of renal function on TEE irrespective of the renal function measure that was used or whether TEE was normalised to body weight. TEE encompasses both REE and physical activity related energy expenditure. As there has been no change in REE across CKD groups, the study data suggests there has been no significant change in physical activity level too with declining renal function. This is supported by the lack of significant difference between CKD groups in physical activity measures measured by DLW method and that estimated from two activity questionnaires. Studies assessing physical activity through direct measurements in patients with CKD are scarce. Two studies carried out in CKD patients with an eGFR > 30 ml/min where physical activity was measured using accelerometers showed low levels of physical activity in this patient group (Robinson-Cohen et al., 2013; Hawkins et al., 2011). However, another study by Wlodarek et al in 24 pre-dialysis CKD patients with a mean eGFR of 18 ml/min showed physical activity levels comparable to general population (Wlodarek,

Glabska, & Rojek-Trebicka, 2011). It may be expected that the physical activity level may decline with worsening renal function but there is no evidence published so far showing reduced total energy requirements with declining renal function.

The study data supports the use of Watson formula for estimation of total body water in comparison to that obtained from bioimpedance analysis. Although the strength of association between TBW_{DLW} and TBW_{BI} was better than that between TBW_{DLW} and TBW_{WF}, Bland-Altman plot between TBW_{DLW} and these two measures showed the Watson formula to have less bias compared to the bioimpedance analysis. Both the measurements overestimated TBW compared to the DLW method. There is no published literature on the accuracy of Watson formula for estimation of TBW in patients with CKD using the DLW method. This data strengthens the current clinical practice of using Watson formula for estimation of total body water in the prescription and monitoring of dialysis dose.

Urea is categorised as a small molecule uraemic toxin and the extent of urea clearance is measured to define adequate dialysis dose. Urea generation rate can be considered a marker of small molecule uraemic toxin production. As shown in chapter 4, estimated TEE from physical activity questionnaires was a significant predictor of urea generation rate. This study is a direct measurement of TEE rather than an estimated one. The study data shows a strong relationship between measured TEE and Urea generation rate with TEE contributing to about 42% of variance in urea generation rate. This lends support to the notion that TEE and thereby, physical activity may play a significant role in uraemic toxin production and may impact on the minimum dialysis dose requirement.

6.6 Limitations

The limitations of the study are mostly related to the assumptions of the doubly labelled water technique as explained in an earlier section of this chapter. However, the methodology applied here is the standard technique and hence, results of any future studies can be compared to this study. This study is also limited by its sample size. Of course, a larger sample size may increase the power of the study but this is largely limited by the time-consuming nature of the study and the costs of the isotope and their analysis. Nevertheless, this study of 80 patients is the only study so far employing doubly labelled water to measure TEE in patients with renal disease.

6.7 Conclusion

In summary, the study data have shown that declining renal function does not have a direct impact on basal and total metabolic rate. Any differences found between TEE levels in subjects with declining renal function are likely to be explained by differences in physical activity energy expenditure – though these were not apparent in this study sample.

The study has also shown that the Watson formula provides a more accurate measure of total body water compared to that obtained from bioimpedance analysis. The study data also confirmed that TEE might exert a significant impact on uraemic toxin generation and hence influence the minimum dialysis dose requirements in patients with advanced renal disease.

Chapter 7

7. Validity of Physical Activity Questionnaires in Chronic Kidney Disease

7.1 Introduction

Individuals with chronic kidney disease (CKD) often have poor appetite, especially in advanced stages, which could potentially contribute to low energy intake and protein-energy wasting (Carrero et al., 2007; Kalantar-Zadeh, Block, McAllister, Humphreys, & Kopple, 2004; Burrowes et al., 2003). Assessing total energy requirements of individuals is vital for appropriate nutritional management of these patients. In routine clinical practice, total energy requirements are estimated by a combined measure of estimated basal metabolic rate (i.e. resting energy expenditure) and estimated physical activity level. There are predictive equations for estimation of resting energy expenditure (REE) such as Harris-Benedict, Mifflin-St Jeor and Schofield equations which are commonly used in clinical practice. Recently, a novel disease-specific predictive equation for REE has been published for use specifically in patients with renal disease (Vilar et al., 2014). This equation was derived and validated in a cohort of patients with renal disease and has been shown to be at least as accurate as the existing ones but with less bias compared to other equations.

The estimation of physical activity is usually carried out by means of self-report physical activity questionnaire although prospectively completed activity diaries are an alternate option. There are many physical questionnaires that are available for use in clinical practice. Most of these questionnaires are derived from young healthy adults and as such, may not be applicable to specific groups of patients. CKD is predominantly a disease of the elderly and these activity questionnaires may not be valid in this patient population. A study by Bonnefoy et al (2001) examined the validity of ten physical activity questionnaires in elderly individuals in general population against doubly labelled water and found that only a few questionnaires were reliable for use in elderly (Bonnefoy et al., 2001). Moreover, the individual variability was high for all the questionnaires which limit their use in these individuals.

None of the physical activity questionnaires used in studies involving patients with kidney disease have been validated against doubly labelled water (DLW) method for estimation of physical activity-related energy expenditure (PAEE). Some of the available questionnaires have been tested against energy expenditure obtained from accelerometers. However, this is not ideal as accelerometer measurements themselves may also have a degree of associated measurement error. This error in itself has not been quantified using DLW method in patients with kidney disease. Whilst accelerometers serve as the next best tool to DLW method, it is important to validate physical activity questionnaires against DLW method in individuals with kidney disease.

Recent Physical Activity Questionnaire (shown in Appendix B) enquires about the performance of various activities and the time spent on each of the reported activity over the preceding 4 weeks. The questionnaire is divided into three sections – activities at home and work and recreational activities. The questionnaire has been validated in healthy individuals against DLW method for categorising physical activity levels and estimation of PAEE (Besson et al., 2010). The calculations employed in the analysis of questionnaire data are discussed in the general methodology chapter (section 3.7.2.1). Two assumptions are made in estimating PAEE. Firstly, duration of sleep is assumed to be 6-8 hours depending on the total duration of the activity. Secondly, for the time not accounted for from the questionnaire, a MET (Metabolic Equivalent of Task) value of 1.3 is assumed if the individual's common mode of travel is by walking or cycling and a value of 1 if any other mode of transport is specified. However, this may not be an appropriate method to calculate PAEE.

Any activity other than sleep, even the energy cost of being just awake, is likely to have a MET value of more than 1. It is reasonable to assume that, in this unaccounted time, individuals are performing some activities at home which are not being specifically enquired by the questionnaire. Assigning an average MET value for this time is essential to have a better approximation of PAEE from the questionnaire. Hence, it is vital to explore the appropriate MET value that has the closest approximation to the measured PAEE and this can be carried out by employing the DLW method to measure PAEE directly.

Stanford 7-day recall questionnaire (shown in Appendix C) was developed for the Stanford Five City Project (Sallis et al., 1985). This questionnaire enquires about time spent on moderate, hard and very hard intensity activities and also sleep, over the preceding seven days. Any time not accounted for is considered to be spent performing light activities.

Availability of a physical activity questionnaire that is validated specifically in patients with kidney disease will be beneficial in many ways. Firstly, it will be useful to estimate total energy requirements of the individuals which is the cornerstone of nutritional management in patients with kidney disease. Secondly, in haemodialysis patients, the estimated physical activity level from the questionnaire could potentially be used for adjusting dialysis dose according to metabolic needs of the individual patient. Finally, the activity data could be used to monitor the physical activity levels at regular intervals to encourage an active lifestyle for patients with kidney disease. Moreover, availability of a validated questionnaire may stimulate its consistent use in future research studies leading to comparable results across various studies.

In view of the above potential benefits, this study was designed with the following objectives:

1. To examine the validity of two physical activity questionnaires – Recent Physical Activity Questionnaire (RPAQ) and Stanford 7-day recall questionnaire – for estimation of TEE in subjects with kidney disease
2. To identify appropriate Metabolic Equivalent of Task (MET) value for unaccounted time in RPAQ that, when applied, will better reflect the measured TEE

As explained in the previous chapter, the DLW data presented here is from 80 patients - 40 patients from the current study and 40 patients from another similar study conducted as part of Dr Vilar's PhD (Dr Enric Vilar, PhD, University of Hertfordshire – degree awarded Feb 2012). However, the RPAQ was not used in the earlier study of Dr Vilar and hence, the validation cohort for RPAQ included only the 40 patients from the current study whereas all 80 patients were included for examining the validity of Stanford questionnaire.

7.2 Methods

7.2.1 Ethical Review

The study was reviewed and approved by Hatfield NHS Research Ethics Committee (ref: 13/EE/0388 and UH Ethics ref: LMS/PG/NHS/00255). All participants provided written informed consent.

7.2.2 Overview of Study Design

This was a prospective cross-sectional study of 80 CKD patients – 40 each in CKD stages 1-3 and CKD stages 4&5 group. Consenting subjects participated in a 14-day study. Subjects performed a 24-hour urine collection beginning the day prior to the study day. On day 0, a comprehensive metabolic analysis was performed and subjects were given a measured dose of doubly labelled water isotope. Participants were then asked to collect a urine sample each day, starting 24 hours after the isotope ingestion for a period of 14 days for measurement of isotopic enrichment and calculation of TEE. Subjects were asked to complete two physical activity questionnaires at the end of the study period for validation of these questionnaires.

7.2.3 Study Population

All subjects were recruited from the outpatient clinics of Lister Hospital. The inclusion and exclusion criteria are as discussed in the previous chapter. Briefly, adult CKD patients older than 18 years of age were recruited. Individuals with any illness that may potentially cause abnormal metabolic rates were excluded from the study.

7.2.4 Study Assessments

7.2.4.1 Data collection and Metabolic Analysis

Demographic, comorbidity and clinical data were obtained as discussed in the previous chapter. On the study day (Day 0), a thorough metabolic analysis was performed including anthropometric measurements, bioimpedance analysis and REE measurement by indirect calorimetry as previously explained.

7.2.4.2 Doubly labelled water administration

The doubly labelled water isotope administration along with calculation of the required doses and subsequent calculation of TEE are explained in detail in the General Methodology section 3.8.1. After ingestion of the measured dose of the sample on Day 0, subjects were asked to collect the first urine sample after 24 hours on Day 1. From then on, they were asked to collect a urine sample each day for a period of 14 days approximately around the same time every day. However, as explained above, only the samples on Day 1, 8 and 14 were used for analysis. If any of these samples were missing, the sample closest to the missing day (±1 day) was used. Samples were collected at the end of the study period and frozen at -20°C until analysis was performed.

7.2.4.3 Physical Activity Questionnaires

At the end of the 14-day period, participants completed two physical activity questionnaires – Recent Physical Activity Questionnaire (RPAQ) and Stanford 7-day recall questionnaire. The questionnaires and the calculations for each of the questionnaire are described in detail in the general methodology chapter (section 3.7.2). A brief description is provided below.

7.2.5 Estimation of Physical Activity Level

7.2.5.1 Recent Physical Activity Questionnaire (RPAQ)

In RPAQ, participants were enquired about performance of various activities and the time spent doing each of them over the preceding 4 weeks. Each activity is then assigned a MET value as per the Compendium of Physical Activities (Ainsworth et al., 2011; Ainsworth et al., 2000; Ainsworth et al., 1993). If the total duration of reported activity exceeded 18 hours per day, then the total and individual activity duration was normalised to 18 hours. Sleep duration was assumed to be a maximum of 6-8 hours depending on the duration of the reported activity. For the time unaccounted for by the questionnaire, different MET values were assigned to explore the most accurate value that will achieve the best estimation of PAEE compared to that measured through DLW method. MET values for the unaccounted time were assigned in 0.05 increments from 1 through to 1.3. In addition, MET values for the

unreported time were also assigned as per the recommendation from MRC (MET (rec)).

Two different methods of physical activity level estimation from RPAQ were explored. The first method involved calculating a mean daily MET value. This was carried out as follows. The MET value for each reported activity was multiplied by the duration of that activity. Similarly, assigned MET value for the unreported time and sleep was multiplied by the respective duration for the day. A total MET value, expressed as MET-hours/day, was calculated as explained in section 3.7.2.1. This was then divided by 24 (hours) to give a mean daily MET. $TEE_{RpaqMET}$ was calculated by multiplying the daily MET by estimated REE derived from equation 1 (section 3.6.1). In this method, the physical activity level is expressed as daily MET. The calculation steps are shown below.

$$\boldsymbol{Mean\ Daily\ MET} = \frac{\boldsymbol{Total\ Daily\ MET}}{\boldsymbol{24}}$$

$$\boldsymbol{TEE_{RpaqMET}\ (kcal/day)} = \boldsymbol{REE\ (kcal/day)} \times \boldsymbol{Mean\ Daily\ MET}$$

The second method involves converting the total daily MET value to PAEE expressed as kcal/day. Energy expenditure from physical activity (MET-hours/day) was calculated by subtracting the basal metabolic rate from the total daily MET value.

$$\boldsymbol{Activity\ related\ MET} = \boldsymbol{Total\ MET} - \left(\boldsymbol{(24 - Sleep\ duration)} \times \boldsymbol{1}\right)$$

One MET is considered equal to 3.5 ml/kg/min of oxygen consumption. In order to convert the Activity related MET values (MET-hours/day) to PAEE expressed as kcal/day, a conversion factor is applied as shown below. The factor “3.5 x 60” converts the MET-hours/day into ml/kg/day. Multiplying this by “Weight / 1000” converts it to “L/day”. One Litre/min of oxygen consumption is considered equivalent to 5 kcal/min. Hence, this is multiplied by 5 to arrive at the final value in kcal/day. This is then added to REE to estimate $TEE_{RpaqPAEE}$ as shown below.

$$\boldsymbol{PAEE\ (kcal/day)} = \frac{\boldsymbol{Activity\ related\ MET} \times \boldsymbol{3.5} \times \boldsymbol{60} \times \boldsymbol{Weight} \times \boldsymbol{5}}{\boldsymbol{1000}}$$

$$\boldsymbol{TEE_{RpaqPAEE}\ (kcal/day) = \frac{(REE + PAEE)}{0.9}}$$

In addition, total activity duration spent in performing sedentary, moderate and vigorous intensity activity was also calculated. Sedentary time was estimated as the combined time spent watching television and that spent using a computer. Any activity with a MET value of 3 to 6 was considered moderate intensity and any activity having a MET value of more than 6 was considered vigorous intensity. Moderate and vigorous intensity activity duration was estimated by calculating the total reported time spent in the respective intensities.

7.2.5.2 Stanford 7-day recall Questionnaire

Stanford 7-day recall questionnaire enquires about time spent on different intensities of activities over the preceding 7 days. Any time not accounted for is considered to be spent performing light activities. Unlike RPAQ, this questionnaire enquires about the average duration of sleep over the preceding week and hence, no separate assumption is needed with regards to the sleep duration. Total daily MET was calculated similar to that carried out for RPAQ. TEE_{StMET} and TEE_{StPAEE} were calculated for this questionnaire in a similar fashion to that of RPAQ.

7.2.6 Statistical Analysis

Normally distributed data are presented as mean ± standard deviation and non-normally distributed data as median (inter-quartile range). Comparison between questionnaire-derived TEE and PAEE and that from DLW method was carried out using Bland-Altman analysis. PAEE and TEE were calculated for each assigned MET value for the unaccounted time in RPAQ which were then compared with DLW measurements using Bland-Altman analysis (Bland & Altman, 1986). A p-value of < 0.05 was considered significant.

7.3 Results

7.3.1 Population Demographics

The demographic and biochemical characteristics of the study participants are discussed in the previous chapter (section 6.4.1) and reproduced here in Table 7-1 below.

Table 7-1: Demographic and Biochemical characteristics of Study Participants

Significance in differences tested by: *unpaired t-test **Fisher's Exact test

	All Patients	CKD Stages 1-3	CKD Stages 4-5	p-value
Number of patients	80	40	40	
Age (years)	56.7 ± 16.2	52.7 ± 17.0	60.7 ± 14.6	0.028*
Male Gender	48 (60%)	21 (52.5%)	27 (67.5%)	0.254**
Weight (kg)	77.0 ± 13.0	77.0 ± 13.8	77.0 ± 12.3	0.990*
Height (cm)	169.0 ± 9.1	168.2 ± 8.0	169.8 ± 10.1	0.435*
CCI	4.0 ± 2.5	2.9 ± 2.5	4.6 ± 2.1	0.001*
Haemoglobin (g/dL)	12.1 ± 1.7	12.8 ± 1.5	11.3 ± 1.5	< 0.001*

7.3.2 Reported activity duration from RPAQ

The reported activity durations for different intensities of activities from RPAQ are shown in Table 7-2. The median reported activity duration per day was 9.2 hours with nearly half of that time spent in sedentary activities (median 4.6 hours). The median time spent in moderate intensity activity was approximately 30 minutes per day. The time spent in vigorous activity intensity was negligible. The median time that was not accounted for by the questionnaire was 6.8 hours. There were no significant differences between sexes in reported activity durations for any of the activity intensity groups.

Table 7-2: Reported daily activity durations from RPAQ

Data presented are Median and inter-quartile range.

	All Patients (n = 40)	Males (n = 22)	Females (n = 18)	p-value
Total reported activity time (hours)	9.2 (5.6)	9.2 (5.2)	8.6 (5.9)	0.63
Sedentary time (hours)	4.6 (2.9)	4.8 (4.6)	4.6 (2.1)	0.91
Moderate intensity activity time (hours)	0.29 (0.41)	0.28 (1.1)	0.33 (0.3)	0.52
Unreported time (hours)	6.8 (5.5)	6.8 (5.2)	7.4 (5.9)	0.64

7.3.3 Reported activity duration from Stanford Questionnaire

The reported activity duration from Stanford questionnaire are shown in Table 7-3. The median daily MET was 1.49 (interquartile range: 0.2). The majority of the time is spent in light activity (median 15.3 hours). The median time spent in moderate activity intensity was 1 hour with negligible time spent in hard and very hard intensity activities.

Table 7-3: Reported daily activity durations from Stanford Questionnaire

Data presented are Median and inter-quartile range.

	All Patients (n = 80)	Males (n = 48)	Females (n = 32)	p-value
Mean Daily MET	1.49 (0.2)	1.47 (0.2)	1.5 (0.2)	0.29
Light Activity Time (hours)	15.3 (3.6)	15.3 (3.5)	15.4 (3.9)	0.84
Moderate intensity activity time (hours)	1.0 (1.4)	0.9 (1.7)	1.1 (1.4)	0.34

7.3.4 Comparison of TEE from Stanford questionnaire and DLW

As explained in the methods section, TEE was calculated in two ways from Stanford questionnaire – TEE_{StMET} from the Daily MET and TEE_{StPAEE} by converting MET into PAEE. The mean (± SD) for both these TEE values and that measured through DLW method are shown in Table 7-4.

Table 7-4: TEE from Stanford Questionnaire and DLW method

	Mean (±SD)
TEE_{DLW} (kcal/day)	2481 ± 476
TEE_{StMET} (kcal/day)	2541 ± 580
TEE_{StPAEE} (kcal/day)	3031 ± 754

The estimated TEE from Stanford questionnaire was compared against TEE_{DLW} by Bland-Altman technique. A Bland-Altman plot of TEE_{DLW} and TEE_{StMET} is shown in Figure 7-1. The X-axis represents the average TEE from these two measures and the Y-axis represents the difference between them. The mean difference between TEE_{DLW} and TEE_{StMET} was -61 kcal/day. The limits of agreement between the two measures were wide with the upper limit being 1003 kcal/day and the lower being -1125 kcal/day.

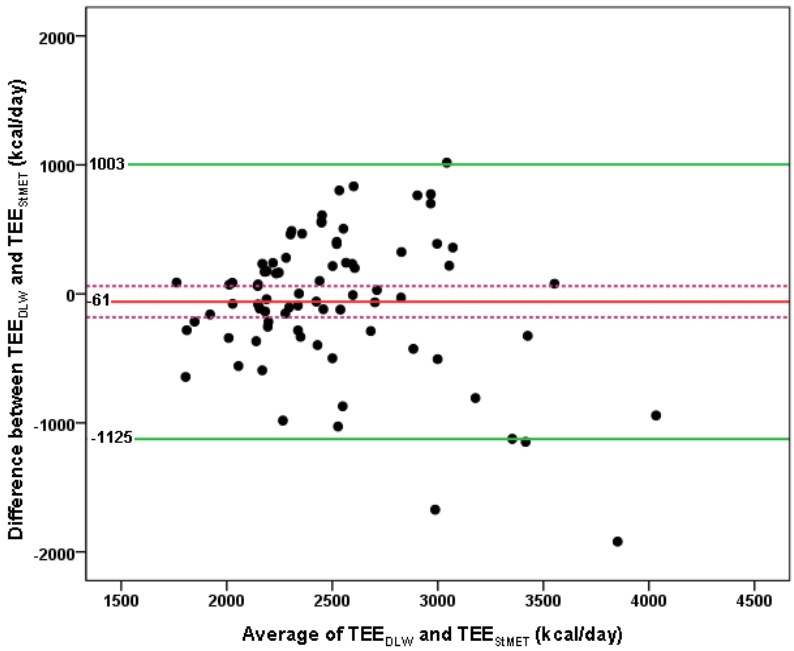


Figure 7-1: Bland-Altman Plot of TEE_{DLW} and TEE_{StMET}

Difference between TEE measured by DLW and Stanford questionnaire (as multiple of REE) plotted against the mean of the two measurements. A negative sign indicates an overestimation and a positive sign indicates an underestimation by the questionnaire.

A Bland-Altman plot of TEE_{DLW} and TEE_{StPAEE} is shown in Figure 7-2. The mean difference between TEE_{DLW} and TEE_{StPAEE} was -551 kcal/day. The limits of agreement between the two measures were wider than that for TEE_{StMET} with the upper limit being 788 kcal/day and the lower being -1889 kcal/day.

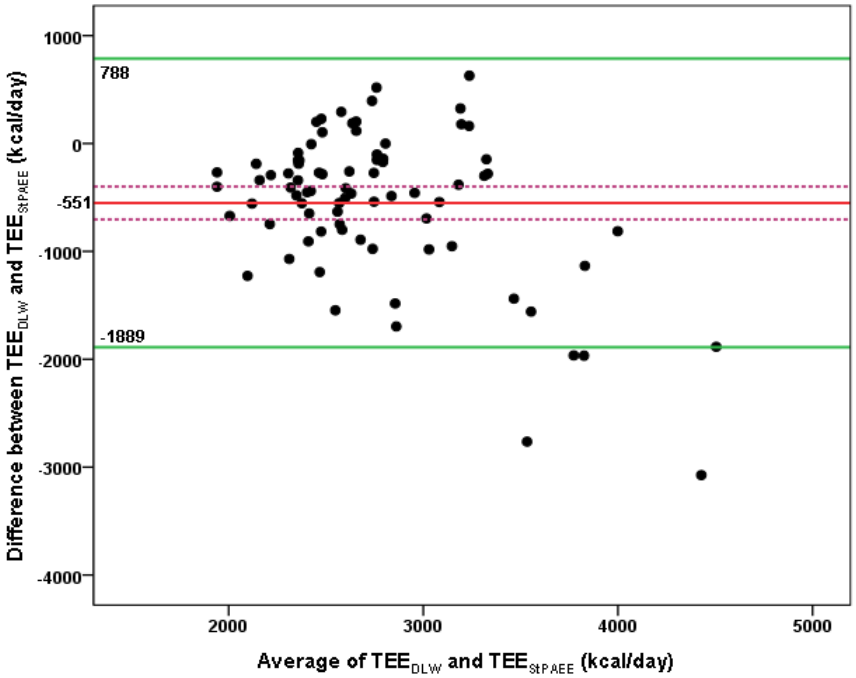


Figure 7-2: Bland-Altman Plot of TEE_{DLW} and TEE_{StPAEE}

Difference between TEE measured by DLW and Stanford questionnaire (after conversion to PAEE) plotted against the mean of the two measurements. A negative sign indicates an overestimation and a positive sign indicates an underestimation by the questionnaire.

7.3.5 Predicting the best approximation of MET for unreported time in RPAQ

As discussed earlier in the chapter, different MET values ranging from 1-1.3 were assigned to the unreported time in RPAQ. TEE was calculated using each of these values in the two different methods described above and bias was calculated for each of these values compared to TEE_{DLW}. The bias and the limits of agreement were examined separately using the measured REE and the estimated REE using the equation. Figure 7-3 shows the mean difference (bias) between TEE_{DLW} and $TEE_{RpaqMET}$ estimated with each of the assigned MET value from 1 to 1.3. MET (rec) is the recommended MET value assigned as described in the methodology section. Figure 7-4 shows the limits of agreement (upper and lower error bars) for these mean bias values.

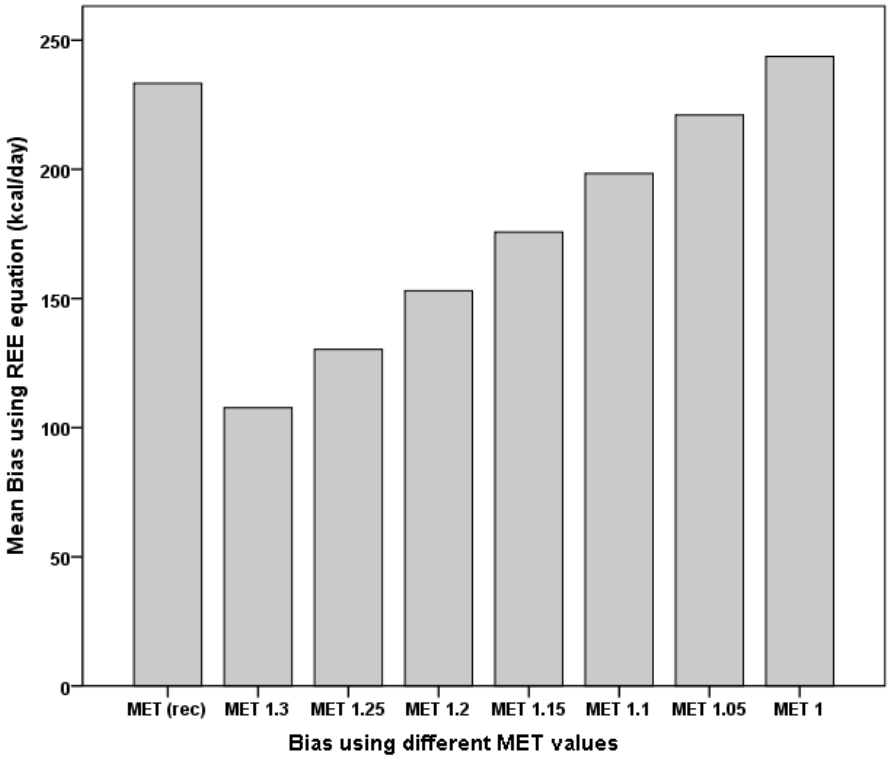


Figure 7-3: Mean difference between TEE_{DLW} and $TEE_{RpaqMET}$ using different MET values for unreported time and estimated REE

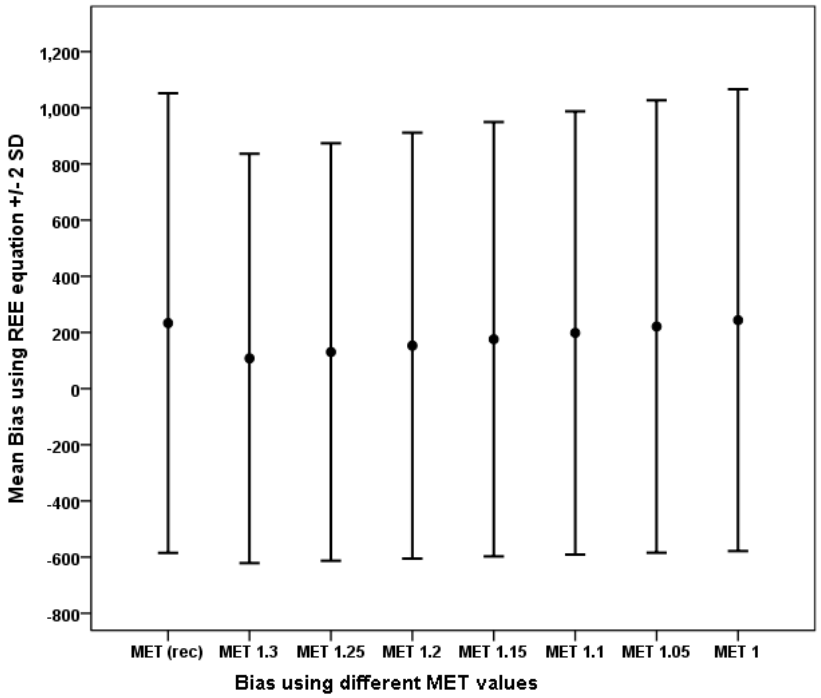


Figure 7-4: Error bars showing the limits of agreement for the bias between TEE_{DLW} and $TEE_{RpaqMET}$ using estimated REE

Upper and lower error bars represent the upper and lower limits of agreement respectively.

A MET value of 1.3 for the unreported time gave the least bias (108 kcal/day) and the best limits of agreement (upper limit: 822 kcal/day; lower limit: -607 kcal/day) compared to other MET values including the recommended MET value.

Similarly, TEE was also calculated as above using the REE measured by indirect calorimetry instead of the estimated REE. Figure 7-5 and Figure 7-6 shows the mean bias and limits of agreement between the two TEE estimates using the measured REE. Again, the MET value of 1.3 had the least bias (171 kcal/day) and the best limits of agreement (upper limit: 1105 kcal/day; lower limit: -764 kcal/day) compared to other MET values.

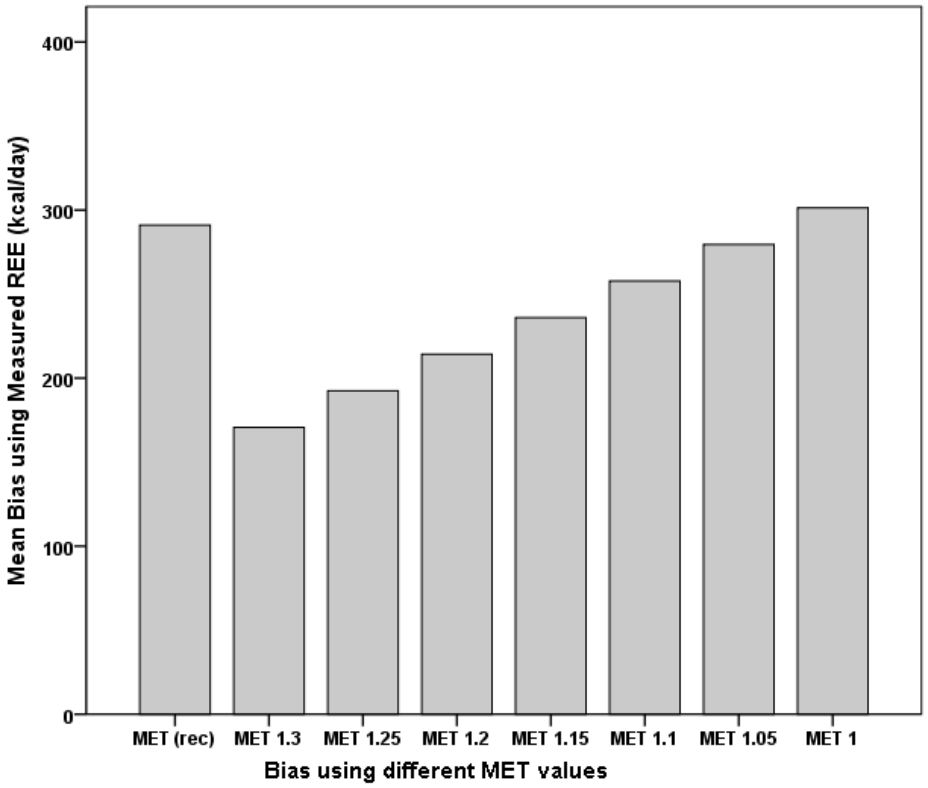


Figure 7-5: Mean difference between TEE_{DLW} and $TEE_{RpaqMET}$ using different MET values for unreported time and measured REE

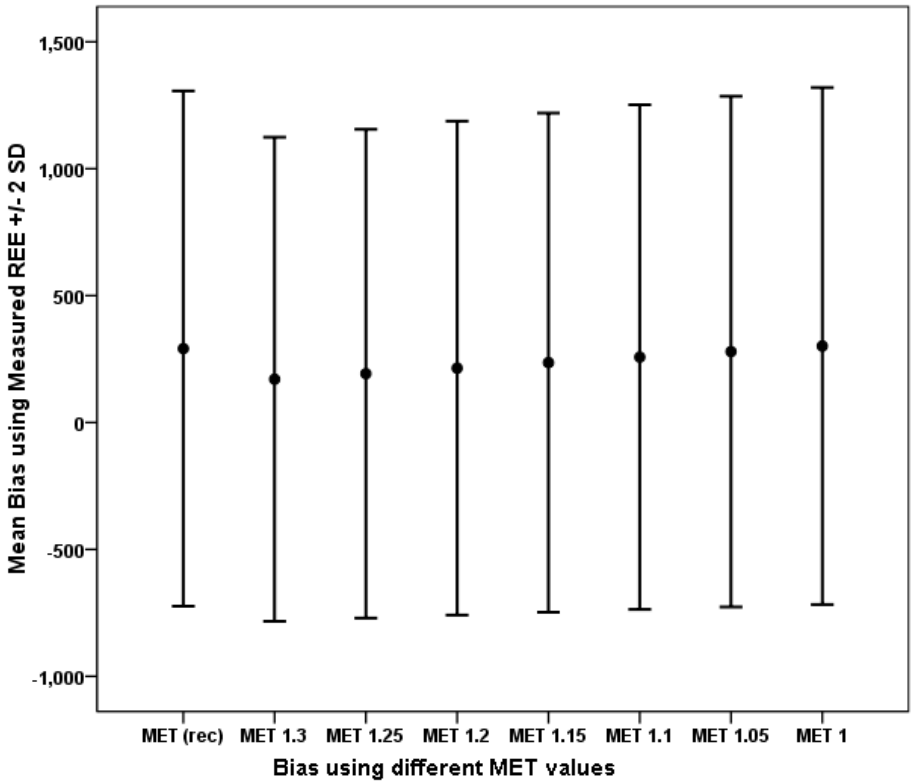


Figure 7-6: Error bars showing the limits of agreement for the bias between TEE_{DLW} and $TEE_{RpaqMET}$ using measured REE

Upper and lower error bars represent the upper and lower limits of agreement respectively.

$TEE_{RpaqPAEE}$ was also calculated as described above using the same assigned MET values. The mean difference between TEE_{DLW} and $TEE_{RpaqPAEE}$ using different MET values are shown in Figure 7-7 and the associated limits of agreement in Figure 7-8.

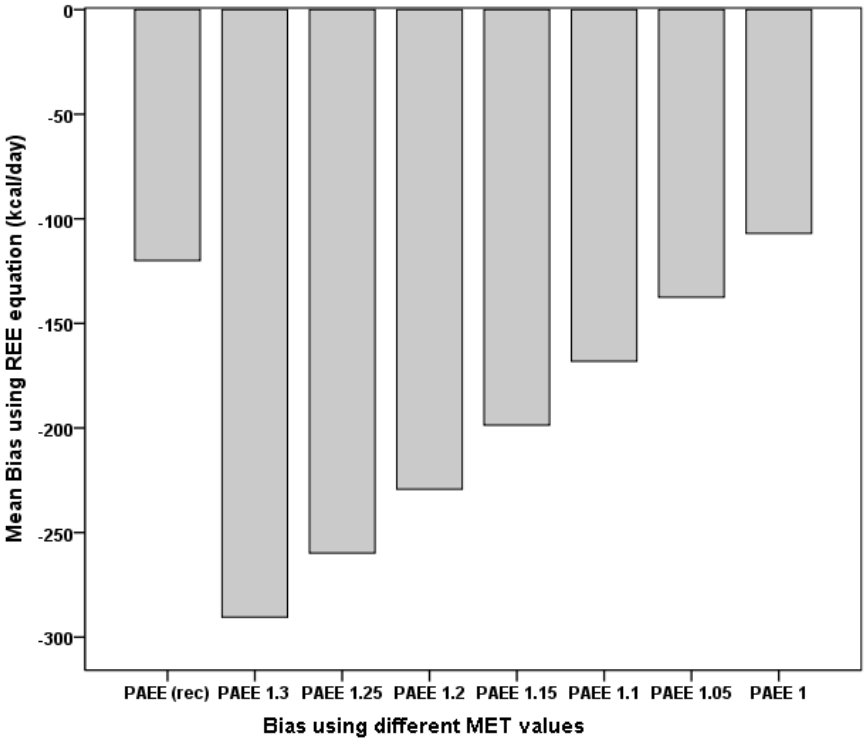


Figure 7-7: Mean difference between TEE_{DLW} and $TEE_{RpaqPAEE}$ using different MET values for unreported time and estimated REE

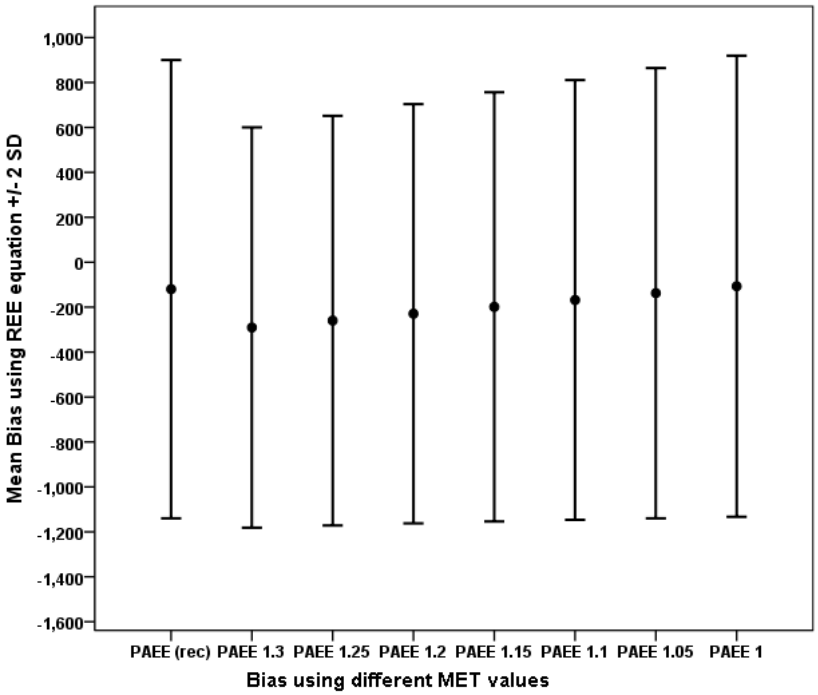


Figure 7-8: Error bars showing the limits of agreement for the bias between TEE_{DLW} and $TEE_{RpaqPAEE}$ using estimated REE

Upper and lower error bars represent the upper and lower limits of agreement respectively.

In contrast to the estimated $TEE_{RpaqMET}$, a MET value of 1 for the unreported time gave $TEE_{RpaqPAEE}$ the least bias (-107 kcal/day) when compared to TEE_{DLW}. However, the limits of agreement for this MET value (upper limit: 899 kcal/day; lower limit: -1113 kcal/day) were wider compared to that of $TEE_{RpaqMET}$.

TEE calculation by this method using the measured REE instead of the estimated REE was also examined. Figure 7-9 and Figure 7-10 shows the mean bias and limits of agreement for TEE estimates using measured REE. Again, a MET value of 1 had the least bias (-42 kcal/day) but had wider limits of agreement (upper limit: 1102 kcal/day; lower limit: -1186 kcal/day).

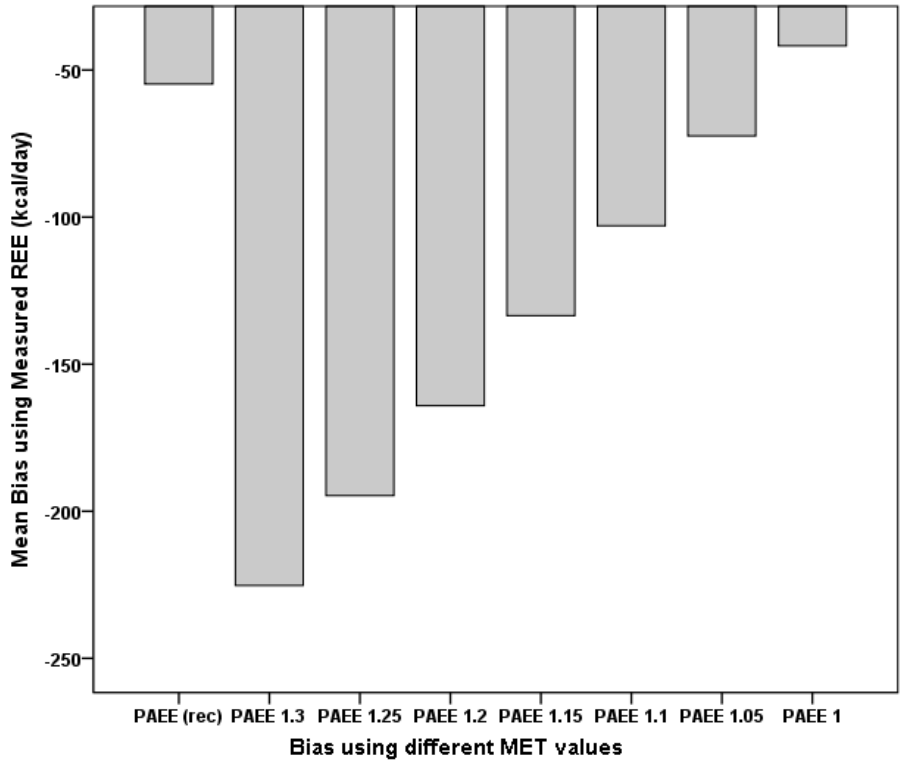


Figure 7-9: Mean difference between TEE_{DLW} and $TEE_{RpaqPAEE}$ using different MET values for unreported time and measured REE

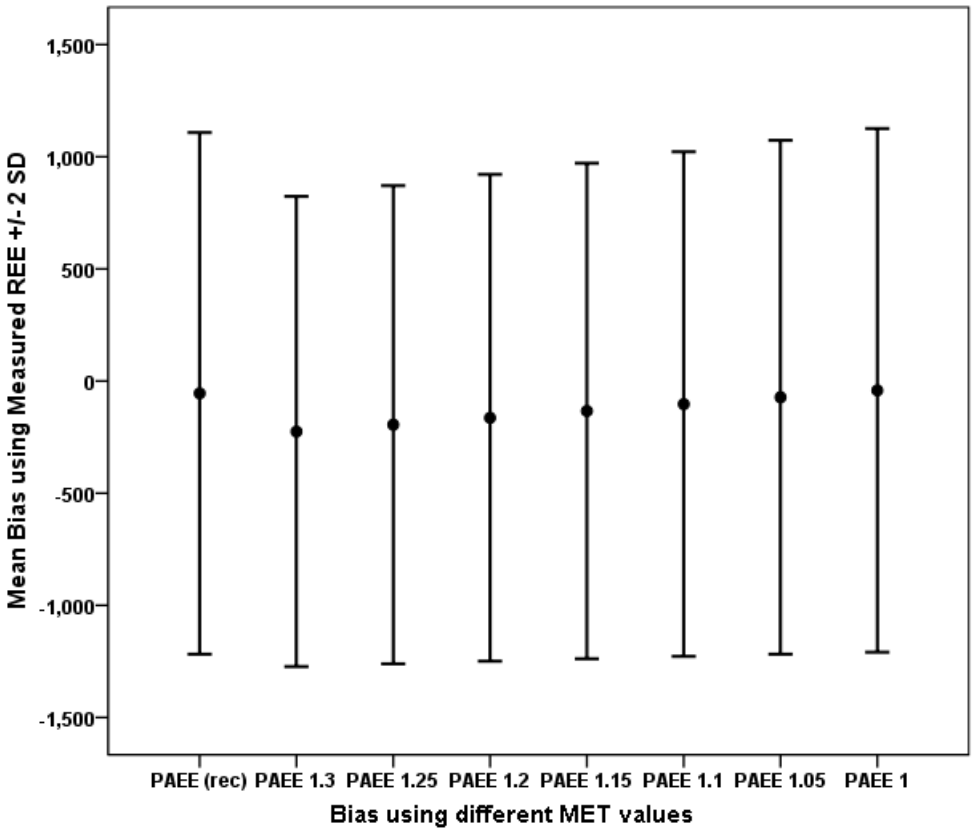


Figure 7-10: Error bars showing the limits of agreement for the bias between TEE_{DLW} and $TEE_{RpaqPAEE}$ using measured REE

Upper and lower error bars represent the upper and lower limits of agreement respectively.

7.3.6 Comparison of TEE from RPAQ questionnaire and DLW

As a MET value of 1.3 for the unreported time was found to be the best approximation of activity intensity for the unreported time, this was employed to estimate $TEE_{RpaqMET}$ and $TEE_{RpaqPAEE}$ which were then compared with TEE_{DLW}. The mean (± SD) for both these TEE values and that measured through DLW method are shown in Table 7-5.

Table 7-5: TEE from RPAQ Questionnaire and DLW method

	Mean (±SD)
TEE_{DLW} (kcal/day)	2481 ± 476
$TEE_{RpaqMET}$ (kcal/day)	2324 ± 538
$TEE_{RpaqPAEE}$ (kcal/day)	2722 ± 667

Both the estimated TEE values from RPAQ questionnaire were compared against TEE_{DLW} by Bland-Altman technique. A Bland-Altman plot of TEE_{DLW} and $TEE_{RpaqMET}$ is shown in Figure 7-11. The mean bias was 108 kcal/day. The upper limit of agreement was 822 kcal/day and the lower limit was -607 kcal/day.

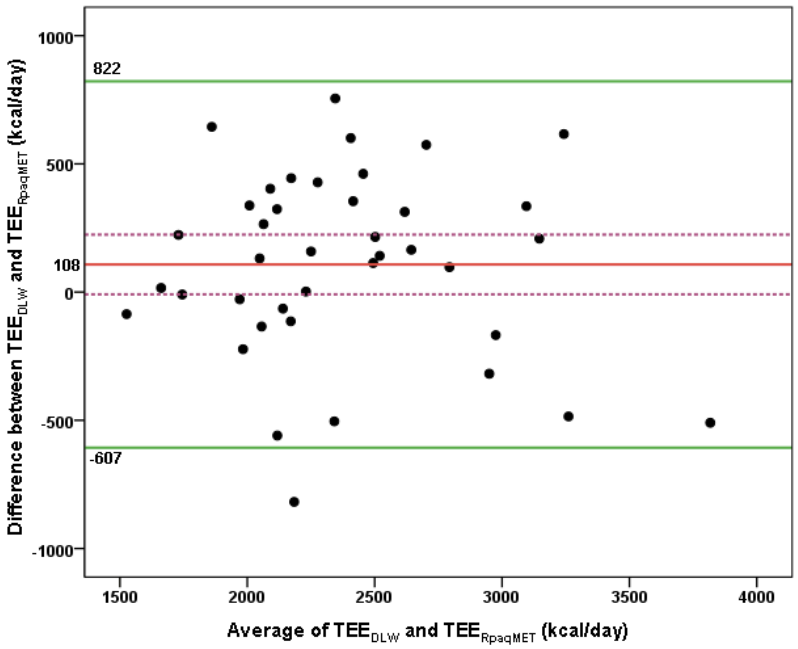


Figure 7-11: Bland-Altman plot of TEE_{DLW} and $TEE_{RpaqMET}$

Difference between TEE measured by DLW and RPAQ (as multiple of REE) plotted against the mean of the two measurements. A negative sign indicates an overestimation and a positive sign indicates an underestimation by the questionnaire.

A Bland-Altman plot of TEE_{DLW} and $TEE_{RpaqPAEE}$ is shown in Figure 7-12. The mean bias was -290 kcal/day with the upper limit of agreement being 583 kcal/day and the lower limit being -1163 kcal/day.

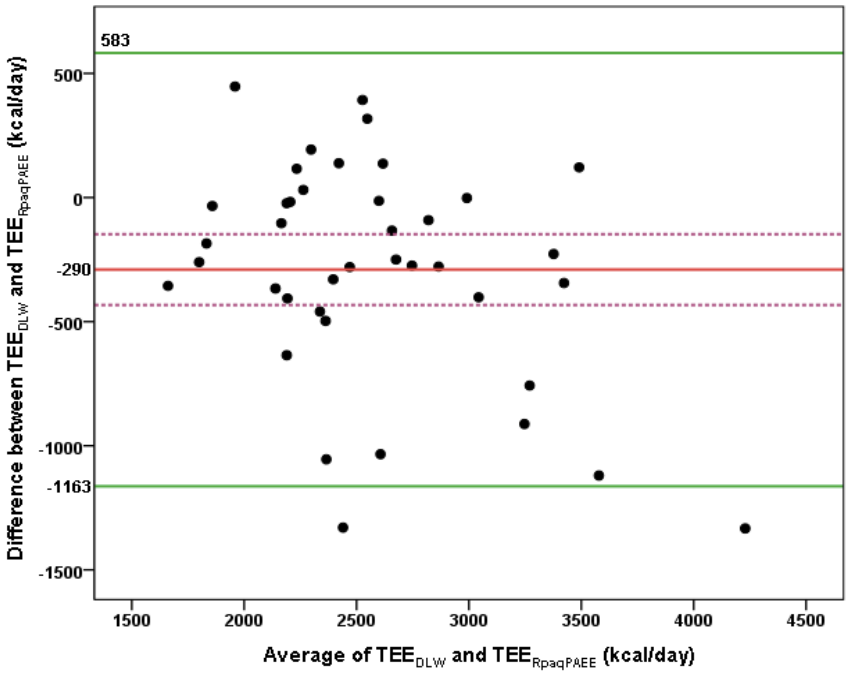


Figure 7-12: Bland-Altman plot of TEE_{DLW} and $TEE_{RpaqPAEE}$

Difference between TEE measured by DLW and RPAQ (after conversion to PAEE) plotted against the mean of the two measurements. A negative sign indicates an overestimation and a positive sign indicates an underestimation by the questionnaire.

7.3.7 Relationship between TEE from the questionnaires and DLW method

The relationship between TEE estimates derived from both the questionnaires and that measured using DLW method are shown in Figure 7-13 and Figure 7-14.

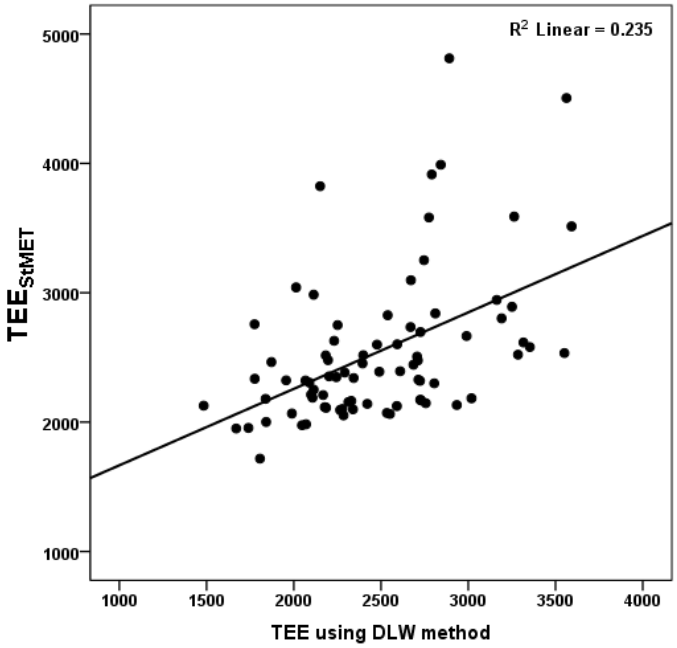


Figure 7-13: Relationship between TEE_{StMET} and TEE_{DLW}

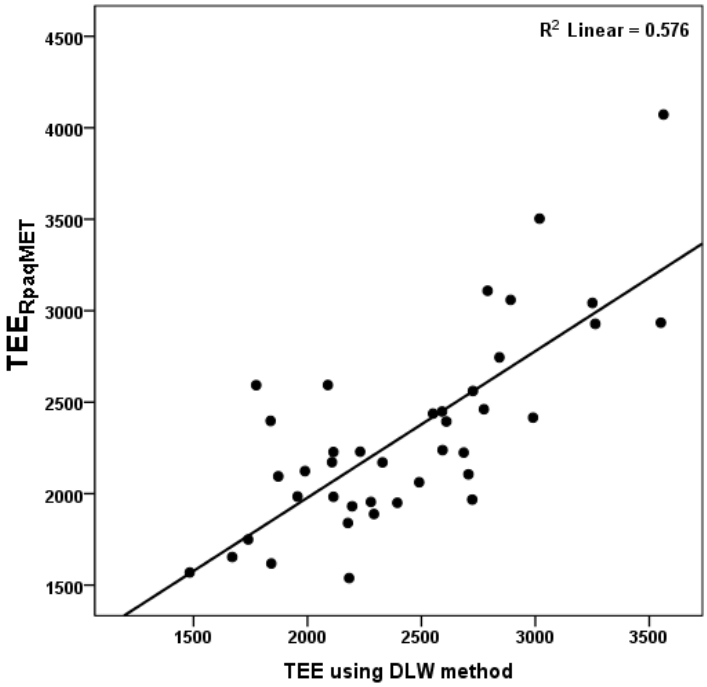


Figure 7-14: Relationship between $TEE_{RpaqMET}$ and TEE_{DLW}

The estimated $TEE_{RpaqMET}$ showed a closer relationship with TEE_{DLW} (R^2 linear – 0.576) compared to that between TEE_{StMET} and TEE_{DLW} (R^2 linear – 0.235).

7.4 Discussion

The primary aim of this study was to examine the validity of the two physical activity questionnaires as tools to estimate TEE in clinical practice. The study showed the RPAQ questionnaire performs better for estimating energy requirements compared to Stanford 7-day recall questionnaire and has a higher correlation to TEE measured using the gold-standard doubly labelled water method. A MET value of 1.3 for the unreported time in RPAQ provides the best approximation of TEE from the questionnaire to the measured TEE.

Assessing physical activity in patients with kidney disease will be beneficial in many ways. Firstly, it will help to characterise patients at risk of poor physical functioning. Secondly, it can be used to monitor the level of physical activity with disease progression and aid early detection of declining physical functioning. Finally, it can also be used to monitor the response to any clinical and psychological interventions related to physical activity levels.

The DLW method does not offer a practical solution for measurement of TEE in clinical practice due to the time-consuming nature and the costs associated with it. Physical activity questionnaires are useful tools to assess activity levels on a routine basis. The commonly available physical activity questionnaires are not generally derived from elderly population or from specific subgroups of patients such as those with kidney disease. Questionnaires developed in younger people are inaccurate when used in elderly population (Washburn, Smith, Jette, & Janney, 1993; Washburn, Jette, & Janney, 1990). Patients with kidney disease are likely to be older adults and to have higher comorbidity and hence, the activity questionnaires need to be validated in this patient group.

Two questionnaires – RPAQ and Stanford 7-day recall questionnaire – were examined for their validity in patients with kidney disease in this study. TEE derived from RPAQ correlated well with that measured from DLW method (R^2 of 0.58). The strong correlation of RPAQ derived TEE with TEE_{DLW} implies the questionnaire is an acceptable tool for use in patients with kidney disease. On the other hand, Stanford questionnaire had a weaker correlation with TEE_{DLW} with the R^2 being 0.24. This is comparable to other questionnaires that have been examined in patients with kidney disease (Johansen et al., 2001). Johansen et al (2001) compared 4 activity

questionnaires against accelerometer derived energy expenditure data and found wide variations in the validity of these questionnaires. Human Activity Profile was the best performing questionnaire in that study with a R^2 of 0.53 for the Adjusted Activity Score from the questionnaire and the Stanford questionnaire had a R^2 of 0.35. In a review by Neilson et al (2008), it was shown that the TEE derived from various questionnaires across many studies had only moderate correlation with TEE measured from DLW method (Neilson, Robson, Friedenreich, & Csizmadi, 2008). This highlights the limited capability of questionnaires for TEE estimation. However, the correlation between RPAQ-derived TEE and that from DLW is better than many of the other currently available questionnaires for energy expenditure estimation.

There is a considerable amount of unreported time from RPAQ (median 6.8 hours per day) which does not include the sleep time. Excluding this time from TEE calculation will grossly underestimate the energy expenditure. The questionnaire captures higher intensity activities well with specific activities listed in detail. However, activities that are routinely carried out at home and low intensity activities are not specifically enquired for in the questionnaire. This may lead to these activities not being reported by patients. It is reasonable to assume that individuals are performing some activity – likely sedentary or low intensity activities – in this unreported time and hence, assigning an average MET value above 1 for this unreported time seems prudent. It is essential to identify an appropriate MET value to assign for this period which will enable better approximation of estimated TEE to the directly measured TEE from DLW method.

A range of MET values (1 to 1.3) for the unreported time in RPAQ was explored. The study data shows that TEE estimated by assigning a MET value of 1.3 for the unreported time was the closest to the measured TEE compared to other MET values. Not only did the MET value of 1.3 showed the least bias but the limits of agreement with TEE_{DLW} were also narrower when compared to other MET values. Intuitively, this would seem the appropriate MET for the unreported time. A MET value of 1.3 is generally considered the threshold between sedentary and light activities. Patients are more likely to under-report sedentary activities especially when not specifically enquired about it and hence, it is possible that patients with kidney disease are performing sedentary activities during this time. In another study measuring activity by accelerometer (chapter 8), it was found that the majority of the

day (>85%) was spent in sedentary activity by patients with kidney disease. Considering the activity data as measured by DLW method in this study and that measured by accelerometer (chapter 8) together, a MET value of 1.3 for the unreported time in RPAQ seems the best approximation to the true activity behaviour of patients with kidney disease.

TEE estimation was also carried out using both measured REE and estimated REE from the specified equation. It was found that the bias and limits of agreement were higher with measured REE compared to that derived from predictive equation. This demonstrates that derivation of TEE from a questionnaire is only an approximation of the true total energy expenditure even when used in conjunction with measured REE values. Nevertheless, the better performance of predictive REE equation with MET value suggests that this is a reliable tool for TEE estimation in clinical practice.

This study also explored two different methods of calculating TEE from the questionnaires. TEE can be expressed by a factorial approach as a multiple of REE using a physical activity factor which is the Mean Daily MET calculated from the questionnaires in this study. The total activity MET value per day can also be converted as energy expenditure related to physical activity (PAEE) in kcal/day. As there is no previous literature to suggest that one method is superior to other with regards to these two questionnaires, the study explored the reliability of both these methods against DLW technique. The study data showed that the factorial approach of estimating TEE was superior compared to that estimated by conversion to PAEE. This was true for both the questionnaires used in this study. Using the factorial approach for both the questionnaires and a MET value of 1.3 for the unreported time in RPAQ, it was seen that RPAQ performed better overall compared to the Stanford questionnaire in estimating TEE. Though the mean bias was slightly lower in Stanford questionnaire, the limits of agreement were much narrower with RPAQ than that with Stanford questionnaire.

It has been suggested that the validity of activity questionnaires should not be assessed solely on correlations alone but be carried out in conjunction with other statistical methods (Schmidt & Steindorf, 2006; Bellach, 1993; Bland & Altman, 1986). Bland-Altman plot is an appropriate way of exploring the validity of a new tool compared to a gold-standard method. This study has employed this method to

compare TEE measures from the questionnaire to DLW technique which strengthens the validity of the results reported here. If the questionnaire measures activity for a different time point than that measured by DLW, this may result in unreliable results from the questionnaire data (Neilson et al., 2008). The questionnaires in this study were administered at the end of the 14-day study period and hence the questionnaires reflect the activity behaviour in the same time frame as that measured by DLW.

7.5 Limitations

As explained in chapter 6, the main limitations of the study are related to the assumptions of the DLW method. As with any questionnaire method, recall bias may have been a confounding factor in the accuracy of the data. This has been negated to some extent by enquiring about specific activities in the preceding weeks which facilitates recall of the activities by the subjects. Moreover, the strength of relationship between questionnaire data and DLW method is also in line with previously published literature. Finally, the study is limited by its relatively small sample size but the costs associated with DLW method restrict the use of this technique in large-scale studies.

7.6 Conclusion

In conclusion, this is the first study to have validated activity questionnaires against doubly labelled water method in patients with kidney disease. This study has shown that RPAQ is a valid tool for assessment of activity level and TEE in CKD patients. Stanford questionnaire, though showing some relationship to the measured TEE, performs worse than RPAQ in estimating TEE in this patient group. A MET value of 1.3 has been shown to be the best estimate of the activity level for the unreported time in RPAQ and use of this value is recommended when using RPAQ for patients with kidney disease.

Chapter 8

8. Influence of Physical Activity on Urea Generation Rate

8.1 Introduction

Individuals with Chronic Kidney Disease (CKD) have increased cardiovascular risk and high mortality from cardiovascular causes (Robinson-Cohen et al., 2009; Johansen, 2005). Physical activity levels are low in patients with CKD and ESRD (Robinson-Cohen et al., 2013; Johansen et al., 2000). Physical inactivity may play a significant role in CKD patients through its association with cardiovascular risk. Physically active lifestyle may reduce the progression of kidney disease(Kouidi, Grekas, Deligiannis, & Tourkantonis, 2004) and also reduce the malnutrition-inflammation complex syndrome prevalent in this patient population (Castaneda et al., 2004).

Exercise induces profound changes in renal haemodynamics and protein excretion. The renal fraction of the cardiac output decreases with increasing exercise intensity (Grimby, 1965) which may lead to a reduction in effective renal plasma flow. This produces a concomitant reduction in Glomerular Filtration Rate (GFR) although the reduction in GFR is proportionately less than that of renal plasma flow(Poortmans, 1984) due to an increase in filtration fraction which may reach up to 67% during heavy exercise (Castenfors, 1967). In addition, there is an increase in proteinuria during exercise and immediate post-exercise period which is also related to the intensity of exercise (Poortmans, Rampaer, & Wolfs, 1989; Poortmans & Labilloy, 1988). This is due to an increased permeability of the glomerular capillaries and also to reduced reabsorption in the tubules.

There is paucity of studies examining GFR levels at rest in physically active individuals. There are no comparative clinical studies comparing GFR in physically active and sedentary individuals. One might expect GFR to be higher in physically active individuals due to increased generation of metabolic waste products and there is circumstantial evidence to support this.

Lippi et al (2008) found that estimated GFR (by Modification of Diet in Renal Disease equation- eGFR-MDRD) was significantly higher in professional cyclists compared to sedentary controls(Lippi, Banfi, Luca Salvagno, et al., 2008), though the cyclists had lower body weight and BMI than sedentary controls, so no conclusions can be drawn about the direct influence of physical activity on GFR. In a similar study comparing renal function among amateur and professional cyclists and healthy sedentary controls, eGFR-MDRD was significantly higher in cyclists compared to sedentary controls (Lippi, Banfi, Salvagno, Franchini, & Guidi, 2008) but non-significantly higher in professional compared to amateur cyclists. A large prospective study in Norway showed that higher levels of physical activity were associated with increase in eGFR-MDRD over time in women but not men (Kronborg et al., 2008). Physical activity data was collected through questionnaires and not directly measured.

There is however a definite and direct link between high body mass index (BMI) and GFR. In a cross-sectional study conducted by Kambham et al (2001) examining the relationship between obesity and glomerular changes, obese individuals were found to have glomerular hypertrophy compared to age- and sex-matched healthy controls (Kambham, Markowitz, Valeri, Lin, & D'Agati, 2001). They also found that creatinine clearance was significantly higher in obese individuals with 41.4% of study patients having a creatinine clearance of >130 ml/min. This effect of BMI on GFR is also seen in individuals with high BMI owing to increased muscle mass. Schwimmer et al (2003) reported findings from 3 male patients whose jobs involved strenuous physical activity (Schwimmer et al., 2003). BMI of these patients ranged from 30.4 – 32.1 kg/m^2 with body fat content of only 12.9-16.8%. All 3 patients had high measured creatinine clearance ranging from 113-208 ml/min and their renal biopsies showed glomerular hypertrophy. These studies provide some evidence to support the hypothesis that physical activity levels influence GFR.

There are a limited number of studies that have measured habitual physical activity levels in haemodialysis patients. These studies have shown physical activity levels to be extremely low in HD patients (Baria et al., 2011; Cupisti, Capitanini, Betti, D'Alessandro, & Barsotti, 2011; Zamojska et al., 2006; Majchrzak et al., 2005; Johansen et al., 2000). A recent multi-centre study conducted by Avesani et al (2012) showed energy expenditure from physical activity in HD patients contributed

only 12-16% to total energy expenditure in comparison to the expected 15-30% in general population (Avesani et al., 2012). Similar results have also been reported in questionnaire-based studies of physical activity (Johansen et al., 2010; Stack & Murthy, 2008).

Whether higher levels of physical activity result in increased metabolic waste generation and mobilisation from peripheral tissues in patients with end-stage renal disease (ESRD) has not yet been studied. The potential effect of physical activity on uraemic toxin generation may be important in this setting as it may influence minimum dialysis requirements. There is considerable debate as to the choice of an ideal uraemic toxin marker. The current adequacy target for dialysis is based on urea clearance. Urea generation rate (G) is an accepted surrogate marker of small solute uraemic toxin generation and can be calculated through formal urea kinetic modelling. However there are limitations to using urea for this purpose as discussed in section 2.2.1.

This study was designed

- To examine the impact of physical activity on urea generation rate and thereby, minimum dialysis requirement
- To explore the relationship between measured physical activity and that estimated by the Recent Physical Activity Questionnaire (RPAQ).

The experimental hypothesis for this study was that increasing physical activity levels contribute to higher uraemic toxin generation in haemodialysis patients. The null hypothesis was that there was no relationship between physical activity and uraemic toxin generation.

8.2 Methods

8.2.1 Ethical Review

The study was approved by Essex Research Ethics Committee (ref: 13/EE/0053 and UH Ethics ref: LMS/PG/NHS/00254). All study participants gave written informed consent.

8.2.2 Study Design

This was a cross sectional study of 120 haemodialysis patients. Physical activity was measured by a wrist-worn accelerometer over a period of 7 days. All study participants prospectively completed a food diary for three days during the study duration and also, the recent physical activity questionnaire (RPAQ) at the end of the study period.

8.2.3 Study Population

A total of 120 maintenance HD patients were recruited from the renal units of East and North Hertfordshire NHS Trust and Royal Free Hampstead NHS Trust. Inclusion criteria were patients older than 18 years of age, those who have been on maintenance haemodialysis for at least 3 months and those who were anuric. Exclusion criteria included patients with untreated hypo- or hyperthyroidism, active malignancy, ongoing sepsis, active vasculitis, history of hospitalisation in the last month, current pregnancy, cardiac pacemakers or defibrillators, unexplained weight loss, limb amputation, immobility due to major physical ailments and those with no capacity to consent. Only anuric patients were included to facilitate estimation of urea generation rate without the need for the inter-dialytic urine collection.

8.2.4 Study Assessments

The following assessments were carried out for the study.

8.2.4.1 Demographic and Anthropometric data

Data comprising of age, sex, ethnicity, weight, height and comorbid conditions were collected for all study participants through direct measurements and individual medical records. Charlson Comorbidity Index was calculated as previously described (Charlson et al., 1987).

8.2.4.2 Dietary Assessment

Participants were asked to prospectively complete a food diary for three days in the week when the activity was measured. They were asked to record all food and drinks consumed on a dialysis day, a non-dialysis day and a weekend day. The dietary

records were assessed by trained renal dieticians to estimate average total protein and energy intake during the study period.

8.2.4.3 Measurement of Physical Activity

Physical activity was measured using a validated wrist-worn tri-axial accelerometer, GENEActiv as explained in section 3.7.1. Study participants were asked to wear the accelerometer on the non-fistula hand for 7 consecutive days. They were asked to wear it for all 24 hours in the day.

8.2.4.4 Recent Physical Activity Questionnaire

The recent physical activity questionnaire (RPAQ) enquires about various physical activities at home, work and during leisure time in the preceding 4 weeks and is explained in detail in the general methodology chapter. Information on physical activity was also collected using the RPAQ questionnaire at the end of the 7-day period as explained in section 3.7.2.1.

8.2.4.5 Metabolic analysis

Resting energy expenditure (REE) was estimated using equation 1 described in section 3.6.1. Total body water was calculated using the Watson formula (section 3.10.1). In addition, body water and composition was analysed using the Inbody 720 bioimpedance device as explained in section 3.10.2.

8.2.4.6 Urea Kinetic Modelling

As explained in section 4.2.4.6, 2-pool Urea generation rate was calculated using Solute Solver. Normalised Protein Catabolic Rate (nPCR) can also be calculated using formal urea kinetic modelling. nPCR is a marker of protein degradation and when measured in-between dialysis sessions, is a marker of dietary protein intake in steady-state conditions.

8.2.5 Measurement of Activity Time

The data from GENEActiv accelerometer was downloaded onto a computer and was analysed as explained in the general methodology chapter (section 3.7.1). The raw data was analysed using a pre-defined algorithm and the outcome variables

obtained for each study day were total wear and non-wear time, time spent in sedentary, light, moderate and vigorous intensity activity. The algorithm does not define sleep time and hence, sleep time is included in the sedentary time.

Analysis of the RPAQ data is explained in the general methodology chapter (section 3.7.2.1). Moderate activity time from RPAQ was calculated by the summation of time spent in activities with a MET (Metabolic Equivalent of Task) value of 3-6. Vigorous activity time was calculated similarly by taking into account the activities with a MET value of higher than 6 as per the Compendium of Physical Activities (Ainsworth et al., 2011; Ainsworth et al., 2000; Ainsworth et al., 1993).

8.2.6 Sample Size and Statistical Analyses

Data from the questionnaire-based study of physical activity (chapter 4) was used to calculate the sample size for this study. A power analysis was performed to estimate the statistical power provided by a study of 120 haemodialysis patients. Assuming $\alpha=0.05$, N=120 patients and the correlation coefficient from the previous study, the power to detect significant relationship between physical activity levels and G was estimated to be more than 0.8 with five to ten covariates in the model demonstrating sufficient power for the study.

Normally distributed data are presented as mean ± standard deviation and non-normally distributed data as median (inter-quartile range). Differences in activity time between groups were compared using unpaired t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data and using ANOVA for multiple group comparisons. Correlations between variables were analysed using Pearson correlation coefficient for normally distributed data and Spearman's for non-normally distributed data.

Specific criteria were applied to accelerometer wear time for inclusion in the study. The study day was considered 'valid' for analysis only when subjects have worn the accelerometer for at least 50% of the day. Patients who have worn the accelerometer for at least 3 'valid' days during the 7-day period were included for analysis. Accelerometer-derived activity time was converted to logarithmic scale for normalisation and was used as such in linear regression models to predict urea generation rate. Collinearity testing was carried out for all the variables included in

the regression model and variables with only low variance inflation factor (<10) were included. A p-value of <0.05 was assumed to indicate statistical significance.

8.3 Results

8.3.1 Population Demographics

A total of 120 patients were recruited for the study. Due to insufficient wear time of the accelerometer as described in statistical analysis section, 15 subjects were excluded and thereby, 105 patients were included in the analyses below. The demographic characteristics of the study population are given in Table 8-1. Body size parameters such as weight and height and REE were significantly higher in males. There were no differences between sexes with regards to age, ethnicity, comorbidity, haemoglobin, serum urea, dietary protein intake and presence of ischaemic heart disease and diabetes mellitus. The delivered dialysis dose (equilibrated Kt/V) was slightly higher in females even though the dialysis session time was less.

Table 8-1: Demographics of Study Participants

Values expressed are mean ± SD. Categorical variables are expressed as percentages. Differences between sexes were analysed by unpaired t-test for continuous variables and by Fisher's Exact test for categorical variables. CCI: Charlson Comorbidity Index; IHD: Ischaemic Heart Disease; REE: Resting Energy Expenditure

	All patients (n = 105)	Males (n = 66)	Females (n = 39)	p-value
Age (years)	64.0 ± 14.3	62.5 ± 15.1	66.5 ± 12.5	0.173
Weight (kg)	75.0 ± 20.8	80.4 ± 20.6	66.0 ± 17.9	< 0.001
Height (cm)	166.9 ± 9.2	171.6 ± 6.3	159.0 ± 7.9	< 0.001
White: Asian: Black Ethnicity (%)	61: 16.2: 22.9	63.6: 18.2: 18.2	56.4: 12.8: 30.8	0.310
Urea (mmol/L)	20.1 ± 5.6	20.2 ± 5.3	19.8 ± 6.0	0.709
Creatinine (µmol/L)	869 ± 236	924 ± 240	776 ± 198	0.001
Haemoglobin (g/dL)	11.4 ± 1.4	11.6 ± 1.5	11.2 ± 1.1	0.175
Dialysis Session Time (minutes)	222 ± 27	227 ± 27	213 ± 24	0.007
Equilibrated Kt/V	1.43 ± 0.30	1.39 ± 0.26	1.51 ± 0.33	0.045
CCI	3.3 ± 2.2	3.3 ± 2.3	3.2 ± 1.9	0.796
IHD (%)	31.4	33.3	28.2	0.666
Diabetes Mellitus (%)	21.9	25.8	15.4	0.235
REE (kcal/day)	1542 ± 284	1655 ± 255	1351 ± 224	< 0.001
Protein Intake (g/kg/day)	0.84 ± 0.31	0.81 ±0.31	0.89 ± 0.31	0.195
nPCR (g/kg/day)	0.92 ± 0.20	0.93 ± 0.21	0.91 ± 0.18	0.666

8.3.2 Accelerometer- and Questionnaire-derived Activity Time

Table 8-2 shows the measured activity time in different intensities by the accelerometer. The measured vigorous intensity activity time was negligible with a median of zero minutes (interquartile range of 0.14 minutes). The estimated moderate and vigorous activity times from the reported data in RPAQ were also negligible with a median of zero minutes for both.

Table 8-2: Measured Time Spent in Different Intensity Activities per day

	Median (interquartile range)
Sedentary Activity Time (minutes)	1149 (118)
Light Activity Time (minutes)	122 (116)
Moderate Activity Time (minutes)	11 (29)

8.3.3 Differences in Activity Level on Dialysis and Non-dialysis Days

The proportion of time spent in different intensity activities on dialysis and non-dialysis days are shown in Table 8-3. As the total wear time might vary between days, the times are shown as proportion of total measured activity time. The sedentary time was significantly higher on dialysis days whereas subjects spent more time on light and moderate activity time on non-dialysis days. There was no significant difference in the vigorous activity time between dialysis and non-dialysis days.

Table 8-3: Comparison of Activity Time on Dialysis and Non-dialysis Days

Values expressed are mean ± SD. Differences between dialysis and non-dialysis days were compared using paired Wilcoxon test.

	Dialysis Days	Non-dialysis Days	p-value
Sedentary Activity Time (%)	89.4 ± 7.4	87.8 ± 8.7	0.013
Light Activity Time (%)	9.8 ± 5.7	10.6 ± 6.3	0.007
Moderate Activity Time (%)	1.6 ± 2.1	1.9 ± 2.7	0.015
Vigorous Activity Time (%)	0.02 ± 0.09	0.02 ± 0.07	0.387

As subjects spent a considerable amount of time sedentarily while receiving dialysis, this time was deducted from the total sedentary time on dialysis days and compared with the sedentary time on non-dialysis days. The proportion of sedentary time was then significantly higher on non-dialysis days compared to dialysis days (69.4% vs. 87.8%, $p < 0.001$ by paired Wilcoxon test) if the time spent on dialysis was not included.

8.3.4 Correlates of Activity Time

As vigorous intensity activity time was very small in the overall study population, the moderate and vigorous activity time was combined to reflect higher intensity activity time. Table 8-4 shows the correlates of M-V (moderate-vigorous) activity time.

Table 8-4: Correlates of Moderate-Vigorous Activity Time

Correlation coefficient shown is Spearman's (rho) correlation coefficient. CCI: Charlson Comorbidity Index; REE: Resting Energy Expenditure

	Correlation coefficient (rho)	p-value
Age	-0.484	< 0.001
Ethnicity	0.168	0.086
Weight	0.075	0.449
CCI	-0.505	< 0.001
REE	0.253	0.009
Fat-free mass (FFM)	0.222	0.028
Muscle Mass	0.246	0.015
Daily Protein Intake	0.370	< 0.001
Protein Intake/kg body weight	0.310	0.002
M-V Activity Time from RPAQ	0.330	0.001

M-V activity time correlated negatively with age and Charlson comorbidity index and positively with REE, fat-free mass (FFM) and muscle mass. The correlation between body weight and M-V activity time was not significant. The measured M-V activity time also correlated significantly with daily protein intake and protein intake normalised to body weight.

8.3.5 Correlates of Urea Generation Rate

The mean urea generation rate (G) was 4.7 (± 1.6) mg/min. Univariate correlation of G with anthropometric, metabolic and activity measures are shown in Table 8-5.

Table 8-5: Correlates of Urea Generation Rate

Correlation coefficient shown is Pearson's (r) correlation coefficient; REE: Resting Energy Expenditure; FFM – Fat-free mass; M-V activity time: Combined moderate and vigorous activity time

	Correlation coefficient (r)	p-value
Age	-0.306	0.002
Weight	0.598	< 0.001
REE	0.641	< 0.001
Watson Volume	0.657	< 0.001
FFM	0.709	< 0.001
M-V Activity time	0.204	0.037
Daily moderate activity time	0.213	0.029
Daily Protein Intake	0.108	0.298

Urea generation rate correlated negatively with age with fat-free mass having the highest correlation coefficient. G also correlated significantly with resting energy expenditure and moderate-vigorous activity time. There was no significant correlation between G and daily protein intake.

8.3.6 Relationship between G and REE and FFM

The relationship between G and REE is shown in Figure 8-1. There was a significant positive relationship between these two variables with a R^2 linear of 0.411.

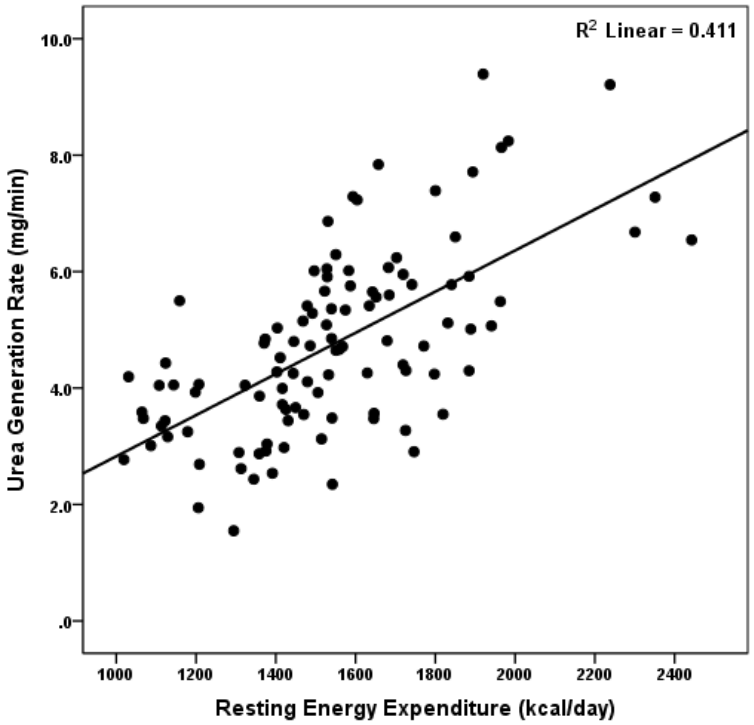


Figure 8-1: Relationship between REE and Urea Generation Rate

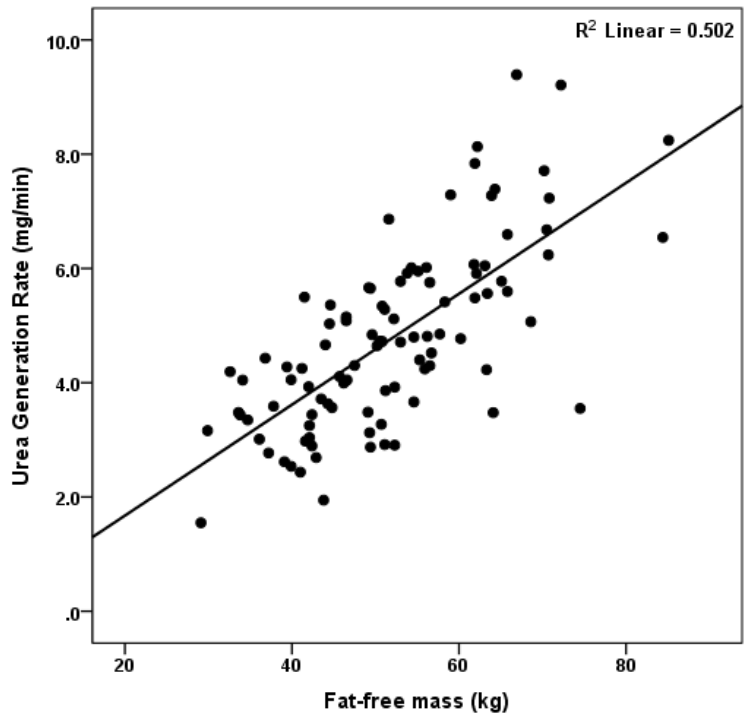


Figure 8-2: Relationship between FFM and Urea Generation Rate

Figure 8-2 shows the relationship between G and FFM which was better than that with REE with a R^2 linear of 0.502.

8.3.7 Relationship between Activity Time and G

Figure 8-3 and Figure 8-4 shows the relationship between M-V activity time with G and G normalised to body weight. In both these cases, there was a trend towards higher urea generation rate between the lowest and the highest quartiles of activity time though it did not reach statistical significance (p = 0.08 for urea generation rate and 0.06 for urea generation rate/kg body weight).

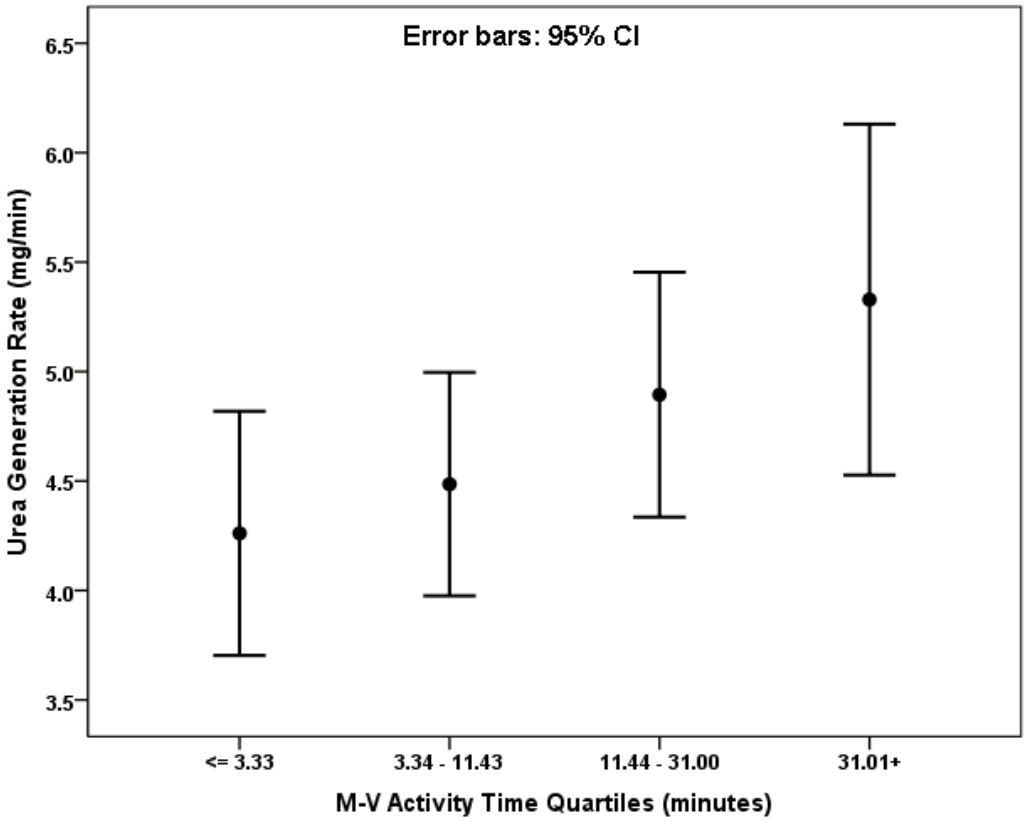


Figure 8-3: M-V Activity time and Urea Generation Rate

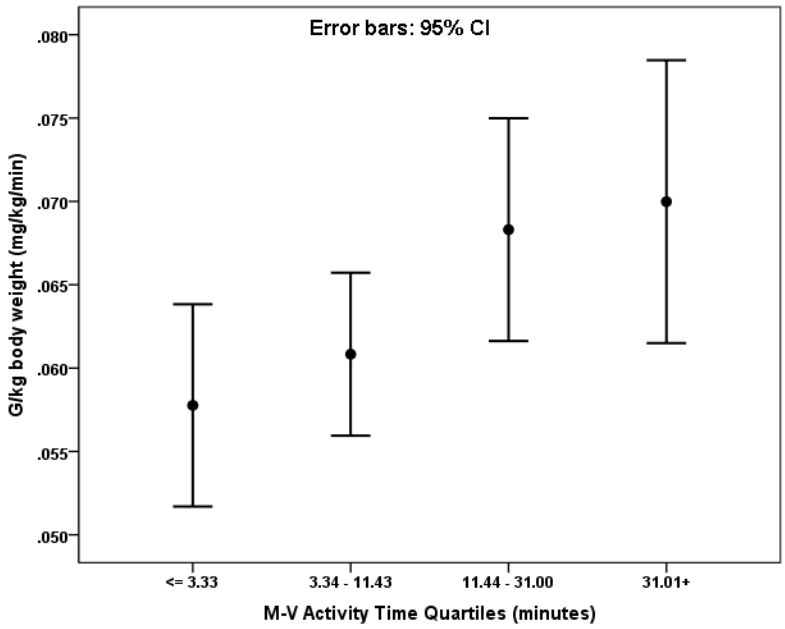


Figure 8-4: M-V Activity time and Urea Generation Rate normalised to body weight

M-V activity time was dichotomised using a cut-off value of 10 minutes. The difference in G and G normalised to body weight between the two groups of M-V activity time is shown in Figure 8-5 and Figure 8-6. The urea generation rate was significantly higher in patients who spent at least 10 minutes in performing moderate-vigorous activity time per day.

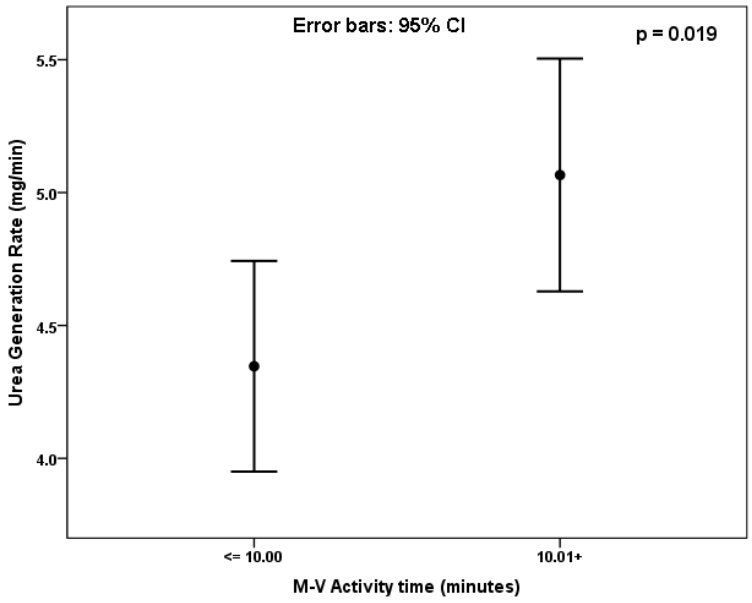


Figure 8-5: Effect of M-V Activity time on Urea Generation Rate

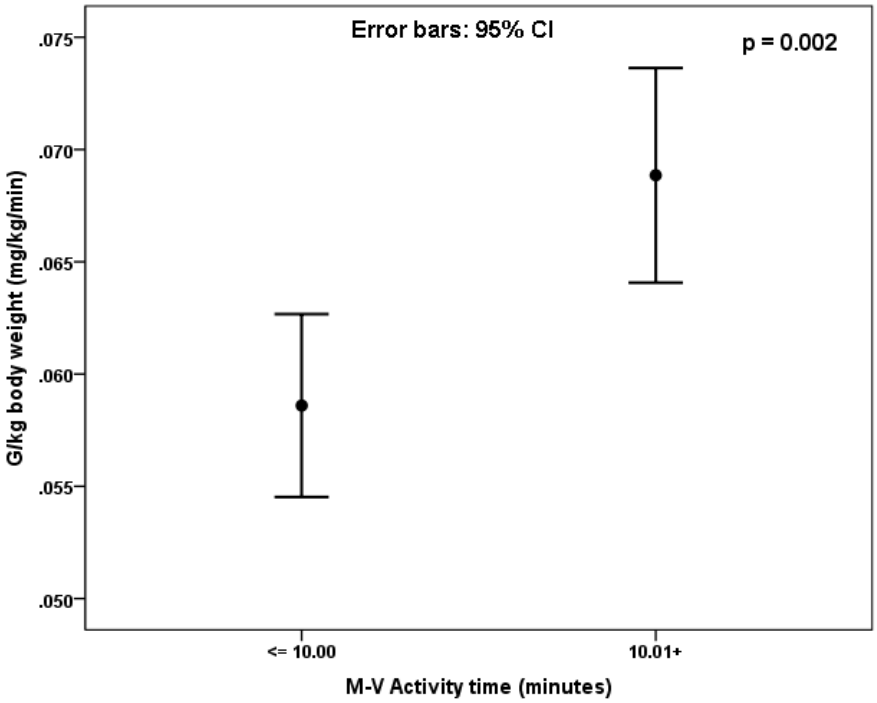


Figure 8-6: Effect of M-V Activity time on Urea Generation Rate normalised to body weight

8.3.8 Relationship between G and Ethnicity

The relationship between G and ethnicity was explored. Urea generation rate was normalised to body weight to adjust for any body size differences among patients of different ethnic origins. Figure 8-7 shows the difference in G/kg body weight between Asians and Non-Asians. Non-Asians had a significantly higher urea generation rate/kg body weight compared to Asians ($p = 0.019$).

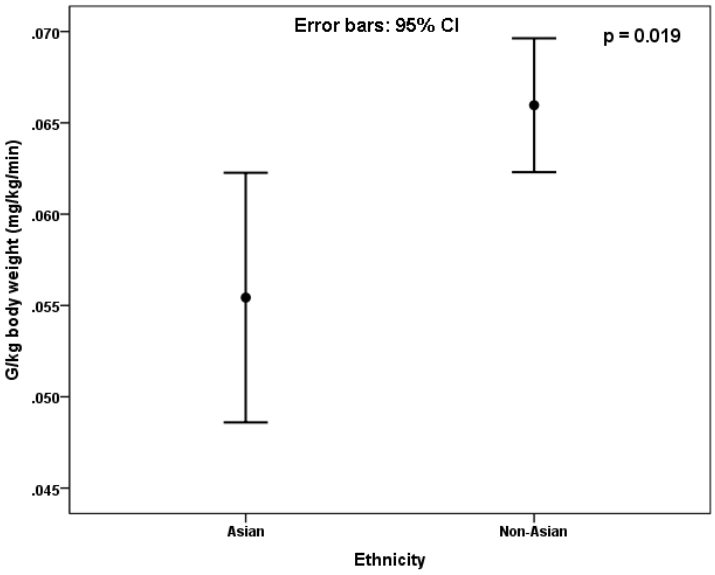


Figure 8-7: Urea Generation Rate normalised to body weight based on Ethnicity

8.3.9 Relationship between G and Protein Intake

Figure 8-8 shows a graph of G/kg body weight against dietary protein intake/kg body weight. There was a weak relationship between these variables with a R^2 linear of 0.068.

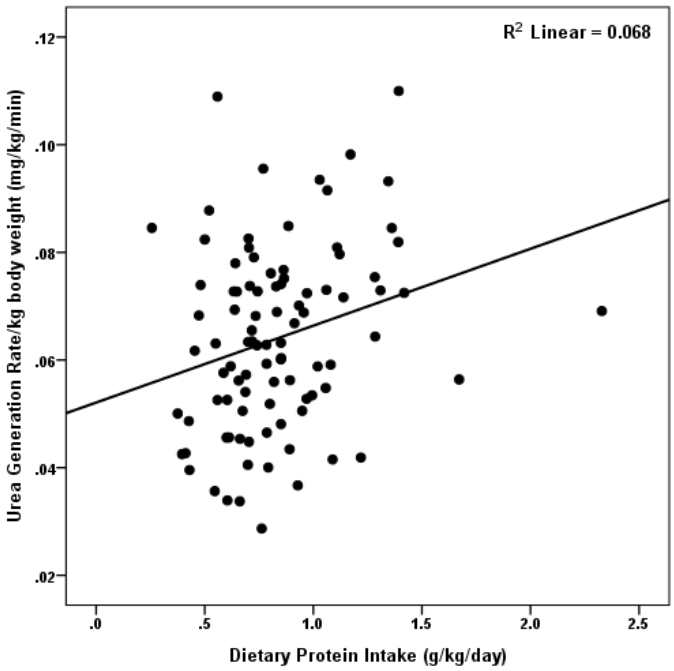


Figure 8-8: Relationship between Protein intake and Urea Generation Rate

8.3.10 Predictors of Urea Generation Rate

In order to explore the predictors of G, multivariate linear regression models were developed. M-V activity time was normalised using logarithmic transformation. The variables considered were age, sex, Asian ethnicity, weight, REE, Log of M-V activity time, dietary protein intake and Fat-free mass. Table 8-6 and Table 8-7 show two regression models of predictors of urea generation rate. Age and dietary protein intake did not contribute significantly to any of the models examined. The best model was the one that included Fat-free mass (adjusted R^2 – 0.486).

Table 8-6: Predictors of G with FFM excluded

Adjusted R^2 – 0.449

	Standardised Coefficient (Beta)	t	p-value
Constant		-0.902	0.369
Male Gender	0.206	2.617	0.010
Asian Ethnicity	0.208	2.837	0.006
Weight	0.543	6.988	< 0.001
Log M-V Activity Time	0.179	2.412	0.018

Table 8-7: Predictors of G with FFM included

Adjusted R^2 – 0.486

	Standardised Coefficient (Beta)	t	p-value
Constant		-0.997	0.321
Male Gender	-0.021	-0.222	0.825
Asian Ethnicity	0.059	0.779	0.438
Log M-V Activity Time	0.044	0.585	0.560
Fat-free mass	0.706	7.293	< 0.001

8.4 Discussion

The experimental hypothesis for the study was that higher physical activity level contributes to increased uraemic toxin generation. The study data showed that higher level of moderate to vigorous intensity activity contributes to increased urea generation rate. Moderate-vigorous activity time was also a significant predictor of urea generation rate in multivariate regression models adjusted for other factors such as age, sex, ethnicity and body weight. These findings lend support in favour of rejecting the null hypothesis and accepting the experimental hypothesis.

Habitual physical activity levels are low in haemodialysis (HD) patients. There are multiple physical and mental health benefits associated with higher activity levels in patients with kidney disease. Firstly, physically active HD patients have reduced mortality risk compared to sedentary patients (Matsuzawa et al., 2012; O'Hare et al., 2003). Secondly, exercise training in patients with kidney disease improves physical performance, skeletal muscle strength, exercise capacity and blood pressure control (Cheema et al., 2007; Cappy, Jablonka, & Schroeder, 1999; Boyce et al., 1997). Thirdly, exercise training is more likely to improve the quality of life and reduce symptoms of insomnia and depression (Anand et al., 2013; van Vilsteren, de Greef, & Huisman, 2005; Suh et al., 2002; Painter, Carlson, Carey, Paul, & Myll, 2000). Finally, increasing physical activity has also been shown to improve signs of

inflammation in patients with kidney disease (Viana et al., 2014; Castaneda et al., 2004). In view of these potential benefits, there has been increasing efforts to encourage physical activity in haemodialysis patients. Besides considering factors such as patient characteristics and feasibility, a holistic exercise programme in HD patients should also consider the direct impact of physical activity on dialysis requirements. But, there is a knowledge gap here in that the influence of PA levels on dialysis requirements has not been explored so far.

This study data showed that physical activity levels are extremely low in HD patients with the majority of the time being spent in sedentary activities. This is in keeping with previous published literature (Zamojska et al., 2006; Majchrzak et al., 2005). The time spent in moderate intensity activity was very low with no significant time spent at all in vigorous intensity activity. The sedentary activity time was higher on dialysis days compared to non-dialysis days. However, there was slight but significant increase in light and moderate activity time on non-dialysis days. The higher sedentary time on dialysis days may, in part, be due to the time spent receiving the dialysis treatment and possibly be secondary to the post-dialysis fatigue. Moderate-vigorous activity time correlated positively with metabolic parameters such as REE and FFM. There was also positive correlation with muscle mass. This suggests that even small increases, as seen in this cohort, in higher intensity activities can favourably impact on muscle mass. As would be expected, there was a negative correlation between activity time and age and comorbidity status. The activity time also correlated significantly with protein intake indicating that physically active individuals are likely to consume higher amounts of dietary protein.

Urea generation rate (G) was used as a surrogate marker of uraemic toxin generation in this study. Urea generation rate correlated positively with parameters such as weight, total body water (Watson volume), REE and FFM. The best correlation was with fat-free mass. Fat-free mass encompasses the muscle mass and visceral organs where the bulk of uraemic toxin production occurs. Hence, it is not surprising that G, a marker of uraemic toxin generation, correlated significantly with FFM. The relationship between these two factors was also evident in regression models where FFM was the single most important determinant of urea generation rate with the model explaining nearly 49% of the variance in G.

Urea generation rate also correlated positively with moderate-vigorous (M-V) activity time. In multivariate regression model adjusted for sex, ethnicity and body size, M-V activity time was found to be a significant predictor of G. Urea generation rate was significantly higher in patients who performed at least 10 minutes of M-V intensity activity per day and the significance persisted even after adjusting the urea generation rate to body weight. This suggests that even small increases in M-V activity time may influence uraemic toxin generation.

Though the cut-off of 10 minutes seems low, the significant contribution of log-transformed activity time in regression model for predicting G suggests that the relationship between activity time and G is likely to be a true effect. In a study by Matsuzawa et al (2012) on 202 HD patients, there was 28% reduction in all-cause mortality with 10-minute increments in daily activity (Matsuzawa et al., 2012). The M-V activity time cut-off of 10 minutes in this study is more likely a marker of overall levels of activity within the study cohort. The relationship between the M-V activity time of at least 10 minutes with G should be interpreted as a marker of the effect of activity on G rather than as an absolute value of time that may provide beneficial effects.

The suggestion from the study data that increased activity contributes to higher metabolic waste generation has important implications on the prescription of dialysis dosing. The current practice does not take into consideration the activity level of patients when deciding the dialysis dose. If the above effect of activity on uraemic toxin generation is confirmed in future studies, then it is important that an adjustment of dialysis dose is carried out depending on activity level of individual patients such that a higher dose is delivered for more active patients. This practice will have the dual benefit of individualising the dialysis treatment for the patient and facilitating better clearance of uraemic toxins according to the metabolic needs of the individual.

The average protein intake of the study participants was 0.84 g/kg/day which is much less than the recommended 1.1 g/kg/day of protein intake for HD patients (Naylor et al., 2013). However, there was no significant correlation found between urea generation rate and dietary protein intake. Although it is difficult to understand the lack of relationship between these variables, it is possible that the dietary records by the participants may not have been complete and did not reflect the entirety of

their diets. The dietary protein intake did not correlate significantly with normalised Protein Catabolic Rate (nPCR) as well. nPCR is estimated from inter-dialytic urea generation by urea kinetic modelling and is considered to be a marker of dietary protein intake. The poor correlation between nPCR and dietary protein intake, again, suggests possible incomplete dietary records and hence, it is difficult to derive any firm conclusions regarding the dietary protein intake of study participants.

The moderate-vigorous activity time estimated from RPAQ correlated significantly with that measured by accelerometer. However, participants spent the bulk of their time in sedentary activities which is not captured adequately by the questionnaire. As discussed in chapter 7, questionnaires derived in general population need to be validated in patients with kidney disease as their activity behaviour is different to that of healthy individuals. It is important to design activity questionnaires specifically designed to capture low intensity and sedentary activity time which will facilitate physical activity data collection in physically inactive patient subgroups such as those with kidney disease. Nevertheless, RPAQ performs relatively well in estimating activity levels as shown by comparison with objectively measured physical activity by doubly labelled water (chapter 7) and by accelerometer in this study.

8.5 Limitations

The study is limited by its cross-sectional nature. Physical activity is measured only at a single time-point. However, the measurements were carried out over a period of 7 days which might adjust for any short bursts of increased activity by individual subjects. Moreover, the activity level of participants is in line with published literature suggesting activity level in this study is representative of the haemodialysis patient population. There are some limitations associated with using urea generation rate as a marker of uraemic toxin generation. It has been suggested that urea kinetics is different to other uraemic toxins in the same class and hence, urea removal on dialysis may not be representative of overall uraemic toxin removal (Eloot et al., 2007; Eloot et al., 2005). No other molecule has been identified as the ideal marker of uraemic toxin till date. Urea clearance on dialysis has been shown to be associated with mortality risk (Bloembergen et al., 1996; Owen et al., 1993) and current dialysis adequacy guidelines are based only on urea clearance. Moreover, many of the uraemic toxins are end products of protein metabolism as is urea.

Hence, at present, urea generation rate is possibly the closest marker of overall uraemic toxin generation.

8.6 Conclusion

In conclusion, the study data supports the hypothesis that increasing physical activity level contributes to higher uraemic toxin generation as measured by urea generation rate. Fat-free mass was found to be the single most important determinant of uraemic toxin generation. Estimated activity time from RPAQ significantly correlated with measured activity time by the accelerometer. Further research is needed to develop physical activity questionnaires that would capture sedentary and low intensity activities.

Chapter 9

9. Relationship of Whole Body Protein Turnover and Resting Energy Expenditure in Chronic Kidney Disease

9.1 Introduction

Kidneys play a major role in protein and amino acid metabolism through involvement in all aspects of whole body protein turnover such as synthesis, degradation and excretion of amino acids. Approximately 50-70 g/day of amino acids is filtered and reabsorbed by the kidneys. As explained in section 2.3.1.2, malnutrition is common in patients with CKD. The mechanisms underlying the specific form of malnutrition that happens in renal failure, termed 'Protein energy wasting', are being increasingly explored in clinical studies. Whatever the underlying mechanisms are, it is clear that there is derangement of protein metabolism in renal failure.

Given the important role of kidneys in protein metabolism, deranged protein metabolism is expected. However, the magnitude of derangement and the specific abnormalities in various components of the protein turnover have not been clearly studied. A detailed review of whole body protein turnover studies carried out in patients with CKD and in those on HD is discussed in section 2.3.1.2 and is not discussed here for reasons of brevity. It can be seen from these studies there has not been a definitive conclusion about the alterations in protein turnover in HD patients.

Protein turnover is an important component of basal metabolic rate and constitutes about 20% of resting energy expenditure in healthy adults (Boirie et al., 2001; Welle & Nair, 1990). However, this relationship has not been explored in the setting of renal failure. Although it is not expected that the energetic cost of protein turnover will be any lower than healthy adults but, given the derangement in protein metabolism, it may be higher in patients with advanced renal disease. The energetic cost of protein turnover in CKD has not been studied previously.

Dietary protein intake has a significant impact on whole body protein turnover but whether this is an important determinant of resting energy expenditure (REE) is not

known. Moreover, many of the uraemic toxins are the product of tissue protein turnover and hence, protein turnover is likely to influence uraemic toxin generation in patients with renal failure.

This pilot study was therefore conducted with the following objectives:

1. To measure whole body protein turnover (WBPT) in maintenance haemodialysis patients and in those with advanced CKD not receiving any form of dialysis therapy

2. To estimate the energetic cost of protein turnover in the above patient population

9.2 Considerations on isotope selection and methodological issues in kidney disease

9.2.1 Measurement Methods for Whole Body Protein Turnover

Measurement methods for whole body protein turnover can be broadly categorised into two categories – precursor method and end product method. Both these methods involve administering a stable isotope of an amino acid orally or intravenously and measuring isotopic enrichment of the amino acid or its immediate transaminated product in various body pools. However, the difference lies in the site of sampling and the molecule that is sampled as discussed below.

In the end-product method, a nitrogen-labelled stable isotope of an amino acid (typically ^{15}N-glycine) is administered orally or intravenously. The nitrogen in the amino acid consumed is then absorbed and is metabolised in a similar way to other proteins and finally incorporated into urea and ammonia which are then excreted in urine and faeces. The amount excreted in faeces is considered to be negligible and hence, the isotopic enrichment of ^{15}N in urea and ammonia in urine is measured to enable calculation of WBPT. The urine samples are collected at regular intervals throughout the 10-hour study period. The advantage of this method is that it is non-invasive (when the isotope is given orally) as the only samples needed are the urine samples. However, this method assumes normal excretion of urea and ammonia within the study duration which may not hold true in patients with kidney disease. Moreover, many of the HD patients are anuric which poses a practical problem in

this population. Even though, this method has been used in a study of WBPT in peritoneal dialysis patients, it has not been validated for use in renal failure or anuric patients (Tjiong et al., 2008).

The other method is the precursor method. Here, the stable isotope of an amino acid is given intravenously, typically as a priming bolus dose followed by an infusion either in the fed or post-absorptive state. Once the steady state is reached, a series of blood (and breath samples in certain studies) samples are taken to measure the isotopic enrichment of the amino acid or a related metabolite of the infused amino acid which will enable calculation of the WBPT. The steady state is the period when the isotopic enrichment has reached plateau level with minimal variation between the readings. The choice of the amino acid used for the study becomes important in patients with kidney disease due to the derangement of amino acid handling in this condition.

Two stable isotopes were considered as potential candidates for our study – $^{2}H_{5}$-phenylalanine and ^{13}C-leucine. The former isotope, when infused, is rapidly converted to tyrosine and measuring the isotopic enrichment of phenylalanine and tyrosine at steady state will enable calculation of WBPT (Thompson et al., 1989). This method is based on the assumption that in-vivo conversion of phenylalanine to tyrosine is not impaired in study subjects. In steady-state conditions, conversion of phenylalanine to tyrosine occurs predominantly in liver and kidneys. It has been shown that this pathway is impaired significantly in patients with renal failure as the contribution of kidneys to the phenylalanine metabolism is very little (Kopple, 2007). Hence, phenylalanine isotope may not be suitable for WBPT studies in renal failure and as such, has not been validated for use in this patient population.

This means that ^{13}C-leucine is the ideal isotope to use for WBPT in patients with kidney disease. The advantages of using leucine isotope for WBPT measurement and the model of leucine turnover are discussed in section 3.9.1.

9.2.2 Safety of the Isotope

^{13}C-leucine is a stable isotope and is non-radioactive. The isotope has been used for WBPT studies in normal healthy adults and in various disease conditions. Sterility and pyrogen testing was carried out by the isotope providers and a certificate of

analysis (shown in Appendix F) was provided for each batch of the isotope purchased. The isotope infusions were prepared in a strict sterile manner as per the local hospital guidelines.

9.2.3 Assumptions in the methodology

There are several assumptions made in the protein turnover study model. The first is that the body pools are homogenous. Although this may not be correct at all times, this assumption is necessary for analysis of any protein turnover studies. The second assumption is that the amino acid exchanges between compartments occur at constant fractional rates. Although this assumption is essential in certain models of protein turnover studies especially those involving compartmental analysis, it is irrelevant in stochastic analysis such as that used in this study where steady state of tracer is achieved.

The third assumption is that the amount of tracee amino acid in each pool remains constant throughout the study period i.e. the tracee is in a steady state. This assumption is reasonable and accurate enough in studies conducted over a period of few hours. Finally, the whole body protein pool is considered to be turning over at a slow enough rate to ensure that there is no recycling of the tracer within the study period. This is dependant on the rate of turnover of the tracer amino acid in a given pool and the duration of the study period. Although the tracer recycling is not to be ignored, it is unlikely that this will cause significant errors in studies conducted over a few hours compared to those carried out over days.

9.2.4 Timing of the samples

It is vital to obtain a series of blood and breath samples after the steady state is reached for measurement of isotopic enrichment. Previous published studies showed the steady state would be reached around 2 hours from the start of the infusion. Samples were collected every 30 minutes for the first 2 hours to establish that the steady state has been reached and every 15 minutes between 2 and 3 hours for measurement of isotopic enrichment.

9.3 Methods

9.3.1 Ethical Review

The study was reviewed and approved by Cambridge East Research Ethics Committee (ref: 13/EE/0158 and UH Ethics ref: LMS/PG/NHS/00256). All study participants provided written informed consent.

9.3.2 Overview of Study Design

A cross-sectional study of 12 patients – 6 of them receiving maintenance haemodialysis and 6 of them with advanced renal disease (eGFR < 20 ml/min/1.73 m^2) – was carried out to measure WBPT. All subjects maintained a prospective food diary for three days prior to the study day. Subjects who were not anuric also performed a 24-hour urine collection starting the day before the study. On the study day, metabolic analysis was performed including indirect calorimetry for REE measurement. Leucine isotope infusion was carried out for 3 hours. Blood and breath samples were collected at specified time points from the start of the infusion. There were no further follow-up visits for the study.

9.3.3 Study Population

Subjects were recruited from the renal unit and the outpatient clinics at Lister Hospital. Potential patients were initially approached in person when attending their regular HD session or the outpatient clinic. A written information sheet about the study was given to them. They were given a minimum of 24 hours to consider participation in the study and were given an opportunity to clarify any queries about the study before they consented. The inclusion and exclusion criteria are given below.

9.3.3.1 Inclusion Criteria

- Adult haemodialysis patients older than 18 years of age and have been on maintenance HD for at least 3 months
- CKD patients with an eGFR of <20 ml/min/1.73 m^2 and not receiving any form of renal replacement therapy

9.3.3.2 Exclusion Criteria

Patients with

- untreated hypo or hyperthyroidism
- active malignancy or receiving chemotherapy/radiotherapy
- ongoing sepsis
- active vasculitis or lupus
- history of hospitalisation in the last month
- current pregnancy
- unexplained weight loss
- metabolic acidosis
- limb amputations
- no capacity to consent for themselves

9.3.4 Study Assessments

9.3.4.1 Study Preparation

All subjects were given verbal and written instructions about the study. They were advised to restrain from strenuous physical activity for 3 days preceding the study day. They were also asked to maintain a food diary and were advised to avoid protein supplements and excessive protein intake for those three days. Participants who were not anuric also performed a 24-hour urine collection beginning the day prior to the study.

9.3.4.2 Demographic and Anthropometric data

Data comprising of age, sex, ethnicity, weight, height and comorbid conditions were collected for all study participants through direct measurements and individual medical records.

9.3.4.3 Study Procedure

Participants were advised to attend the research facility in an empty stomach after overnight fasting. Body composition analysis was performed using bioimpedance as described previously. Isotope administration and sampling was carried out as described in section 3.9.1.1. Indirect calorimetry was performed at baseline and at steady state to obtain direct measurements of REE and VCO_2. All participants were also asked to complete the RPAQ questionnaire to obtain information regarding habitual physical activity data.

9.3.5 Calculation of Protein Turnover and Energetic Cost of WBPT

The individual components of WBPT – synthesis, breakdown and oxidation rates and the total flux – were calculated as explained in the general methodology chapter (section 3.9.1.3).

The best-fit regression line equation was identified using a plot of leucine flux and REE which was of the form

$$\boldsymbol{REE = a \times Leucine\ Flux + Constant}$$

Using this equation energy expenditure of protein turnover for each patient was estimated using the leucine flux. Energetic cost of protein turnover is then the proportion of REE that has been contributed by the protein turnover.

9.3.6 Statistical Analysis

Leucine flux at steady state and the rate of whole body protein synthesis, oxidation and breakdown calculations are described in detail in section 3.9.1.3. Continuous variables are expressed as mean ± SD. Differences between CKD groups were compared using unpaired t-test. Correlations between variables were examined using Pearson's correlation coefficient.

9.4 Results

9.4.1 Demographics of Study Participants

A total of 12 patients with kidney disease were recruited – 6 patients with advanced CKD (eGFR < 20ml/min/1.73 m^2) not receiving dialysis therapy (CKD group) and 6

patients on chronic maintenance haemodialysis (HD group). However, one of the CKD patients was found to have abnormally high CRP on the day of the study and hence, has been excluded from analyses.

The demographic and clinical data of the study participants are shown in Table 9-1. The mean eGFR in the CKD group was 15.4 (±2.8) ml/min/1.73m^2.There were no differences between CKD and HD groups in age, body size measures and haemoglobin. There were also no differences in REE, daily protein and energy intake between the groups. Serum albumin was significantly lower in HD group ($p = 0.015$). Physical activity, expressed as Daily MET from RPAQ, was significantly lower in HD group.

Table 9-1: Demographic and clinical data of Study participants

Values expressed are mean ± SD. Differences between the groups were examined by unpaired t-test; nPCR: Normalised Protein Catabolic Rate; REE: Resting Energy Expenditure

	All patients (n = 11)	CKD group (n = 5)	HD group (n = 6)	p-value
Age (years)	65.3 ± 8.2	65.7 ± 9.7	65.0 ± 7.6	0.891
Males: Females	5:6	2:3	3:3	
Height (cm)	166.8 ± 9.3	168.0 ± 9.9	165.8 ± 9.6	0.721
Weight (kg)	73.8 ± 8.9	75.0 ± 8.1	72.8 ± 10.2	0.706
Haemoglobin (g/dL)	11.2 ± 1.7	10.5 ± 1.5	11.9 ± 1.7	0.194
Albumin (g/L)	38.9 ± 2.7	40.8 ± 2.3	37.2 ± 1.7	0.015
Urea generation rate (mmol/min)	0.18 ± 0.06	0.19 ± 0.09	0.18 ± 0.04	0.785
nPCR (g/kg/day)	0.92 ±0.25	0.96 ± 0.34	0.90 ± 0.18	0.707
Protein Intake (g/kg/day)	0.94 ± 0.21	1.03 ± 0.27	0.87 ± 0.15	0.249
Energy Intake (kcal/day)	1743 ± 338	1853 ± 336	1651 ± 341	0.351
Daily MET	1.27 ± 0.11	1.34 ± 0.09	1.21 ± 0.10	0.038
REE (kcal/day)	1317 ± 225	1237 ± 217	1383 ± 228	0.309

9.4.2 Measures of Whole Body Protein Turnover

Table 9-2 shows the mean (± SD) values of different components of whole body protein turnover for 11 patients included in the analysis. The breakdown rate was higher than the protein synthesis rate and as a result, there was negative balance (Synthesis rate minus Breakdown rate) in terms of whole body protein turnover.

Table 9-2: Components of Whole Body Protein Turnover

	Mean ± SD
Leucine Flux (μmol/kg/hr)	92.3 ± 11.1
Synthesis rate (μmol/kg/hr)	83.3 ± 11.0
Oxidation rate (μmol/kg/hr)	9.0 ± 3.7
Breakdown rate (μmol/kg/hr)	92.3 ± 11.1
Net balance (μmol/kg/hr)	-9.0 ± 3.7

9.4.3 Clinical Correlates of WBPT

Univariate correlation between clinical parameters and the net balance of WBPT are shown in Table 9-3.

Table 9-3: Clinical Correlates of WBPT Net Balance

The coefficient shown is Pearson's correlation coefficient; nPCR: Normalised Protein Catabolic Rate

	Correlation Coefficient	p-value
Age	0.048	0.889
Serum Albumin	-0.461	0.154
Urea Generation Rate	-0.663	0.026
nPCR	-0.624	0.040
Daily Protein Intake per kg body weight	0.373	0.259
Daily Energy Intake	-0.641	0.033
Fat-free mass	-0.216	0.523

Urea generation rate, nPCR and daily energy intake were the only parameters which correlated significantly with WBPT net balance. Both synthesis and breakdown rates did not correlate significantly with any of these parameters.

9.4.4 Correlation between WBPT and Physical Activity Measures

Table 9-4 shows the correlation between net turnover balance and physical activity measures derived from activity questionnaire RPAQ. Mean Daily MET estimated from RPAQ correlated highly with net turnover balance. As with the clinical parameters, neither the breakdown rate nor the synthesis rate correlated significantly with any of these activity measures.

Table 9-4: Correlation between Net Turnover Balance and Activity Measures

The coefficient shown is Pearson's correlation coefficient; MET: Metabolic Equivalent of Task

	Correlation Coefficient	p-value
Daily MET	-0.839	0.001
Moderate Activity Time	-0.403	0.220
Vigorous Activity Time	0.150	0.660

9.4.5 Comparison of WBPT between CKD and HD Groups

The differences in various components of whole body protein turnover between the CKD and HD groups are shown from Figure 9-1 to Figure 9-3. The protein breakdown and synthesis rates were significantly higher in HD group compared to that of CKD group. However, there was no significant difference in the net balance of protein turnover between the groups.

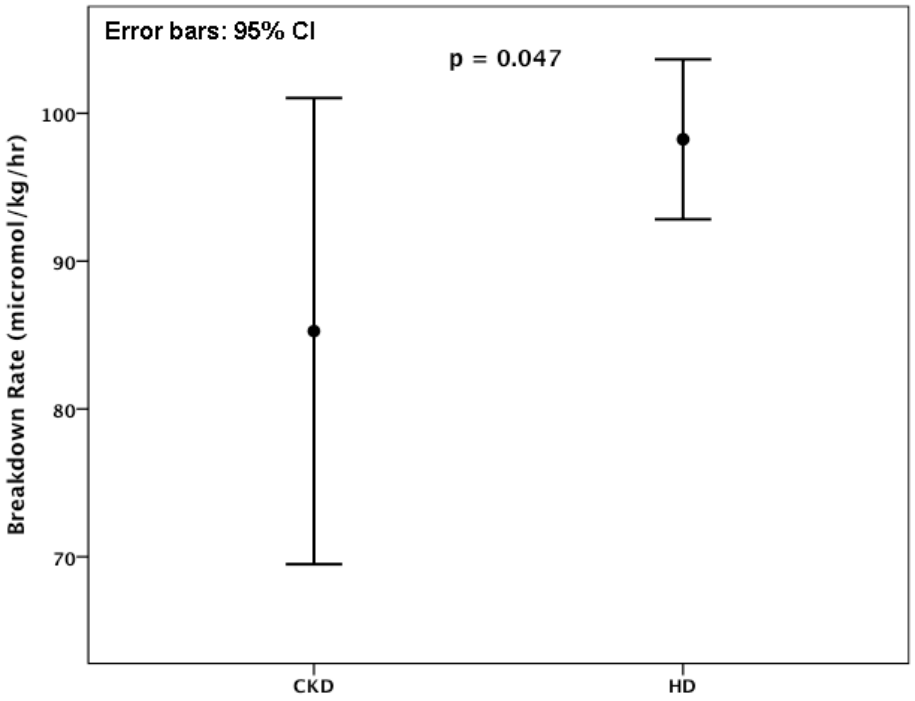


Figure 9-1: Protein Breakdown rate in the CKD and HD groups

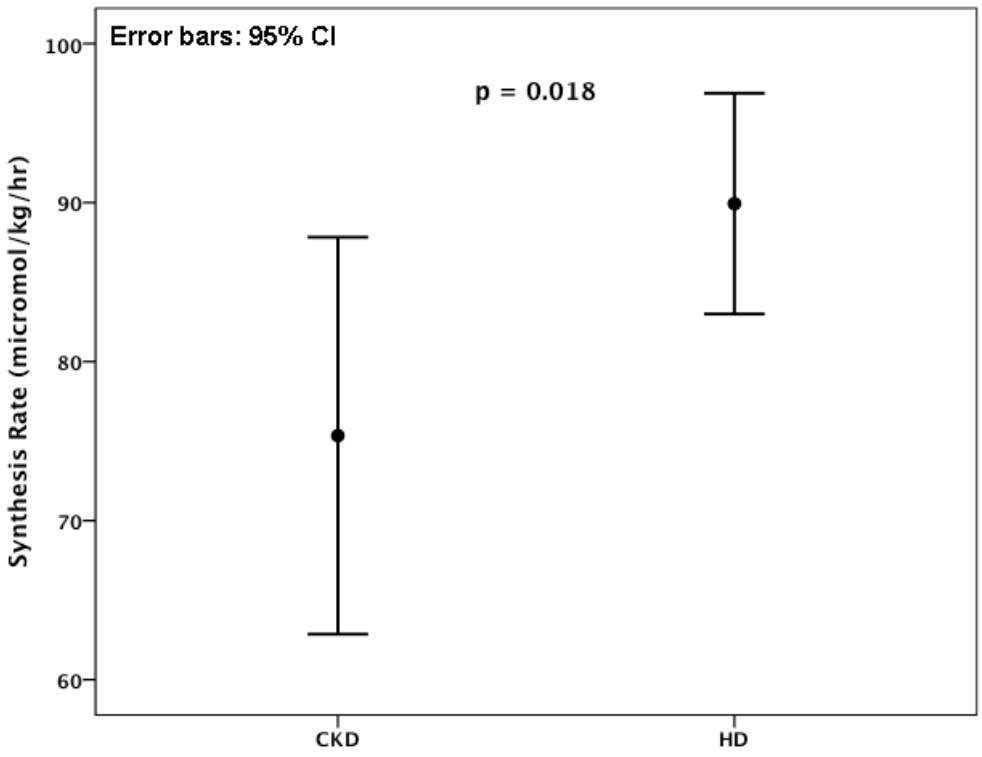


Figure 9-2: Protein Synthesis rate in CKD and HD groups

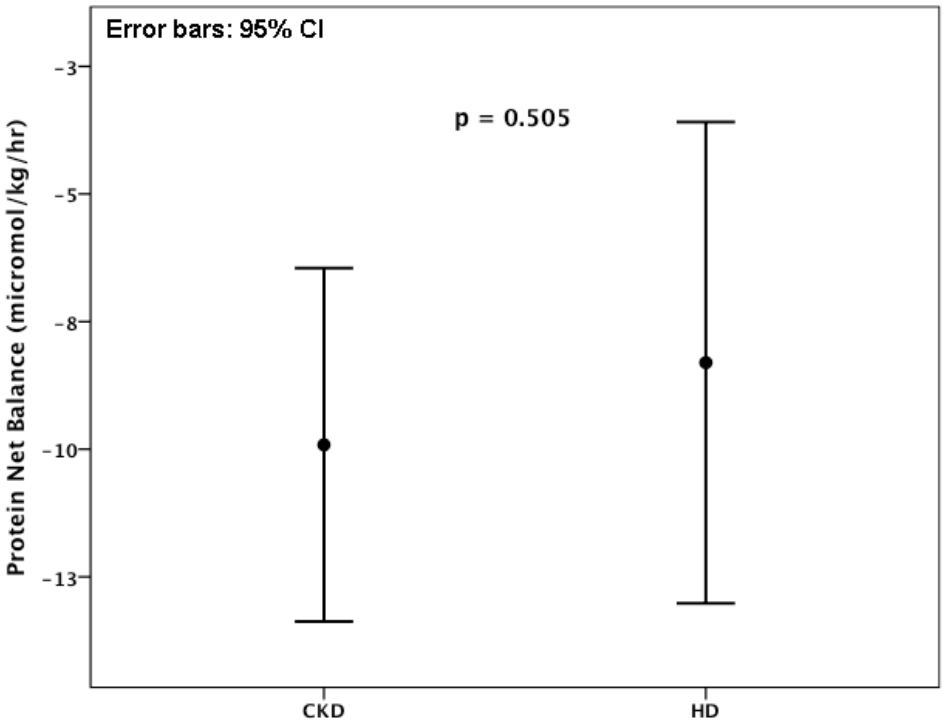


Figure 9-3: Net Balance of Protein Turnover in CKD and HD groups

9.4.6 Energetic Cost of Protein Turnover

The relationship between leucine flux and REE is shown in Figure 9-4. There was a moderate relationship between the two variables with a R^2 value of 0.143. The equation of the best-fit regression line is as shown in the figure. The energy expenditure associated with WBPT was calculated from the regression equation. Energy cost of WBPT was then calculated as a percentage of measured REE. The mean energy expenditure associated with WBPT was 705 (± 85) kcal/day. This equates to a mean energetic cost of 55% of the resting energy expenditure per day for Whole Body Protein Turnover.

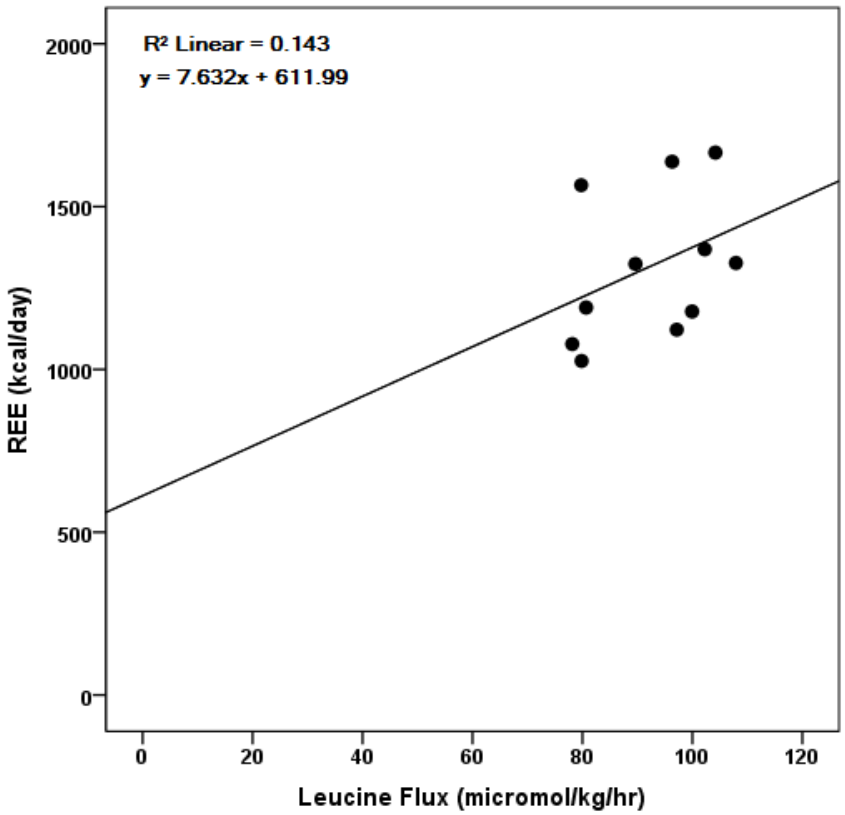


Figure 9-4: Relationship between Leucine flux and REE

9.4.7 Relationship between Protein Turnover and Urea Generation Rate

The relationship between urea generation rate and protein breakdown rate is shown in Figure 9-5. There was a moderate positive relationship between these two variables with a R^2 value of 0.263.

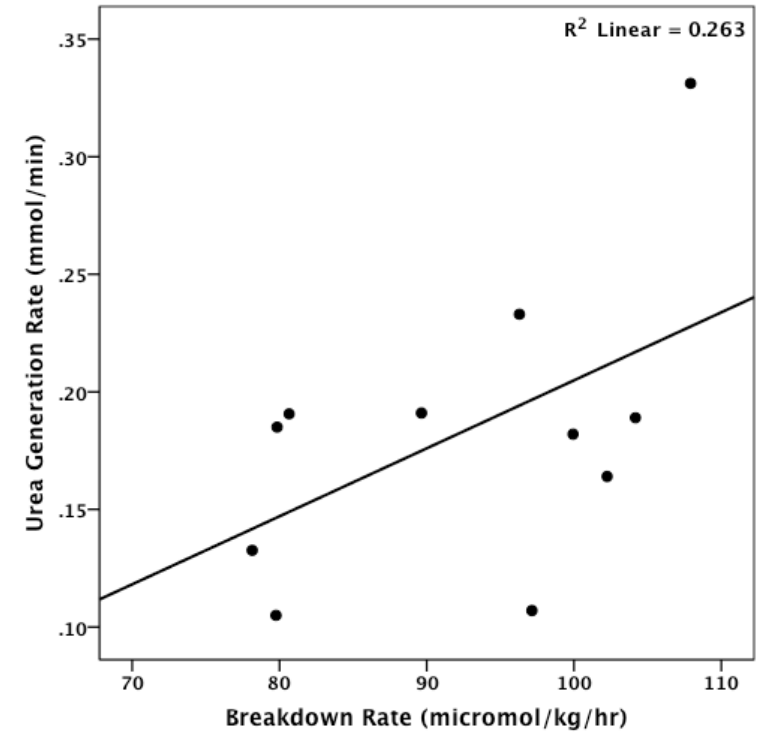


Figure 9-5: Relationship between Protein Breakdown and Urea Generation Rate

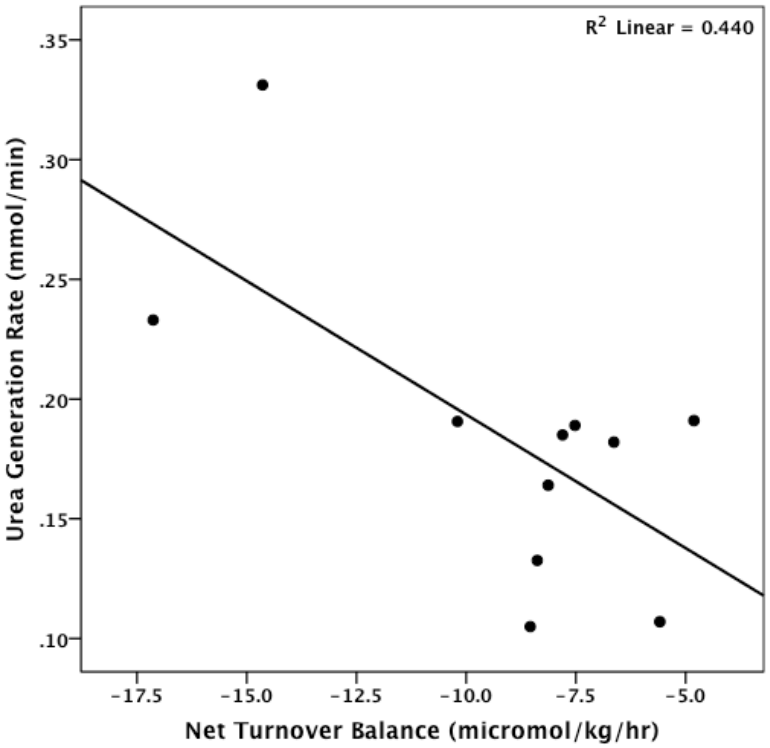


Figure 9-6: Relationship between Net Turnover Balance and Urea Generation Rate

Figure 9-6 shows a graph of urea generation rate against net turnover balance. The variables correlated negatively with more negative net balance of protein turnover contributing to higher urea generation rate.

9.5 Discussion

The primary aim of this study was to measure whole body protein turnover in pre-dialysis CKD patients and in chronic haemodialysis patients. The study showed that both pre-dialysis and HD patients are in negative protein balance. The study also showed that dialysis patients have a significantly higher protein turnover compared to pre-dialysis CKD patients i.e. a higher rate of breakdown and synthesis. But, the balance between the synthesis and breakdown is of similar magnitude between the groups. The energetic cost of WBPT per day in this cohort of patients was around 55% of the daily resting energy expenditure.

It is widely believed that uraemia is a catabolic state. In animal models, an increase in protein degradation and reduction in protein synthesis has been demonstrated in the presence of uraemia (Arnold & Holliday, 1979; Harter, Karl, Klahr, & Kipnis, 1979; Holliday, Chantler, MacDonnell, & Keitges, 1977). This has not been replicated universally in human studies. A few studies have compared protein turnover in CKD patients and healthy controls and have come to contrasting conclusions. A study by Adey et al (2000) on 12 CKD patients and 10 healthy controls showed that there was no difference between different components of WBPT between CKD patients and healthy controls (Adey et al., 2000). Similarly, Goodship et al (1990) showed no differences in WBPT in non-dialysis CKD patients compared to healthy controls irrespective of whether they were on a high or low protein diet (Goodship et al., 1990). However, some other studies have shown reduced turnover, oxidation and synthesis in CKD patients compared to healthy controls (Lim & Kopple, 2000; Castellino et al., 1992).

Comparative studies examining whole body protein turnover in pre-dialysis CKD and HD are scarce. The only such study was published by Lim et al (1998) in which WBPT was measured in a same cohort of patients before initiation of dialysis therapy and 8-10 weeks after initiation of haemodialysis (Lim et al., 1998). In this study, though there was an increase in protein breakdown rate, there was a relatively

higher increase in protein synthesis rate after HD initiation compared to pre-dialysis period.

The study data compares WBPT between pre-dialysis CKD patients and HD patients. The protein breakdown rate was significantly higher in HD patients compared to CKD patients which was also matched by the increase in synthesis rate in HD group. This implies that the turnover rate is much higher after initiation of haemodialysis therapy. Studies have shown that the dialysis treatment itself induces increased protein catabolism and higher protein loss and this effect might even last for many hours after the completion of a dialysis session (Raj et al., 2004; Ikizler et al., 2002). Though there was a net negative balance of protein turnover in HD patients, there was no difference in the magnitude of the protein loss compared to pre-dialysis patients. There was a 19% increase in synthesis rate and 15% increase in breakdown rate in HD patients compared to CKD group. This is comparable to the previously published data showing approximately a 19% increase in synthesis and breakdown rates after initiation of haemodialysis therapy (Lim & Kopple, 2000).

The study also aimed to explore the energetic cost of protein turnover in patients with kidney disease. It was found that protein turnover contributes to about 55% of the basal metabolic rate (i.e. REE). This is in line with previously published literature in normal individuals which showed the energetic cost of protein turnover to be approximately 50% of the metabolic rate (Nair & Halliday, 1985). However, if the metabolic rate was adjusted for active cell mass, the energetic cost reduces significantly to around 20% of metabolic rate (Welle & Nair, 1990). It is worth noting that these previous data are derived from young healthy individuals. Though there is a suggestion that the energetic cost of protein turnover is similar between young and elderly individuals (Boirie et al., 2001), no previous data are available for comparison in patients with kidney disease. The small numbers of patients did not permit calculation of energetic cost in each group in this study and hence, any potential difference in energetic cost between CKD and HD groups was not explored.

The study also showed a significant negative correlation between urea generation rate and the net turnover balance. As discussed in previous chapters, urea generation rate can be considered a surrogate marker of uraemic toxin generation. Given that many of the uraemic toxins are products of protein metabolism, it is not

surprising that protein turnover contributes significantly to uraemic toxin generation. However, this relationship between whole body protein turnover and uraemic toxin generation has not been demonstrated before. This implies that individuals with higher protein turnover rates may generate higher amounts of uraemic toxins thereby necessitating dialysis dose adjustments based on metabolic needs.

The study has several strengths. All studies published on this topic to date have been carried out on younger individuals, typically in their mid-40s. The mean age of patients in this study was 65 years which makes it the first study to examine protein turnover in patients with kidney disease above the age of 65. Apart from one published study on this topic, there have been no studies comparing WBPT between pre-dialysis CKD and HD patients. The patients in the two groups were well matched in terms of age, body size and dietary parameters which allowed better comparison of the groups with regards to protein turnover. By comparing these two groups, this study has contributed to a better understanding of protein turnover changes that occur after initiation of haemodialysis therapy. This is also the first study that had explored the energetic cost of protein turnover in patients with kidney disease. And finally, this study has also demonstrated a significant positive relationship between whole body protein turnover and uraemic toxin generation in patients with advanced kidney disease which has not been shown in any studies to date.

9.6 Limitations

The study is limited by its small sample size. The intensive nature of the whole body protein turnover study combined with patient factors such as older age and comorbidity that are prevalent in patients with advanced kidney disease restricts the sample size of such studies to small numbers. This is evident in majority of protein turnover studies in patients with kidney disease where the sample size is restricted to around 6-12 patients. Whilst these studies, including the current study, provide valuable information on whole body protein turnover in renal failure, any definitive conclusions based on these study data need to be made cautiously. The small sample size had also restricted the comparison on energetic cost of protein turnover between the CKD and HD groups.

9.7 Conclusion

In summary, the rate of protein turnover was significantly higher in chronic haemodialysis patients compared to pre-dialysis patients with advanced kidney disease. The net turnover balance was negative, albeit to a similar magnitude, in both the pre-dialysis and haemodialysis patient groups. The energetic cost of protein turnover in these patients was around 55% of resting energy expenditure. There was also a direct relationship between protein turnover rate and uraemic toxin generation.

Chapter 10

10. Determinants of Habitual Physical Activity in Haemodialysis Patients

10.1 Introduction

One of the primary functions of dialysis is to remove metabolic waste products. Judging minimal requirements for dialysis is difficult. The metabolic waste removal is not the only function of dialysis. Other aspects of renal replacement therapy such as excess fluid removal, nutrition, anaemia management and calcium and phosphate management do need to be taken into account when defining adequate dialysis. However, in practice, the dialysis dose is quantitatively measured based on the effectiveness of urea clearance during a dialysis session.

The urea-centric model of dialysis adequacy has some disadvantages to it as discussed in section 2.2.1. Although urea removal has been shown to correlate with better survival (Owen et al., 1993), urea in itself is not toxic even at high concentrations. A study by Johnson et al (1972) in patients with end-stage renal disease showed that patients did not have any adverse symptoms until the urea concentration was as high as 107 mmol/L (normal range: 2.5 – 7.8 mmol/L) (Johnson, Hagge, Wagoner, Dinapoli, & Rosevear, 1972). Moreover, even uraemic toxins that are similar in size to urea do not share the same kinetics in vivo and hence, may not be cleared to the same extent as urea. In addition, larger sized toxins and the protein bound uraemic solutes also have dissimilar kinetics to urea.

In current practise, dialysis dose is prescribed by a dimensionless parameter termed Kt/V where K is the dialyser clearance of urea in a single dialysis session, t is the duration of the session and V is the urea distribution volume which is equal to total body water. This dose prescription is not based on the rate of metabolic waste production but on the presumed volume in which the toxins are distributed. This practise of normalising dialysis dose to total body water may disadvantage some groups of dialysis patients as explained below.

Total body water (V), sometimes referred to as Watson volume, is routinely estimated using Watson formula (Watson et al., 1980). This formula was derived by

pooling total body water data from multiple studies in healthy adults. For any given age, height and weight, women will have lower estimated V compared to men. Similarly, men with small body size will have lower V compared to their larger counterparts. This implies that for a given Kt/V target, women and small men will receive less dialysis dose compared to larger men. This may be the reason behind the survival advantage noticed in women when given higher dialysis doses (Depner et al., 2004; Port et al., 2004). Moreover, it has been shown that for a given Kt/V, patients with low V have as much as two-fold higher relative risk of mortality (Wolfe et al., 2000). Whilst it can be argued that individuals with low V also are prone to malnutrition, it is likely that the current dialysis practice of using V as a normalising factor also contributes to this effect. This may be due to the differences in body composition and potentially a higher toxin load per unit of body weight in small individuals as discussed in sections 2.2.3 and 2.3.1.1.

As a result of this, a number of parameters have been suggested as potential alternatives for scaling the dialysis dose. A review by Daugirdas et al (2008) examining the theoretical aspects of these parameters concluded that Body Surface Area (BSA) is likely to be the best alternative parameter. However, as explained in section 2.3.1, metabolic rate (i.e. resting energy expenditure) could be a more physiological parameter to use for scaling dialysis dose. This may be due to the fact that metabolic rate and GFR scale to body weight by approximately the same exponent. Singer and Morton also hypothesised that the metabolic rate drives the GFR in those with normal renal function. It has been shown that GFR is reduced in patients with untreated hypothyroidism and the opposite effect prevails in those with hyperthyroidism. These changes could be due to corresponding changes in metabolic rate in these conditions (Ford et al., 1961; Davies et al., 1952; Mackay & Sherrill, 1943). Moreover, as resting energy expenditure (REE) represents the sum total of all metabolic activity at rest, it may be a better marker of metabolic waste generation.

Total energy expenditure (TEE) is a combination of REE, energy expenditure from physical activity and the thermic effect of food. TEE encompasses more potential sources of metabolic waste production and hence, may potentially be a better marker of total metabolic activity. In the study described in chapter 4, it has been shown that TEE influences urea generation rate which is a marker of uraemic toxin

generation. Moreover, as physical activity is the most variable component of TEE between individuals, scaling dialysis dose to TEE may also have the benefit of individualising the dialysis dose as per the metabolic needs.

There have been no published studies exploring the applicability of these three parameters to clinical practice for scaling dialysis dose. The benefits of these three parameters in relation to patient outcomes are also not known. Ramirez et al (2012) showed that BSA-normalised dialysis dosing will deliver higher dose to women and may have a favourable impact on survival of women receiving haemodialysis (Ramirez et al., 2012). However, REE and TEE has not been examined in a similar way nor has there been a comparative study to explore all three parameters together.

In order to address this issue, a large-scale, multi-centre prospective study was designed with a primary aim of exploring the applicability of these parameters to clinical practice and to examine the impact on patient survival with potential use of these parameters for scaling dialysis dose. As there are multiple elements to this study, the study results are presented in three separate chapters for clarity and ease of reading. The self-report comorbidity questionnaire, described in chapter 5, was developed as a part of this study and was used in this study for comorbidity data collection. This chapter discusses the physical activity profile of the study participants and determinants of physical activity in this cohort of haemodialysis patients. The next chapter discusses the potential benefit of scaling dialysis dose using BSA, REE and TEE. The following chapter discusses the potential impact of using these parameters on patient survival and derivation of dialysis dose adjustments to the current clinical practice based on these parameters. The methodology explained in this chapter applies to all the three chapters related to this study.

The aim in this section is to examine the physical activity profile of haemodialysis patients and to explore the independent predictors of physical activity-related energy expenditure in this patient population.

10.2 Methods

10.2.1 Overview of the Study Design

This was a prospective, multi-centre study of 1500 chronic haemodialysis patients. Anthropometric data was collected through direct measurements. Information on comorbidity and physical activity was collected through self-report questionnaires. Dialysis-related data was collected from medical records at baseline and at various follow-up time points. The study cohort was followed up for a period up to 24 months from recruitment. Besides the dialysis-related data, survival data was also collected at regular time points during the follow-up.

10.2.2 Ethical Review

The study was approved by the North Wales Research Ethics Committee (ref: 12/WA/0060 and UH Ethics ref: LMS/PG/NHS/00257). All subjects provided written informed consent to take part.

10.2.3 Study Participants

Chronic adult HD patients older than 18 years and with dialysis vintage greater than 3 months were recruited from different centres. Renal units of five hospitals – Lister, Southend, Wirral, Royal Free and Royal London hospitals – participated in the study. Exclusion criteria included patients dialysing for other than thrice weekly frequency, those with amputated limbs and those with no capacity to consent. Study information sheet, consent forms and questionnaires were translated into Bengali and Urdu to facilitate data collection from non-English speaking patients in the participating units.

10.2.4 Study Protocol

The following data were collected from each patient.

1. Demographic data including age, sex, dialysis vintage, employment status
2. Anthropometric data including height and weight were collected by direct measurement as mentioned in the general methodology.
3. Routine pre- and post-dialysis biochemistry and haematology results were obtained from the local pathology system of each participating unit. The

results collected were serum urea, creatinine, haemoglobin and haematocrit values. Single-pool Kt/V (spKt/V) was calculated using the Daugirdas formula (Daugirdas, 1993).

4. Physical activity data was obtained through Recent Physical Activity Questionnaire (RPAQ) which codes data on activity performed at home, work and leisure in the preceding 4 weeks. It has been validated against doubly labelled water technique in general population (Besson et al., 2010). Detailed explanations about the questionnaire and energy expenditure calculation from it are discussed in General Methodology chapter (section 3.7.2.1).

5. Comorbidity data was collected using the self-report questionnaire explained in chapter 5. This scale is based on self-reporting of the presence and severity of 7 potential comorbidities - arthritis, cancer, diabetes, heart disease, lung disease, liver disease, and stroke. The questionnaire enquired whether participants had any of these conditions and if they did, whether they were receiving treatment and whether it limited their daily activities. Each positive response was given a score of 1 and hence, the maximum score for the questionnaire could range from 0–21. High comorbidity is designated as a composite self-report comorbidity score (CSCS) > 3.

10.2.5 Estimation of Alternative Scaling Parameters

Body surface area (BSA, Haycock equation) and Watson Volume (V) were derived from the following equations (Watson et al., 1980; Haycock et al., 1978). Height is expressed in cm and weight is expressed in kg in both these equations.

$$\boldsymbol{BSA = 0.024265 \times Weight^{0.5378} \times Height^{0.3964}}$$

$$\boldsymbol{V = 2.447 - (0.09516 \times Age) + (0.1074 \times Height) + (0.3362 \times Weight)}$$ (males)

$$\boldsymbol{V = -2.097 + (0.1069 \times Height) + (0.2466 \times Weight)}$$ (females)

Resting Energy Expenditure (REE) was estimated from a validated predictive equation which was derived in a cohort of HD patients (Vilar et al., 2014). This disease-specific equation was found to be at least as accurate as the existing general equations and was associated with less bias. The equation is given below.

$$REE = \left[-2.497 \times Age(years) \times Factor_{age}\right] + \left[0.011 \times Height^{2.023}(cm)\right] + \left[83.573 \times Weight^{0.6291}(kg)\right] + \left[68.171 \times Factor_{sex}\right]$$

where $Factor_{age}$ is 0 if age <65 and 1 if ≥65 and $Factor_{sex}$ is 0 if female and 1 if male.

Physical activity data - Each reported activity was assigned a Metabolic Equivalent of Task (MET) value as per the Compendium of Physical Activities (Ainsworth et al., 2011). For subjects reporting more than 18 hours of total activity duration per day, duration of each activity was proportionately adjusted so that the maximum reported total activity duration was 18 hours per day. Sleep duration per day was assumed to be 6-8 hours. Any remaining time not accounted for by the questionnaire was assumed to be spent in performing light activity and was assigned a MET value of 1.3. Mean daily MET value was calculated as explained in section 3.7.2.1.

Total Energy Expenditure (TEE) was estimated from the following equation.

$$TEE = REE \times Mean\ Daily\ MET$$

Physical Activity-related Energy Expenditure (PAEE) was calculated by subtracting the total energy expenditure from resting energy expenditure.

10.2.6 Statistical Analysis

Statistical analysis was carried out using SPSS ® version 19 (SPSS Software, IBM Corporation, New York, USA). Normally distributed data are presented as mean ± SD, and non-normally distributed as median [± interquartile range]. The significance of differences between means was determined by Student's t-test and of differences between medians by the Mann-Whitney U test. The significance of difference in PAEE between multiple group means was assessed by Kruskal-Wallis test. Multivariate regression models to examine predictors of PAEE were developed using linear regression. The variables used in the model were age, sex, ethnicity, comorbidity, employment status, dialysis adequacy (Kt/V), body weight and haemoglobin. Collinearity testing was carried out for all the variables included in the regression model and variables with only low variance inflation factor (<10) were included. A p-value of <0.05 was assumed to indicate statistical significance.

10.3 Results

10.3.1 Demographics

A total of 1500 patients (910 men and 590 women) were recruited. Demographic, anthropometric and biochemical characteristics are shown in Table 10-1. Females were significantly younger, a higher proportion of them classifying themselves as black, and fewer self-classifying as employed. All body size parameters except BMI were significantly greater in men than women. Men had slightly higher serum urea, creatinine and haemoglobin levels compared to women.

Table 10-1: Demographic, anthropometric and biochemical characteristics of Study patients

Values expressed are mean ± SD. Proportions of categorical variables are expressed as percentages.

	All patients (n = 1500)	Males (n = 910)	Females (n = 590)	p-value
Age (years)	62.9 ± 15.5	63.8 ± 15.6	61.6 ± 15.1	0.007
Weight (kg)	75.2 ± 18.3	78.4 ± 17.3	70.4 ± 18.6	<0.001
Height (cm)	165.9 ± 10.0	170.6 ± 8.2	158.7 ± 8.2	<0.001
Ethnicity (% South Asian: Black: White)	27.9: 26.7: 45.5	27.3: 24.0: 48.7	28.6: 30.9: 40.5	0.003
Body Mass Index (kg/m^2)	27.3 ± 6.0	26.9 ± 5.3	27.9 ± 7.0	0.002
High Comorbidity (%)	29.5	28.7	30.7	NS
Employed (%)	11.5	13.1	9.2	0.021
Serum Urea (mmol/L)	19.3 ± 5.7	19.6 ± 5.6	18.7 ± 5.8	0.004
Serum Creatinine (µmol/L)	802 ± 239	839 ± 252	744 ± 203	<0.001
Haemoglobin (g/dL)	10.9 ± 1.2	11.0 ± 1.3	10.8 ± 1.2	0.002

Energy expenditure and physical activity levels expressed as Mean Daily MET are given in Table 10-2. The reported total activity time, sedentary time and activity time are shown in Figure 10-1.

Table 10-2: Energy expenditure and Physical Activity Level of study patients

Values expressed are Mean ± SD (* Median and inter-quartile range). REE: resting energy expenditure, MET: metabolic equivalent of task, PAEE: Physical activity-related energy expenditure, TEE: total energy expenditure

	All patients (n = 1500)	Males (n = 910)	Females (n = 590)	p-value
REE (kcal/day)	1545 ± 250	1621 ± 230	1429 ± 236	<0.001
Daily MET	1.19 ± 0.13	1.20 ± 0.14	1.17 ± 0.10	<0.001
PAEE (kcal/day) *	243 (159)	262 (185)	221 (132)	<0.001
TEE (kcal/day)	1841 ± 388	1948 ± 390	1676 ± 322	<0.001

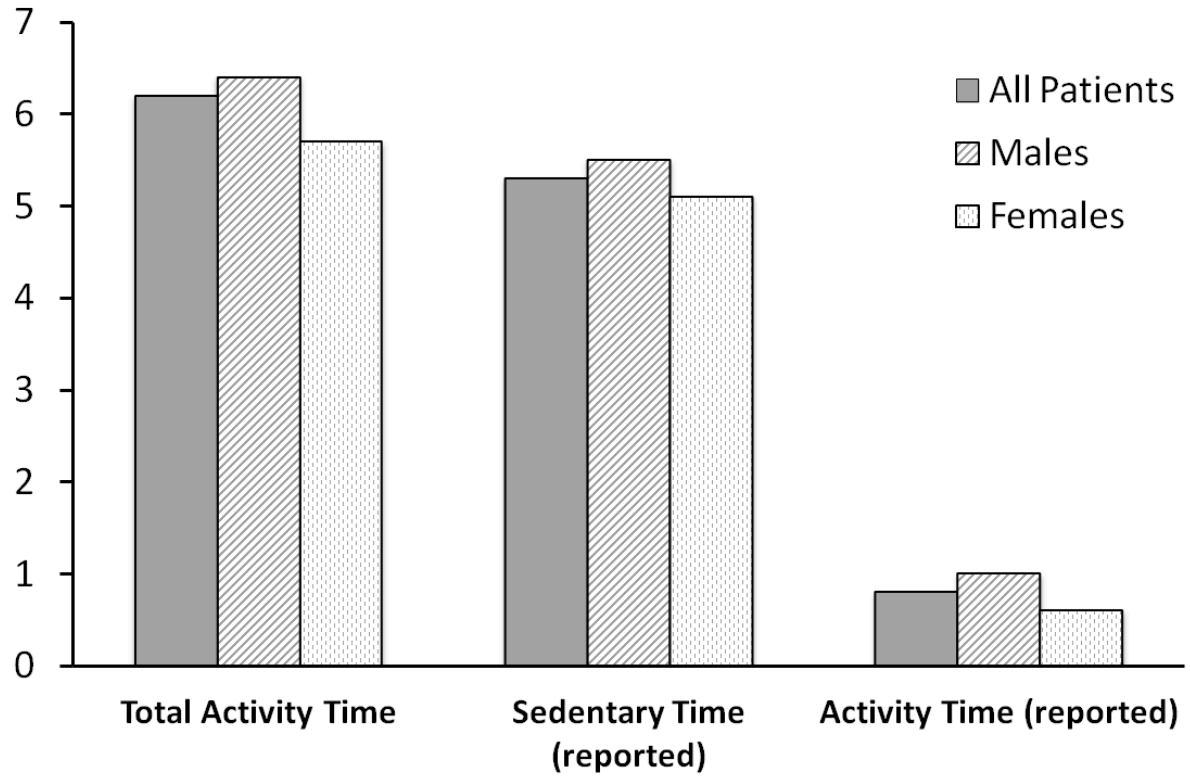


Figure 10-1: Reported Activity Time in Study Participants

Both REE and TEE were significantly lower in females and the physical activity levels were very low with a mean daily MET for the overall study population of only 1.19. The mean total reported activity time was 6.2 hours per day with females reporting slightly lesser time compared to males (5.7 vs. 6.4 hours). However, the majority of this time was spent in sedentary activity (mean - 5.3 hours) with only a small proportion being spent in other intensities of activities (mean - 0.8 hours).

10.3.2 Gender and Physical Activity

Figure 10-2 shows the gender differences in PAEE which was significantly higher in males compared to females (327 vs. 247 kcal/day; $p < 0.001$).

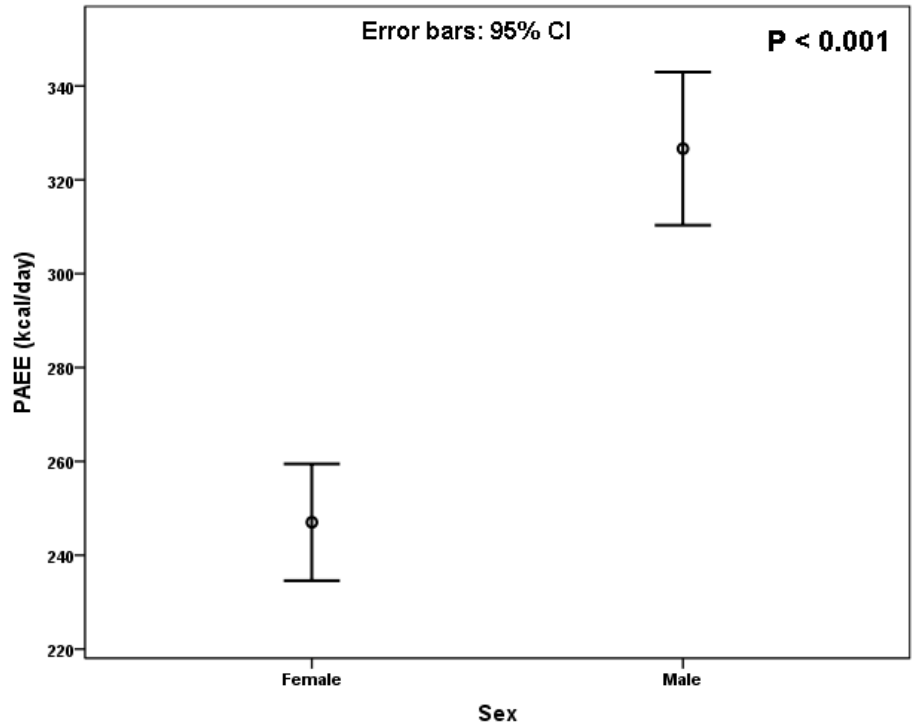


Figure 10-2: Gender differences in Physical Activity Level

10.3.3 Age and Physical Activity

Figure 10-3 shows the difference in PAEE between younger (age < 65) and older (age ≥ 65) subjects. Younger patients had higher activity-related energy expenditure compared to their older counterparts (367 vs. 226 kcal/day; $p < 0.001$).

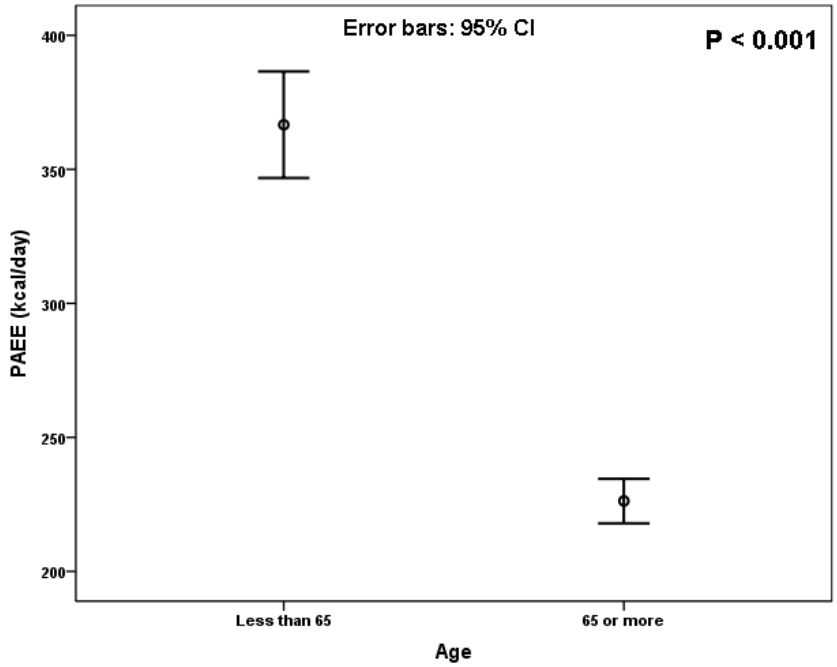


Figure 10-3: Influence of Age on Physical Activity Level

10.3.4 Ethnicity and Physical Activity

PAEE in patients of different ethnic origins is shown in Figure 10-4. There was no significant difference in physical activity between patients of different ethnic origins.

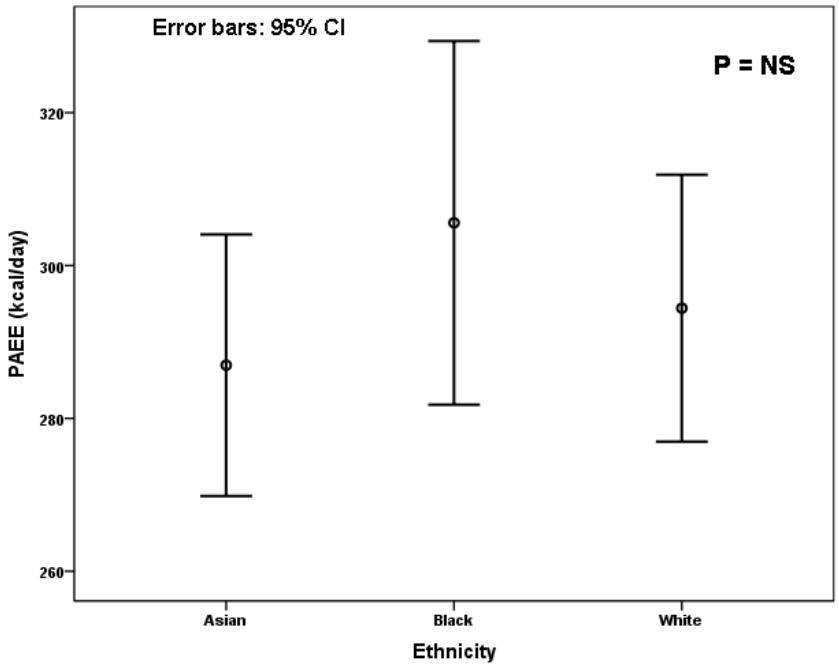


Figure 10-4: Influence of Ethnicity on Physical Activity

10.3.5 Comorbidity and Physical Activity

As shown in Figure 10-5, physical activity-related energy expenditure was significantly higher in patients with low comorbidity (comorbidity score ≤ 3) compared to those with high comorbidity (317 vs. 244 kcal/day; p < 0.001).

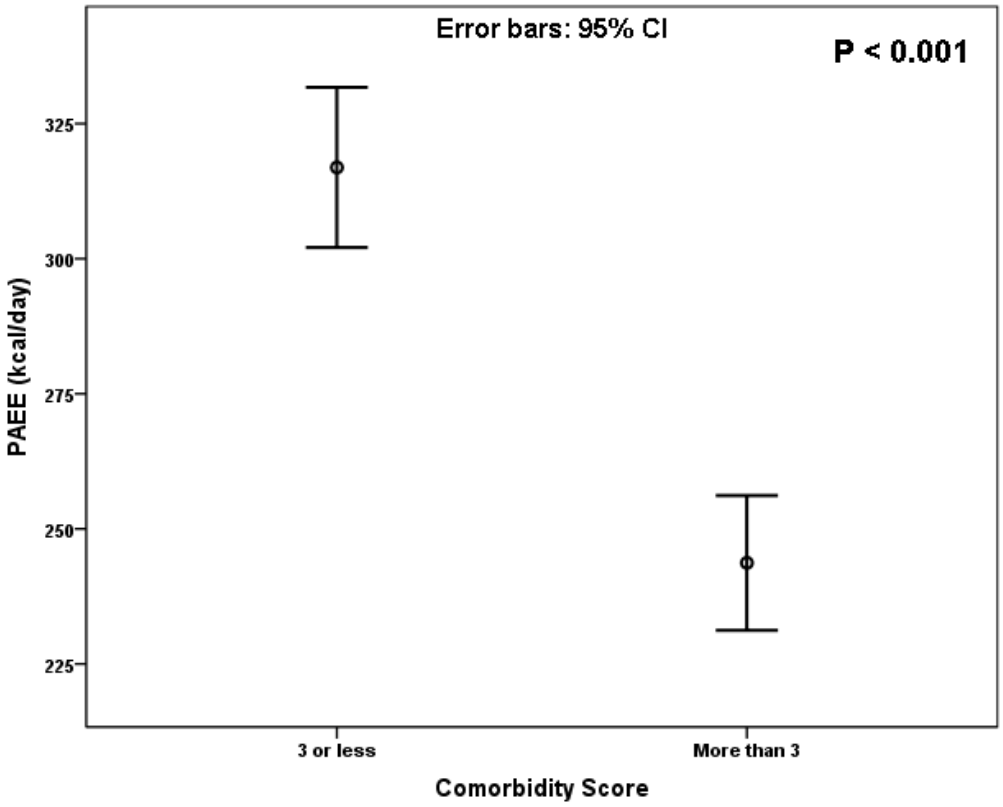


Figure 10-5: Physical Activity level differences based on Comorbidity

10.3.6 Employment and Physical Activity

Figure 10-6 shows PAEE between employed (n = 173) and unemployed subjects. As expected, working patients had higher total activity-related energy expenditure compared to those who were not working (707 vs. 242 kcal/day; p < 0.001). A majority of the employed patients (91%) were working in a sedentary or a light activity occupation with only a handful of them doing a manual job.

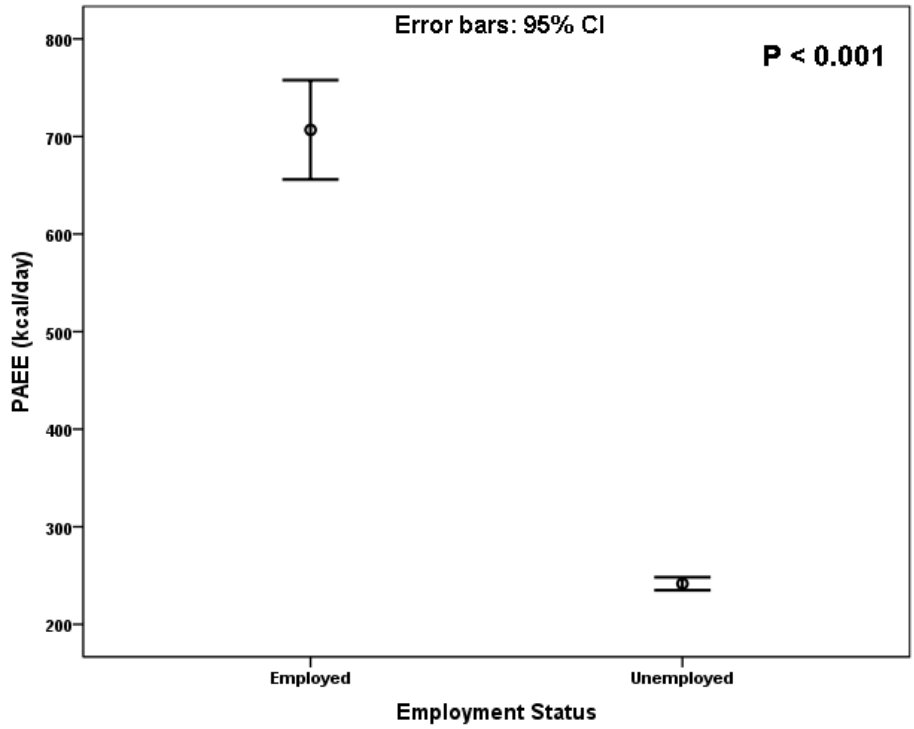


Figure 10-6: Effect of Employment Status on Physical Activity level

10.3.7 Body size and Physical Activity

The relationship between PAEE and body weight is shown in Figure 10-7. PAEE progressively increased with the heavier patients having higher PAEE compared to their smaller counterparts. There were significant differences across the group ($p < 0.001$, Kruskal-Wallis test).

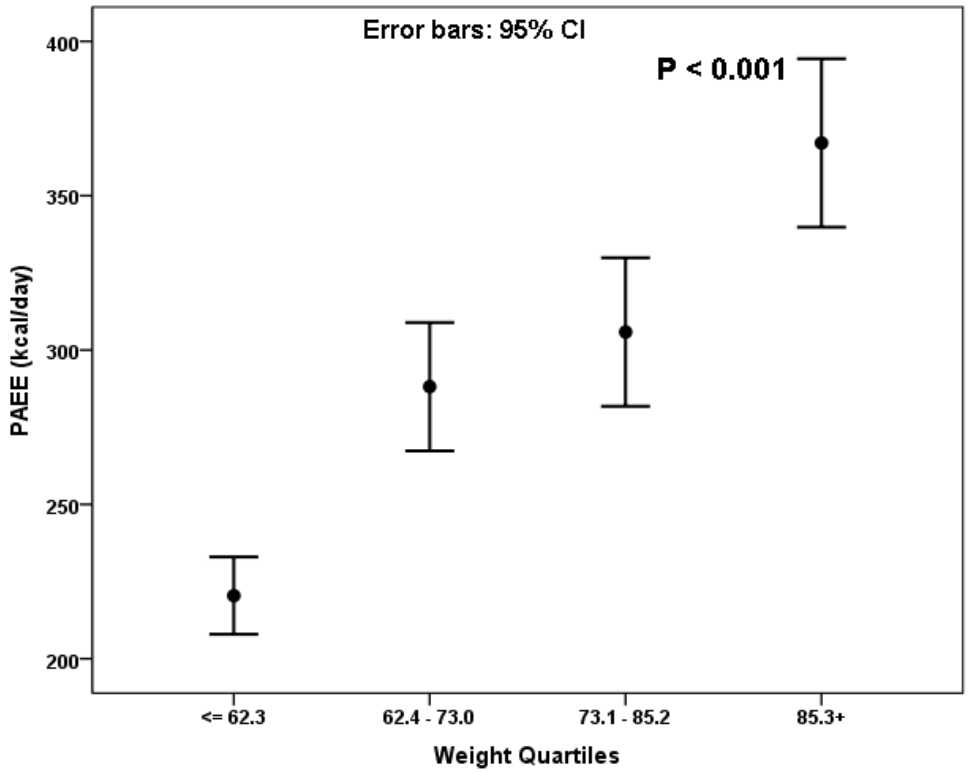


Figure 10-7: Relationship between Physical Activity and Body Size

10.3.8 Relationship of Physical activity to comorbid illnesses and biochemical parameters

Univariate correlations of PAEE with specific comorbid conditions and biochemical parameters are shown in Table 10-3. PAEE correlated positively with body weight, serum urea and creatinine. Presence of cardiac disease, diabetes mellitus and arthritis were negatively correlated with PAEE. Other comorbid conditions specified in the questionnaire did not have significant correlations with PAEE.

Table 10-3: Univariate Correlations of Physical Activity-related Energy Expenditure

Spearman's correlation coefficients are stated in the table. Comorbid conditions mentioned refer to the presence of these diseases.

Variable	Correlation Coefficient	p-value
Watson Volume	0.326	< 0.001
Body Weight	0.254	< 0.001
Serum Urea	0.142	< 0.001
Serum Creatinine	0.232	< 0.001
Haemoglobin	0.047	NS
Cardiac disease	- 0.075	0.004
Diabetes Mellitus	- 0.064	0.012
Arthritis	- 0.149	< 0.001

10.3.9 Predictors of Physical Activity

Table 10-4 shows a linear regression model with PAEE as dependent variable. The variables considered were age, sex, ethnicity, comorbidity, employment status, dialysis adequacy (Kt/V), body weight and haemoglobin. Ethnicity, dialysis adequacy and haemoglobin were not significant in the model.

Table 10-4: Linear regression model of Physical Activity-related Energy Expenditure

Adjusted R^2 for the model: 0.513

	Standardised coefficient (Beta)	t	p-value
Age	- 0.152	- 7.544	< 0.001
Sex	0.123	6.475	< 0.001
Comorbidity Score	- 0.045	- 2.294	0.022
Employment Status	- 0.602	- 31.452	< 0.001
Weight	0.145	7.189	< 0.001

10.4 Discussion

The main aim of this study was to examine the physical activity profile of the study participants and to explore the determinants of their physical activity-related energy expenditure in this section. It was found that physical activity levels were very low across the whole of the study population. Gender, age, comorbidity and employment status have significant impact on physical activity-related energy expenditure. Given their effect on physical activity, these variables along with weight were found to be significant predictors of PAEE in linear regression model.

Physical activity levels have been reported to be low in haemodialysis patients even when compared to sedentary individuals (Avesani et al., 2012; Zamojska et al., 2006; Johansen et al., 2000). Dialysis patients are exposed to a number of factors that may predispose to low physical activity. Firstly, these patients are likely to have significant comorbidities which may restrict their activities. Uraemia per se may lead to sarcopenia thereby reducing the ability to perform regular exercise. Indeed, a study carried out on dialysis patients showed 30% of incident and 39% of prevalent dialysis patients to have signs of muscle atrophy (Carrero et al., 2008). Moreover, renal failure is predominantly a disease of the elderly and factors related to aging may also contribute to reduced physical activity (Brown et al., 2005; Johansen et al.,

2000). Finally, studies have shown markedly diminished physical functioning capacity in CKD patients even in earlier stages of kidney disease (Leikis et al., 2006; Castaneda et al., 2001; Boyce et al., 1997).

The study findings showing reduced physical activity is in line with previously reported studies mentioned above in the haemodialysis patient population. There were also a number of factors associated with reduced physical activity. Females, elderly and those with high comorbidity have even lower physical activity levels compared to the rest of the cohort. This is in line with the potential causes underlying physical inactivity as explained above. Perhaps, it is not surprising that patients who are employed have higher activity-related energy expenditure compared to their unemployed counterparts. However, it is worth noting that the time reportedly spent on sedentary activities at home is only marginally less in employed compared to those unemployed (4.7 vs. 5.4 hours).

Physical activity-related energy expenditure correlated positively with body weight, Watson Volume and Serum urea and creatinine. Urea and creatinine are classified as small molecule uraemic retention solutes. This association between physical activity and these molecules indicates that there may be a relationship between physical activity and uraemic toxin generation. This is merely an association and by no means, implies causality. Physical activity also correlated inversely with three specific comorbid conditions – cardiac disease, diabetes mellitus and arthritis. Individuals with cardiac disease and diabetes derive health benefits from increased activity level (Jonker et al., 2013; Koivula, Tornberg, & Franks, 2013; Myers, 2003). The study results show that these are the very individuals who have higher risk of reduced physical activity. The reduced physical activity in this high-risk group might predispose them to increased cardiovascular mortality risk.

Physical activity levels showed a significant impact on body weight with more active individuals being heavier compared to the relatively inactive patients. Whilst this finding in itself is expected, it does highlight an interesting relationship with regards to dialysis dosing. As referred to at various points in this thesis, the relative effect of metabolic wastes in heavier individuals is likely to be less compared to the smaller patients implying that heavier patients need relatively less dialysis dose. However, it is likely, as shown in chapter 4, that increased physical activity may contribute to

higher uraemic toxin generation and thereby, necessitating higher dialysis doses in these heavier active individuals. Hence, it is essential to individualise the dialysis dose based on body size and activity level to deliver appropriate dialysis dose rather than based on body weight alone as is the current practice.

Given the well-documented poor physical activity levels of dialysis patients, significant efforts are being made by many dialysis units to encourage patients to perform intra-dialysis exercise and also to improve habitual physical activity levels between dialysis sessions. Whilst every effort should be made to improve activity levels across the haemodialysis patient population, it is worthwhile focusing more on groups of patient at risk of reduced activity such as females, elderly and those with high comorbidity. However, the effect of increased physical activity on uraemic toxin generation has not been studied so far. In order to move forward with holistic exercise programs for haemodialysis patients, the relationship of physical activity to uraemic toxin generation needs to be explored in future studies.

10.5 Limitations

This study has some limitations. The physical activity data was collected through an activity questionnaire. The gold standard method of calculating total energy expenditure (and thereby, physical activity-related energy expenditure) – the Doubly Labelled Water method – is expensive, time-consuming and is not feasible to be carried out in large scale studies. The assessment of physical activity using accelerometers, though easier than the doubly labelled water method, is still difficult to perform in large cohort of patients such as this. Hence, questionnaire-based methods were deemed a useful alternative. As with any questionnaire method, there is a risk of recall bias from the participants. The questionnaire enquires regarding activities carried out in the preceding 4 weeks which is assumed to represent the overall activity levels which may not be true in all patients. Nevertheless, the overall activity levels found in this study are in line with previously published literature in this field (Avesani et al., 2012; Zamojska et al., 2006; Johansen et al., 2000).

10.6 Conclusion

In conclusion, physical activity level is very low in haemodialysis patients with some groups of patients performing even lower levels of activity compared to the

population as a whole. Further studies are needed to examine the impact of increased physical activity on uraemic toxin generation. In addition, the relationship between body size and physical activity should be explored with regards to the dosing of dialysis. The potential benefits of scaling dialysis dose based on body size and activity levels and the impact of such a practice on patient survival will be discussed in subsequent chapters.

Chapter 11

11. Scaling Haemodialysis Dose to Reflect Metabolic Activity: Potential Benefits

11.1 Introduction

The current recommended minimum Kt/V target is 1.2 per dialysis session for both men and women ("Clinical Practice Guidelines for Hemodialysis Adequacy, Update 2006," 2006). As discussed in the previous chapter, parameters such as body surface area (BSA), resting energy expenditure (REE) and total energy expenditure (TEE) could be considered as potential scaling factors for normalising dialysis dose. Apart from BSA, the other two parameters have not been explored as a scaling factor for dialysis dosing in clinical studies to date. As REE and TEE are likely to be better markers of metabolic activity, using these parameters may deliver appropriate dialysis doses with respect to individual metabolic needs. In order to examine the reliability and explore the practical application of these parameters for dialysis dose scaling, it is essential to identify the equivalent dialysis dose using these parameters corresponding to current minimum Kt/V target. Although previous studies have explored the impact of using BSA for scaling dialysis dose (Ramirez et al., 2012; Daugirdas, Depner, et al., 2008), no study to date has suggested an equivalent dialysis dose using any of the alternate scaling parameters.

The primary aim of this study was

- To explore the equivalent dialysis dose that would be delivered using the above parameters for scaling corresponding to the current recommended minimum dose target
- To identify patient characteristics that would be associated with risk of sub-optimal delivered dialysis doses with current dosing practice.

The experimental hypothesis of this study was that current minimum Kt/V target would deliver sub-optimal dialysis dose compared to that estimated using one of the alternate scaling parameters and the null hypothesis was that there would be no difference in dialysis dose delivered with any of these scaling parameters.

11.2 Methods

11.2.1 Ethical Review

The study was approved by the North Wales Research Ethics Committee (ref: 12/WA/0060 and UH Ethics ref: LMS/PG/NHS/00257). All subjects provided written informed consent to take part.

11.2.2 Study Protocol

1. The study population consisted of 1500 chronic haemodialysis patients. The inclusion and exclusion criteria are explained in chapter 10.
2. Demographic, anthropometric and biochemical data was collected for each study participant as detailed in chapter 10.
3. Physical activity data was collected using Recent Physical Activity Questionnaire (RPAQ) and comorbidity data from a self-report comorbidity questionnaire. Both the questionnaires were administered once only at the time of recruitment.

11.2.3 Estimation of Alternative Scaling Parameters

Estimation of all relevant parameters – Watson volume, body surface area, resting energy expenditure, physical activity-related energy expenditure and total energy expenditure – are explained in the methodology section in chapter 10.

11.2.4 Scaling of Dialysis Dose

KDOQI guidelines recommend a minimum target dose of single-pool Kt/V (spKt/V) of 1.2 per dialysis session for a thrice-weekly schedule. Hence, in order to compare minimum dialysis targets using alternative scaling parameters, Kt was calculated for each patient as below.

$$\boldsymbol{Kt = 1.2 \times V}$$

where V is Watson Volume. Corresponding target values of Kt/BSA, Kt/REE and Kt/TEE were calculated by dividing Kt by the respective parameters for each individual patient.

11.2.5 Sample Size and Statistical Analysis

A power analysis was performed to estimate the statistical power provided by a study of 1500 patients on haemodialysis, assuming a study drop-out (attrition) rate of 15% per annum due to loss from follow-up or transplantation. Assuming $\alpha=0.05$, and N=1500 patients, and the estimated Hazard ratios in a previous study in our unit, the power to detect group differences (between the middle and lower tertiles) for the different methods of estimating dialysis dose are as follows: $1-\beta=0.91$ Kt/V dose; $1-\beta=0.98$ for Kt/REE; and $1-\beta>0.99$ for Kt/BSA. This provides strong evidence that the study is likely to have sufficient power to demonstrate differences between the relevant groups for each parameter.

Statistical analysis was carried out using SPSS ® version 19 (SPSS Software, IBM Corporation, New York, USA). Normally distributed data are presented as mean ± SD, and non-normally distributed as median [± interquartile range]. The significance of differences between means was determined by Student's t-test and of differences between medians by the Mann-Whitney U test. The significance of differences between multiple group means was assessed by ANOVA with differences between individual groups being assessed using the Bonferroni test. Multivariate regression models to examine predictors of Kt/TEE were developed using stepwise linear regression. The variables used in the model were age, sex, employment status, ethnicity, body weight and comorbidity score. Ethnicity was used as a categorical variable as belonging to White ethnic origin or not. Collinearity testing was carried out for all the variables included in the regression model and variables with only low variance inflation factor (<10) were included. A p-value of <0.05 was assumed to indicate statistical significance.

11.3 Results

11.3.1 Demographics

A total of 1500 patients (910 men and 590 women) were recruited. Some selected anthropometric details are shown in Table 11-1. For other variables, please refer to the demographic section in chapter 10. All body size parameters were significantly greater in men than women.

Table 11-1: Demographic, anthropometric and biochemical characteristics of Study patients

Values expressed are mean ± SD. Proportions of categorical variables are expressed as percentages.

	All patients (n = 1500)	Males (n = 910)	Females (n = 590)	p-value
Age (years)	62.9 ± 15.5	63.8 ± 15.6	61.6 ± 15.1	0.007
Weight (kg)	75.2 ± 18.3	78.4 ± 17.3	70.4 ± 18.6	<0.001
Height (cm)	165.9 ± 10.0	170.6 ± 8.2	158.7 ± 8.2	<0.001
Ethnicity (% South Asian: Black: White)	27.9: 26.7: 45.5	27.3: 24.0: 48.7	28.6: 30.9: 40.5	0.003
High Comorbidity (%)	29.5	28.7	30.7	NS
Employed (%)	11.5	13.1	9.2	0.021

Body size and energy expenditure measures are shown in Table 11-2. Watson Volume, BSA, REE, Mean daily METs and TEE were all significantly lower in women than men.

Table 11-2: Body size and energy expenditure characteristics of Study patients

Values expressed are Mean ± SD. REE: resting energy expenditure, MET: metabolic equivalent of task, TEE: total energy expenditure

	All patients (n = 1500)	Males (n = 910)	Females (n = 590)	p-value
Watson Volume (L)	37.5 ± 7.4	41.0 ± 6.6	32.2 ± 4.9	<0.001
Body Surface Area (m^2)	1.87 ± 0.26	1.93 ± 0.24	1.77 ± 0.26	<0.001
REE (kcal/day)	1545 ± 250	1621 ± 230	1429 ± 236	<0.001
Mean Daily MET	1.19 ± 0.13	1.20 ± 0.14	1.17 ± 0.10	<0.001
TEE (kcal/day)	1841 ± 388	1948 ± 390	1676 ± 322	<0.001

11.3.2 Relationship between Watson Volume and Body Surface Area

The relationship between Watson volume (V) and BSA is shown in Figure 11-1. As can be seen, for any given V, females had higher BSA compared to males.

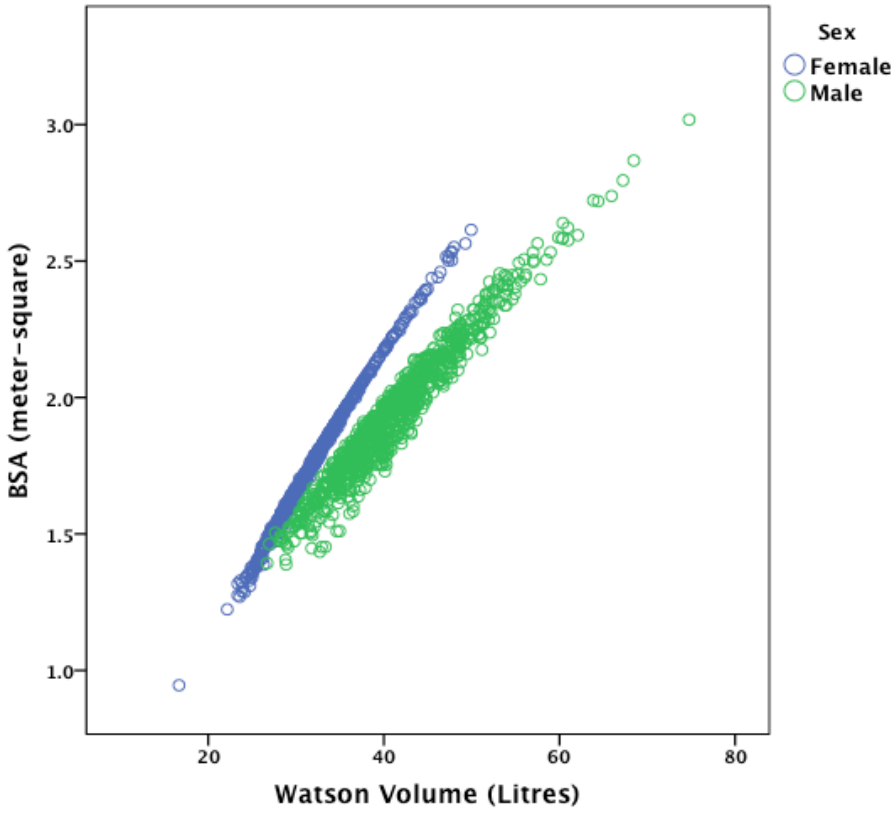


Figure 11-1: Relationship between Watson Volume and BSA

11.3.3 Relationship between Watson Volume and Energy Expenditure

The relationship between Watson volume and REE and TEE are shown in Figure 11-2 and Figure 11-3. Similar to that with BSA, females had higher REE and TEE for a given V although the magnitude of difference is smaller than that seen in comparison with BSA.

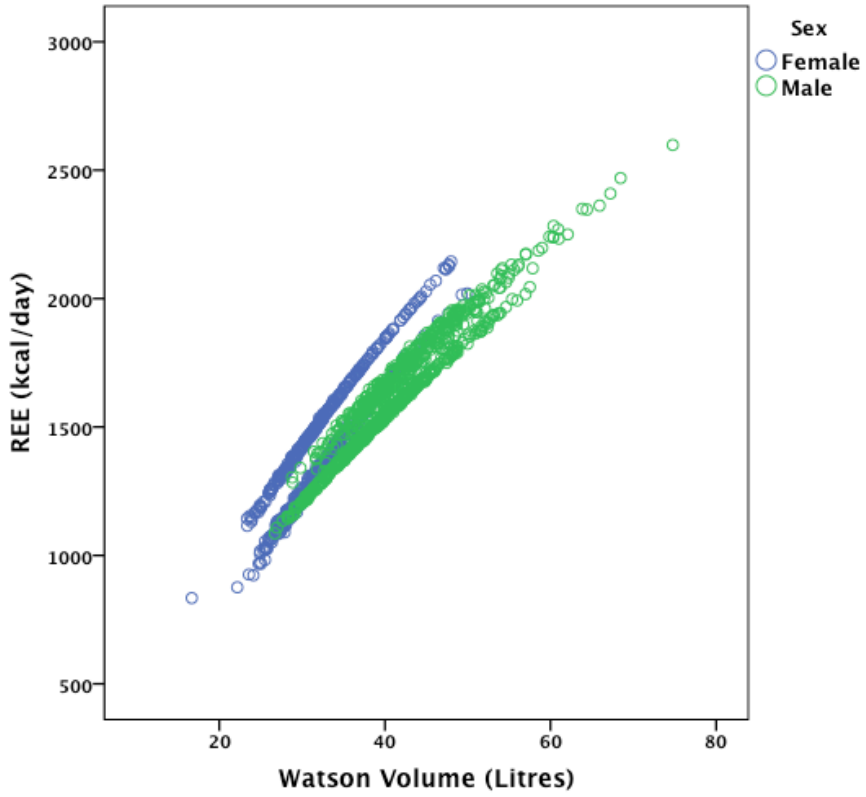


Figure 11-2: Relationship between Watson Volume and REE

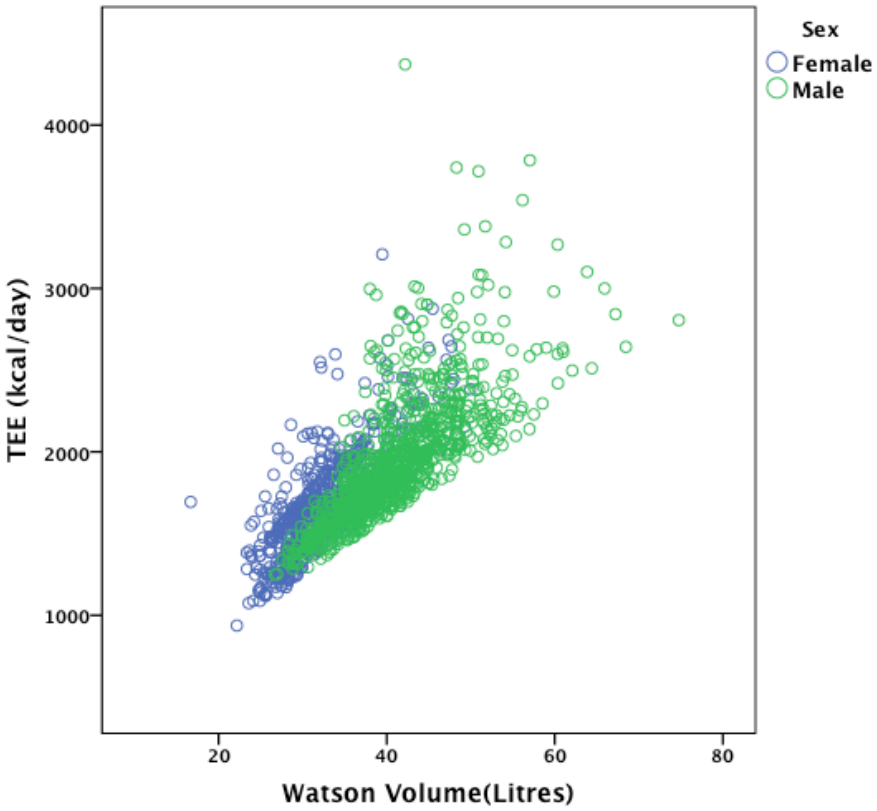


Figure 11-3: Relationship between Watson Volume and TEE

11.3.4 Gender differences in Metabolically Normalised Doses

The mean values of metabolically normalised dialysis doses (Kt/BSA, Kt/REE, Kt/TEE) equivalent to a Kt/V of 1.2 are shown in Table 11-3. The equivalent dose expressed as Kt was markedly less in females than males (38,662 ± 5,940 v 49,251 ± 7,937: $p < 0.001$). There were also marked gender differences in the metabolically normalised parameters. Kt/BSA was 21,851 ± 246 ml/m^2 for women and 25,392 ± 1,208 ml/m^2 for men ($p < 0.001$). For Kt/REE these were 27.18 ± 1.87 ml/kcal for women and 30.34 ± 1.25 ml/kcal for men ($p < 0.001$) and for Kt/TEE, 23.37 ± 2.60 ml/kcal for women and 25.60 ± 2.73 ml/kcal for men ($p < 0.001$).

Table 11-3: Dialysis dose equivalent to a Kt/V of 1.2 expressed as Kt/BSA, Kt/REE and Kt/TEE

BSA: body surface area, REE: resting energy expenditure, TEE: total energy expenditure; *significant difference by unpaired t-test ($p<0.001$), **significant difference by unpaired t-test ($p<0.01$), † significant differences across group by one-way ANOVA

	NUMBER	Kt(ml)	Kt/BSA (ml/m^2)	Kt/REE (ml/kcal)	Kt/TEE (ml/kcal)
All Patients	1500	45,086 ± 8,879	29,999 ± 1,976	29.10 ± 2.17	24.72 ± 2.89
GENDER					
Male	910	49,251 ± 7,937*	25,392 ± 1,208*	30.34 ± 1.25*	25.60 ± 2.73*
Female	590	38,662 ± 5,940	21,851 ± 246	27.18 ± 1.87	23.36 ± 2.60
WEIGHT QUARTILES (kg)					
≤ 62.3	375	35,616 ± 3850†	22,833 ± 1,526†	28.08 ± 2.11†	24.08 ± 2.65†
62.4 to 72.7	375	42,244 ± 3,876	23,823 ± 1,787	28.90 ± 1.86	24.50 ± 2.65
72.8 to 84.5	375	46,906 ± 4,377	24,258 ± 1,786	29.31 ± 1.96	24.80 ± 2.91
>84.5	375	55,578 ± 7,433	25,082 ± 2,072	30.11 ± 2.24	25.48 ± 3.17
AGE QUARTILES (years)					
≤ 52	375	46,932 ± 10,185†	24,734 ± 2,507†	28.15 ± 2.45†	22.98 ± 3.13†
52.1 to 65.3	375	45,980 ± 9,222	24,133 ± 2,024	27.60 ± 1.99	23.28 ± 2.60
65.4 to 75.5	375	45,218 ± 8,451	23,780 ± 1,673	30.36 ± 1.43	26.22 ± 2.06
>75.5	375	42,215 ± 6,579	23,351 ± 1,198	30.29 ± 0.86	26.38 ± 1.62
ETHNICITY					
Asian	418	42,692 ± 8,099†	23,861 ± 1,975	28.60 ± 2.26†	24.16 ± 2.70†
Black	400	46,456 ± 8,617	24,047 ± 2.124	28.87 ± 2.18	24.57 ± 2.93
White	682	45,750 ± 9,209	24,055 ± 1,882	29.54 ± 2.02	25.14 ± 2.93
COMORBIDITY					
Low	1058	44,964 ± 8,911	24,091 ± 2,033**	28.98 ± 2.19*	24.40 ± 3.00*
High	442	45,377 ± 8,804	23,780 ± 1,815	29.38 ± 2.11	25.47 ± 2.47
EMPLOYMENT					
Working	173	48,597 ± 9,132*	24,922 ± 2,181*	28.83 ± 2.18	20.61 ± 2.68*
Not working	1337	44,628 ± 8,745	23,879 ± 1,916	29.13 ± 2.17	25.25 ± 2.17

11.3.5 Body-size and Age-related Differences in Metabolically Normalised Doses

There were also marked differences in these parameters with respect to body size. Table 11-3 shows the influence of the body weight (expressed in quartiles). For each parameter there was a significant difference between the means across the quartiles as judged by one-way ANOVA. Smaller patients received a much lower overall dose irrespective of whatever the metabolically normalised parameter that was used. The mean dose of Kt/BSA ranged from 22,876 (±1,549) ml/m^2 in the lowest weight quartile group to 25,101 (±2,047) ml/m^2 in those in the highest quartile. For Kt/REE, this ranged from 28.14 (±2.08) ml/kcal to 30.15 (±2.23) ml/kcal and for Kt/TEE, 24.15 (±2.60) ml/kcal to 25.47 (±3.17) ml/kcal.

Patient age also influenced these parameters (Table 11-3). For Kt there was a significant reduction across age quartiles by ANOVA. The magnitude of the reduction was smaller for Kt/BSA, but still significant, reducing from a mean of 24,734 (± 2,507) ml/m^2 in the youngest group to 23,351 (± 1,198) ml/m^2 to the oldest group. However both Kt/REE and Kt/TEE increased with increasing age. The mean Kt/REE in the youngest age quartile was 28.15 (± 2.45) ml/kcal which progressively increased to 30.29 (± 0.86) ml/kcal in the oldest age quartile. For Kt/TEE, the corresponding values were 22.98 (± 3.13) ml/kcal and 26.38 (± 1.62) ml/kcal.

11.3.6 Effect of Ethnicity, Comorbidity and Employment on Dialysis Dosing

Table 11-3 also shows the differences between these parameters with respect to ethnicity. In general the equivalent dose for Asians was lower than that for Blacks which was lower than that for Whites. For Kt, Kt/REE, Kt/TEE there was a significant difference in means between ethnic groups by one-way ANOVA. For Kt/BSA the differences were not significantly different.

Patients with high comorbidity (CSCS >3) had slightly but significantly higher Kt/REE and Kt/TEE levels than their counterparts with lower comorbidity, though values of Kt/BSA were slightly lower. The corresponding values are shown in Table 11-3. Values of Kt/REE and Kt/TEE were consistently higher in those with arthritis, cancer, diabetes and heart disease than in those without these conditions.

Patients in employment had lower levels of Kt/TEE than those not employed, though levels of both Kt and Kt/BSA were higher (Table 11-3). This reflects the significantly higher weight (79.4 ± 18.4 vs. 74.7 ± 18.2; p = 0.001) and physical activity expressed as Mean Daily MET (1.42 ± 0.19 vs. 1.16 ± 0.07; p < 0.001) of employed individuals.

11.3.7 Impact of Gender-specific Body Size Differences on Dialysis Dosing

Given the major influence of gender on these parameters, the within gender differences in relation to body weight, age, ethnicity, comorbidity and employment status were examined. Figure 11-4 to Figure 11-7 depicts the effect of weight on these parameters. For women, there was no significant difference in the dose that would be delivered across the weight quartiles irrespective of what the scaling parameter was. However, in males, the delivered dose would be significantly lower in smaller men compared to their larger counterparts with any of the three parameters (p < 0.001 for all).

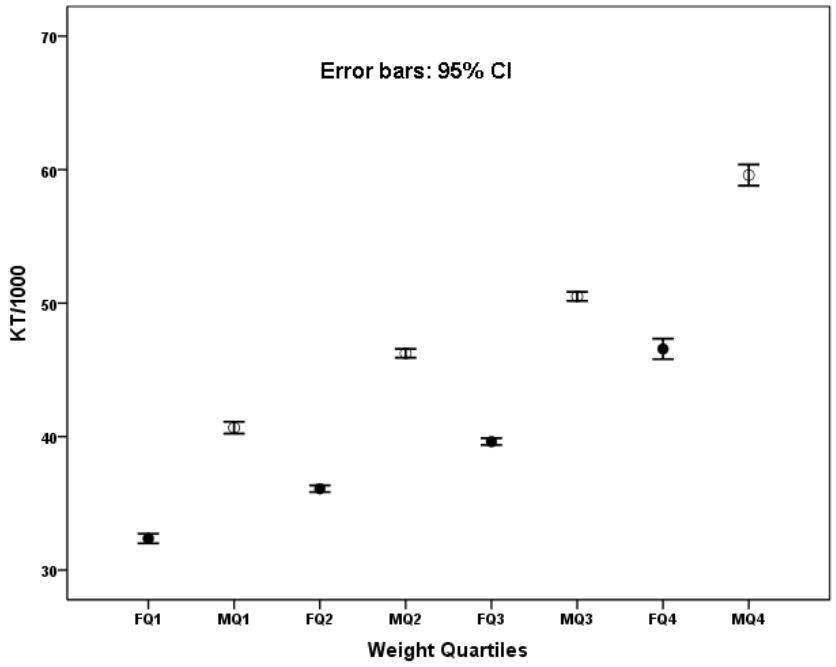


Figure 11-4: Predicted delivered minimum dialysis dose in relation to gender-specific weight quartiles using Kt

FQ1 to FQ4 – Weight quartiles lowest to highest for women; MQ1 to MQ4 – Weight quartiles lowest to highest for men; Males (open circles) and Females (filled circles) (for Figure 11-4 to Figure 11-7)

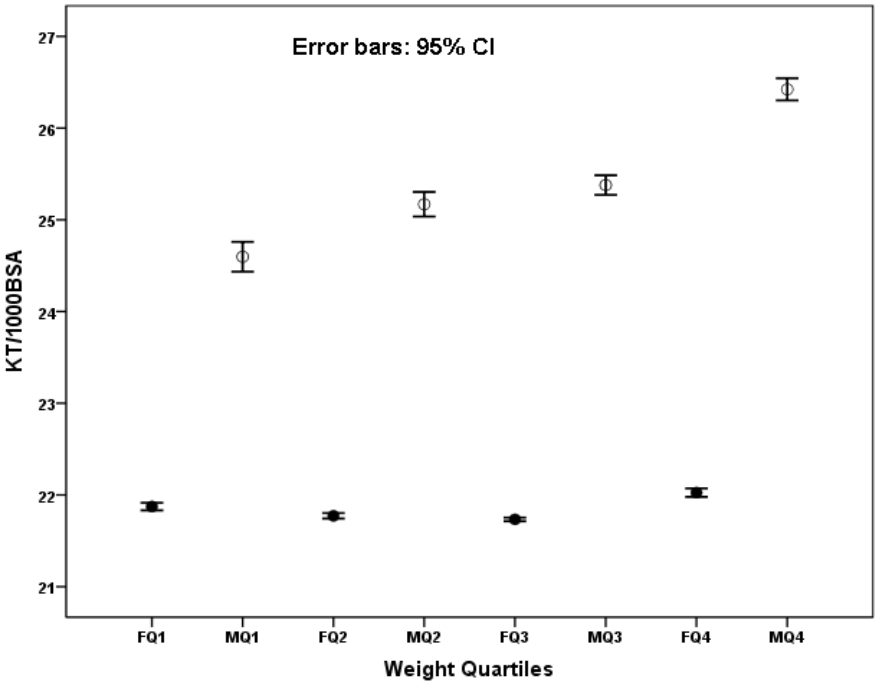


Figure 11-5: Predicted delivered minimum dialysis dose in relation to gender-specific weight quartiles using Kt/BSA

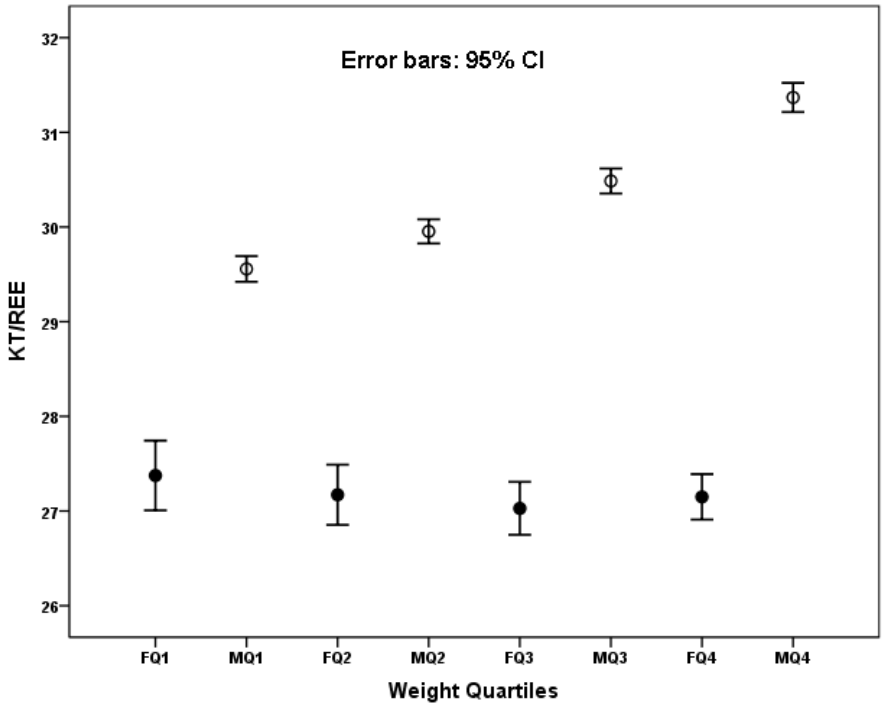


Figure 11-6: Predicted delivered minimum dialysis dose in relation to gender-specific weight quartiles using Kt/REE

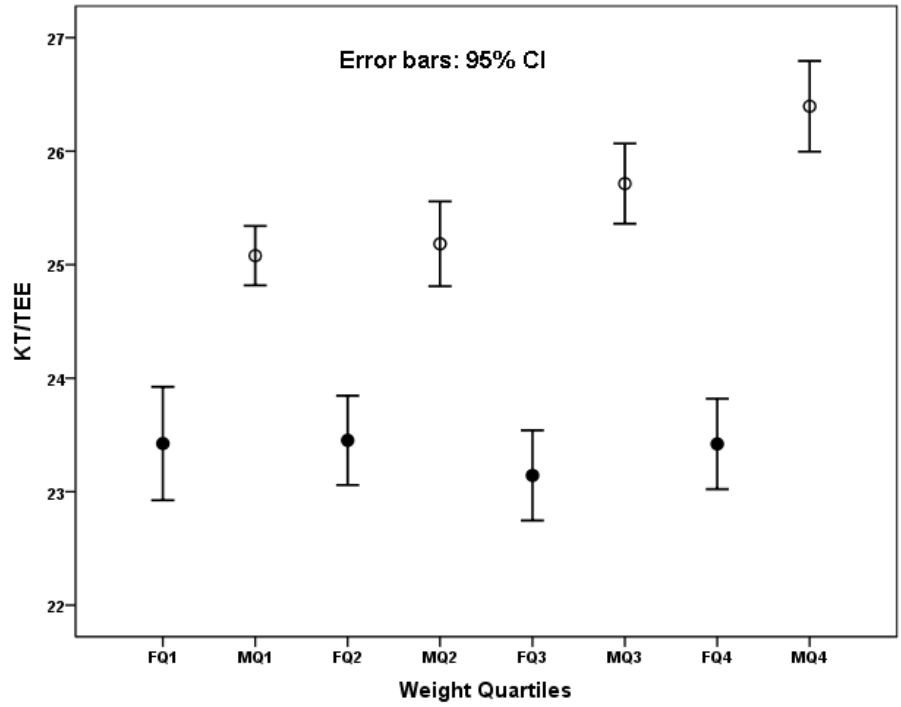


Figure 11-7: Predicted delivered minimum dialysis dose in relation to gender-specific weight quartiles using Kt/TEE

11.3.8 Gender-specific Dosing with Kt/TEE in Patient Subgroups

As shown in Table 11-3, the differences between the groups were most consistent with Kt/TEE. Hence, this parameter was chosen to study the effect of age, ethnicity, comorbidity, and employment status on dialysis dosing. This is shown in Figure 11-8 to Figure 11-11. In both males and females Kt/TEE was lower for younger patients, for those with low comorbidity, and for those in employment. In males, there seemed little influence of ethnic group on Kt/TEE, whereas in Asian females Kt/TEE was slightly but significantly lower than in other females (22.45 ± 2.42 v 23.72 ± 2.59; $p < 0.001$).

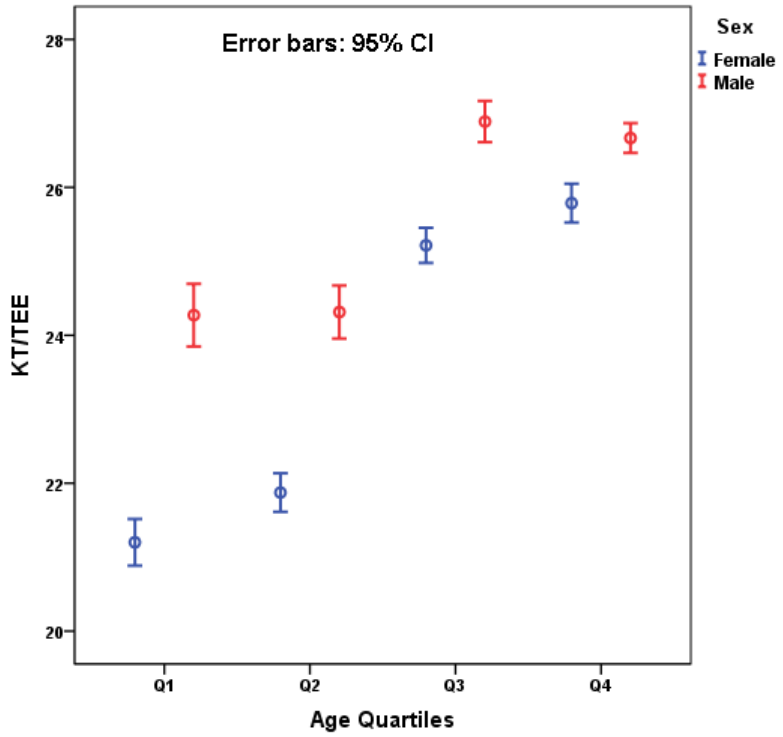


Figure 11-8: Effect of age on predicted delivered minimum Kt/TEE

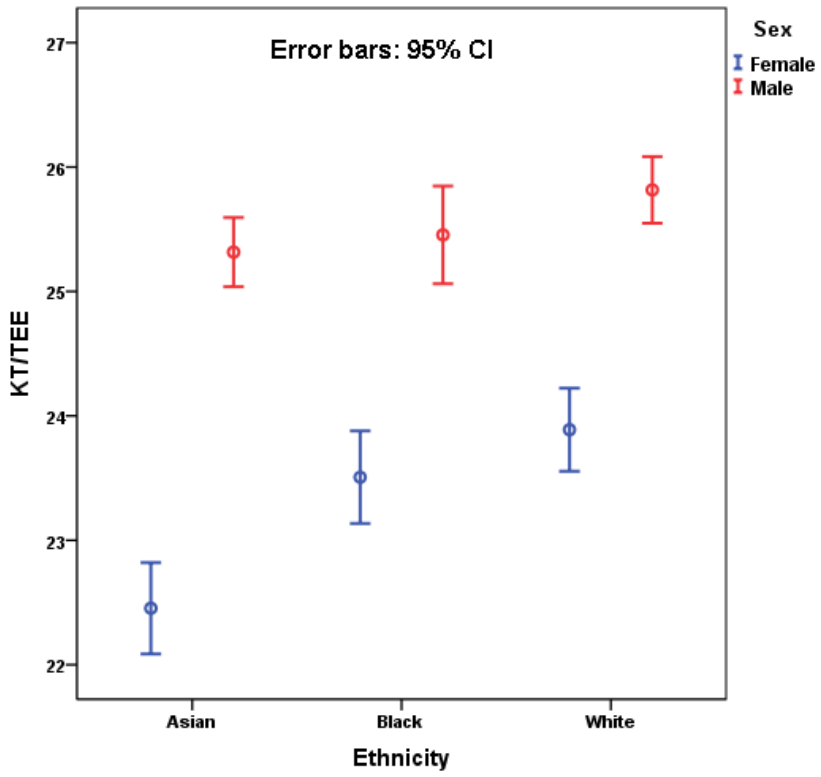


Figure 11-9: Effect of ethnicity on predicted delivered minimum Kt/TEE

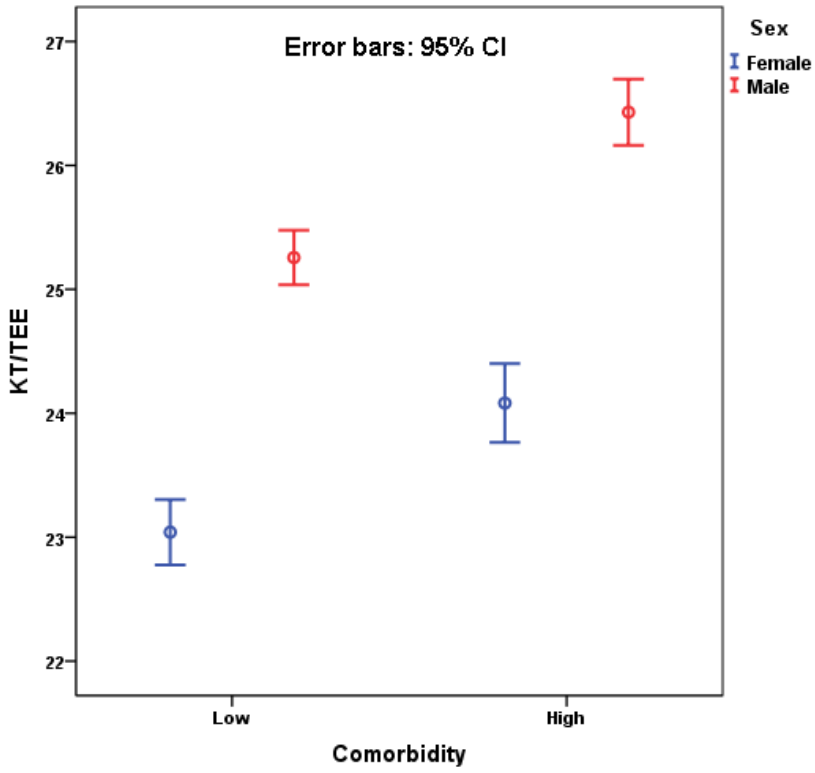


Figure 11-10: Effect of comorbidity on predicted delivered minimum Kt/TEE

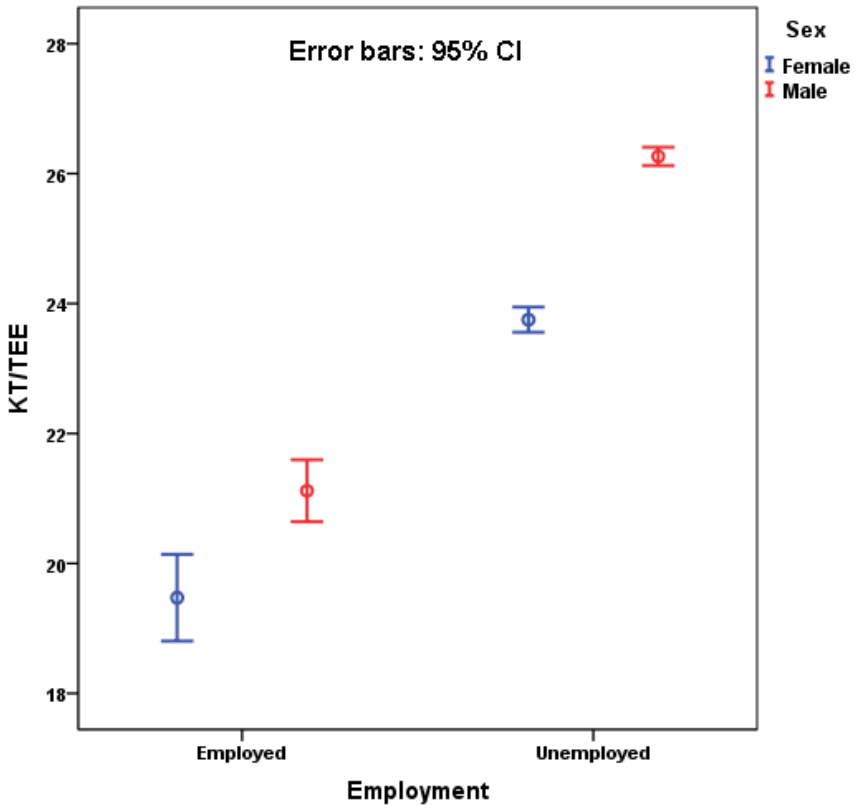


Figure 11-11: Effect of employment on predicted delivered minimum Kt/TEE

The best linear regression model (adjusted R square 0.573) of independent predictors of Kt/TEE is shown in Table 11-4. Age, gender, weight, Asian ethnicity, and employment status were the variables found to predict Kt/TEE.

Table 11-4: Independent Predictors of Kt/TEE

Model	Standardised Coefficients (Beta)	t	p-value
Constant		23.789	< 0.001
Age	0.340	19.109	< 0.001
Sex	0.340	19.552	< 0.001
Weight	0.187	10.571	< 0.001
Asian Ethnicity	-0.056	-3.251	0.001
Employment	0.458	25.954	< 0.001

11.4 Discussion

This study aimed to explore the minimum dialysis dose, corresponding to current recommended Kt/V target of 1.2, that would be delivered using some of the alternate scaling parameters and also to identify the characteristics of patients who are at risk of under-dialysis with current dosing practice. The study results demonstrated that the predicted minimum delivered dialysis dose would be significantly lower in women compared to men if any of the three parameters – BSA, REE or TEE – are used though they all would have had identical Kt/V values. The study findings are in favour of rejecting the null hypothesis and accepting the experimental hypothesis that current dialysis practice delivers sub-optimal dose to some subgroups of patients.

Small women would have received lower doses compared to larger women if Kt/BSA was used. However, small men would have received significantly lower doses compared to their larger men irrespective of whatever the scaling parameter was. Besides sex, younger age, employment, Asian ethnicity and comorbidity status also have an impact on dosing based on these metabolic factors.

There has been an ongoing debate on what is the best parameter for dialysis dosing. Some authors have argued that Kt/V is the best parameter while others have refuted it (Gotch, 2000; Lowrie, 2000). Daugirdas et al (2008) have shown that BSA-based dialysis dosing will result in higher dialysis doses to women and small men (Daugirdas, Depner, et al., 2008). A recent study has also shown a better relationship with survival for the BSA-based dosing compared to current practice (Ramirez et al., 2012). Morton and Singer (2007) have argued that the dialysis dose should be based on metabolic rate because of the non-linear correlation between body mass and metabolic rate (Morton & Singer, 2007). Previous findings have also shown that women have relatively higher urea generation rate and TEE is an independent predictor of urea generation rate (Sridharan, Vilar, Berdeprado, & Farrington, 2013). This would mean that TEE could also be considered a potential scaling parameter for dialysis dosing.

As shown in Figure 11-1 to Figure 11-3, for any given Watson volume, women have higher level of any of the metabolic parameters examined here. This would mean that using these parameters would meet the higher requirements for dialysis dose in females compared to males.

There were gender, body size and age related differences in metabolically normalised dialysed doses based on these parameters. The current study demonstrates that with V-based dosing, women of all body sizes are at risk of under-dialysis compared to similar-sized men if equivalent doses are estimated using metabolic parameters such as BSA, REE or TEE as denominators. As shown in Figure 11-4 to Figure 11-7, females have a requirement for a greater dialysis dose and this overrides any effect of weight. This shows that V-based dosing targets need to be gender-specific, unlike current recommendations. Not only was there significant gender difference, but there were also dialysis dose differences based on body weight. Smaller males require a greater relative dose than larger males. The study also found that women and small men would receive significantly lower dialysis dose with BSA-based dosing compared to their larger counterparts as per the current minimum dialysis dose target. This implies that V-based dosing targets also need to be body-size specific. Alternately, using parameters such as BSA, REE or TEE may inherently adjust for these gender and body size differences thus negating the problem of having multiple dose targets.

Dialysis dose expressed as Kt/REE or Kt/TEE was significantly lower in patients with low comorbidity compared to those with higher levels of comorbidity. This would mean that individuals with low comorbidity are at risk of under-dialysis and may have a requirement for higher dose. However, it is worth noting that these findings are based mainly on body size and estimated physical activity and do not take into account the potential differences in energy metabolism which may be associated with specific comorbidities.

The current study also identified other possible patient characteristics that may be associated with risk of under-dialysis. Both Kt/REE and Kt/TEE increased with increasing age, suggesting a need for higher dialysis doses in younger age groups. Kt/TEE was also significantly lower in those who are in employment compared to those who are not, thereby, implying a greater dialysis dose requirement in employed individuals. Patients of Asian ethnicity were also found to be at risk of under-dialysis if REE or TEE were considered as scaling parameters. However, it has to be emphasised that these subgroup differences are likely to be secondary to differences in body weight and physical activity levels rather than a direct effect of these characteristics on metabolic needs. Given this relationship between the above characteristics and energy metabolism, these factors were shown to be significant in predicting Kt/TEE in a linear regression model.

There is dearth of comparative studies using these 3 parameters in clinical studies and hence, it is difficult to ascertain if one of these parameters is superior to others in providing dialysis dose based on metabolic needs of the individual. Daugirdas et al (2008) have argued that theoretically using REE, unlike BSA, will not result in substantial increase in dialysis dose to women (Daugirdas, Levin, et al., 2008). However, there is suggestion from a previous study that metabolic rate drives the glomerular filtration rate (GFR) (Singer, 2001). Also, GFR and metabolic rate scale to body mass with virtually the same exponent (Singer & Morton, 2000). Hence, metabolic rate i.e., REE could be a potential scaling parameter. Physical activity contributes to increased metabolism and thereby, higher metabolic waste production. It could be argued that TEE, incorporating both REE and physical activity, could be a better parameter reflecting the metabolic activity. However, these are theoretical arguments and there is need for comparative outcome-based studies employing standardised forms of these parameters to examine this issue.

11.5 Conclusion

In conclusion, the current study has demonstrated that metabolic parameters such as BSA, REE and TEE could be used to scale dialysis dose and that current V-based dosing risk under-dialysing women and small men. Our study findings suggest a gender-, body size- and physical activity-specific V-based dosing or dosing based on these alternate metabolic parameters. Scaling dialysis dose based on these metabolic parameters may benefit some subgroups of patients by providing more dialysis and may in turn impact on their survival outcome. The potential impact on survival of using these metabolic parameters for dialysis dosing will be examined in the next chapter.

Chapter 12

12. Metabolic Activity-Based Haemodialysis Dosing: Impact on Survival

12.1 Introduction

As discussed in previous chapters, parameters reflecting metabolic activity such as body surface area (BSA), resting energy expenditure (REE) and total energy expenditure (TEE) have a potential role as scaling factors for haemodialysis dosing. The arguments put forward in support of these energy expenditure parameters have been theoretical ones and there has not been any published study examining REE or TEE in relation to patient outcomes. As explained in chapter 10, this prospective multi-centre study was designed with a primary aim of examining the impact of using these metabolic parameters for scaling dialysis dose on patient survival. The previous chapters have shown some subgroups of patients to be at risk of under-dialysis with current dosing practice and may benefit from use of one of these metabolic parameters.

The main aim of the study was to compare dialysis doses prescribed using each of these parameters in relation to survival outcomes. The study also aimed to develop possible adjustments to the current recommended minimum dialysis dose that would be needed to better reflect the metabolic needs of various subgroups of patients. The experimental hypothesis for this study was that one or more of these parameters, when used to scale dialysis dose, might predict survival better than the current practice of using Kt/V. The null hypothesis was that there would be no difference in predictive power with regards to survival between these scaling parameters.

12.2 Methods

12.2.1 Ethical Review

The study was approved by the North Wales Research Ethics Committee (ref: 12/WA/0060 and UH Ethics ref: LMS/PG/NHS/00257). All subjects gave written informed consent to take part.

12.2.2 Study Protocol

1. The study population consisted of 1500 maintenance haemodialysis patients. The inclusion and exclusion criteria are explained in chapter 10.

2. Demographic, anthropometric and biochemical data was collected for each study participant as detailed in chapter 10.

3. Physical activity data was collected using Recent Physical Activity Questionnaire (RPAQ) and comorbidity data from a self-report comorbidity questionnaire. Both the questionnaires were administered once only at the time of recruitment.

4. Study participants were followed up at pre-specified time points – at 3 months from recruitment, and then, at 6, 12 and 18 months. Patients from the renal units of Lister hospital were followed up till 24 months from recruitment.

5. The following data were collected at follow-up – patient survival; change in renal replacement therapy modality such as transplantation, home haemodialysis or peritoneal dialysis or transfer to a different dialysis unit not included in the study; data pertaining to haemodialysis session to enable calculation of delivered dialysis doses at each time point.

6. Study subjects were censored for transplantation, change of renal replacement therapy from in-centre haemodialysis (i.e. home haemodialysis or peritoneal dialysis) and recovery of renal function or if their care was transferred to another centre.

12.2.3 Scaling Dialysis Dose

The alternate scaling parameters – Body Surface Area (BSA), Resting Energy Expenditure (REE) and Total Energy Expenditure (TEE) – were calculated as explained in chapter 10.

Delivered dialysis dose based on current practice (Delivered Kt/V) was calculated for study subjects at each time point and a mean delivered Kt/V for the entire study duration was calculated. Delivered Kt was calculated for each patient as below.

$$\boldsymbol{Delivered\ Kt\ =\ Delivered\ \frac{Kt}{V}\times V}$$

where V is Watson volume. Corresponding values of delivered Kt/BSA, Kt/REE and Kt/TEE were calculated by dividing Delivered Kt by the respective parameters for each patient at each time point. Similar to the delivered Kt/V, corresponding mean delivered doses for the entire duration of the study was calculated.

12.2.4 Standardising the Dialysis Dose

In order to allow comparison between Kt/V, Kt/BSA, Kt/REE and Kt/TEE, it is essential to standardise these parameters. All the parameters were standardised to within one standard deviation of each parameter by the following method.

For each parameter, mean and standard deviation of the study population was calculated. Then, the individual value for each patient was subtracted from the mean and divided by the standard deviation. For example, the Standardised Kt/TEE was calculated as below.

$$\boldsymbol{Standardised\ \frac{Kt}{TEE}\ =\ \frac{\left(\frac{Kt}{TEE}-\ \mu\right)}{\sigma}}$$

where Kt/TEE is the parameter value for each patient, μ is the population mean of Kt/TEE and σ is the standard deviation of the population. Standardised Kt/V, Kt/BSA and Kt/REE were calculated similarly using respective parameter means and standard deviations.

12.2.5 Statistical Analysis

Statistical analysis was carried out using SPSS ® version 19 (SPSS Software, IBM Corporation, New York, USA). Normally distributed data are presented as mean ± SD, and non-normally distributed as median [± interquartile range]. The significance of differences between means was determined by Student's t-test and of differences between medians by the Mann-Whitney U test. The significance of differences between multiple group means was assessed by ANOVA with differences between individual groups being assessed using the Bonferroni test. Survival differences between groups were analysed using Kaplan-Meier plot with Log-rank test.

Independent predictors of survival were assessed using Cox regression models. A p-value of <0.05 was assumed to indicate statistical significance.

12.3 Results

12.3.1 Demographics

The demographic and anthropometric details of patients at baseline were discussed in chapter 10. Some selected anthropometric information and energy expenditure details are shown in Table 12-1. For other variables, please refer to the demographic section in chapter 10. For weight, Watson volume and the three metabolic parameters, a mean value was calculated by taking an average of the respective measurements at each time point during the follow-up. These mean values are shown in Table 12-1. All measures of body size and energy expenditure were generally lower in females compared to males.

Table 12-1: Anthropometric and Energy Expenditure Characteristics of Study Patients

	All patients (n = 1500)	Males (n = 910)	Females (n = 590)	p-value
Age at Baseline (years)	62.9 ± 15.5	63.8 ± 15.6	61.6 ± 15.1	0.007
Mean Weight (kg)	74.9 ± 18.3	78.0 ± 17.0	70.2 ± 19.0	<0.001
Height (cm)	165.9 ± 10.0	170.6 ± 8.2	158.7 ± 8.2	<0.001
Ethnicity (% South Asian: Black: White)	27.9: 26.7: 45.5	27.3: 24.0: 48.7	28.6: 30.9: 40.5	0.003
High Comorbidity (%)	29.5	28.7	30.7	NS
Employed (%)	11.5	13.1	9.2	0.021
Mean Watson Volume (Litres)	37.5 ± 7.4	40.9 ± 6.5	32.2 ± 5.0	<0.001
Mean BSA (m^2)	1.86 ± 0.26	1.93 ± 0.24	1.77 ± 0.26	<0.001
Mean REE (kcal/day)	1542 ± 250	1617 ± 229	1426 ± 236	<0.001
Mean TEE (kcal/day)	1837 ± 388	1944 ± 391	1673 ± 321	<0.001

The mean delivered dose of Kt/V and estimated mean delivered doses using the alternate metabolic parameters are shown in Table 12-2. The delivered Kt/V dose was significantly higher in females compared to males ($p < 0.001$). The delivered dose expressed as Kt or Kt/BSA was significantly lower in females ($p < 0.001$ for both). However, there is no statistically significant gender difference in the delivered dose expressed as Kt/REE or Kt/TEE.

Table 12-2: Gender Differences in the Delivered Dialysis Dose

	All patients (n = 1500)	Males (n = 910)	Females (n = 590)	p-value
Mean Kt/V	1.37 ± 0.22	1.32 ± 0.20	1.44 ± 0.22	<0.001
Mean Kt (ml)	47,046 ± 8,494	49,698 ± 8,404	42,954 ± 6,854	<0.001
Mean Kt/BSA (ml/m^2)	27,161 ± 4,120	27,732 ± 4,101	26,280 ± 3,993	<0.001
Mean Kt/REE (ml/kcal)	32.97 ± 5.11	33.14 ± 4.81	32.71 ± 5.54	NS
Mean Kt/TEE (ml/kcal)	28.0 ± 5.0	27.93 ± 4.84	28.1 ± 5.23	NS

The median follow-up time was 18.5 months (range: 18 – 25 months). There were a total of 263 deaths during the study period. 192 patients were censored for various reasons of which 143 were due to renal transplantation.

12.3.2 Survival Based on Gender

Kaplan-Meier analysis showed significantly higher survival rates in women compared to men (p = 0.003, Log-rank test) as shown in Figure 12-1.

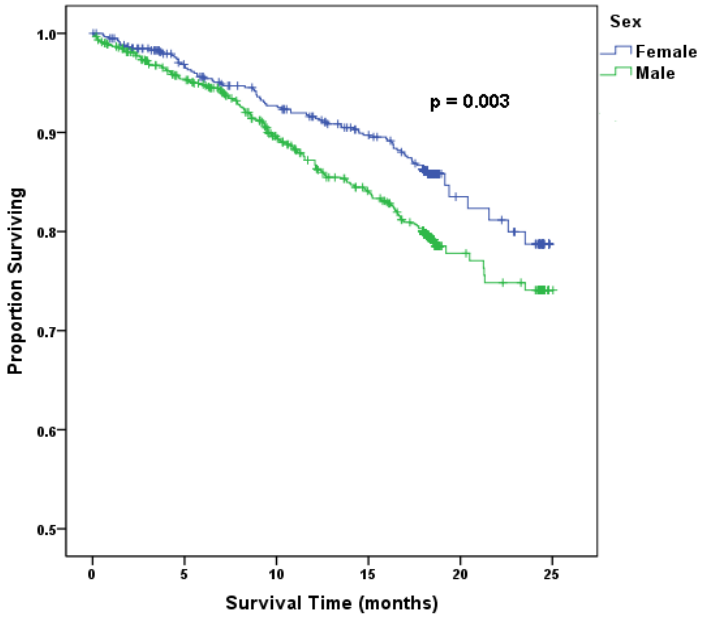


Figure 12-1: Kaplan-Meier plot of Gender difference in Survival

12.3.3 Survival based on Ethnicity

Using a Kaplan-Meier analysis, it can be seen that there was significantly higher survival rate in Non-Whites compared to those of White ethnic origin ($p < 0.001$, Log-rank test; Figure 12-2).

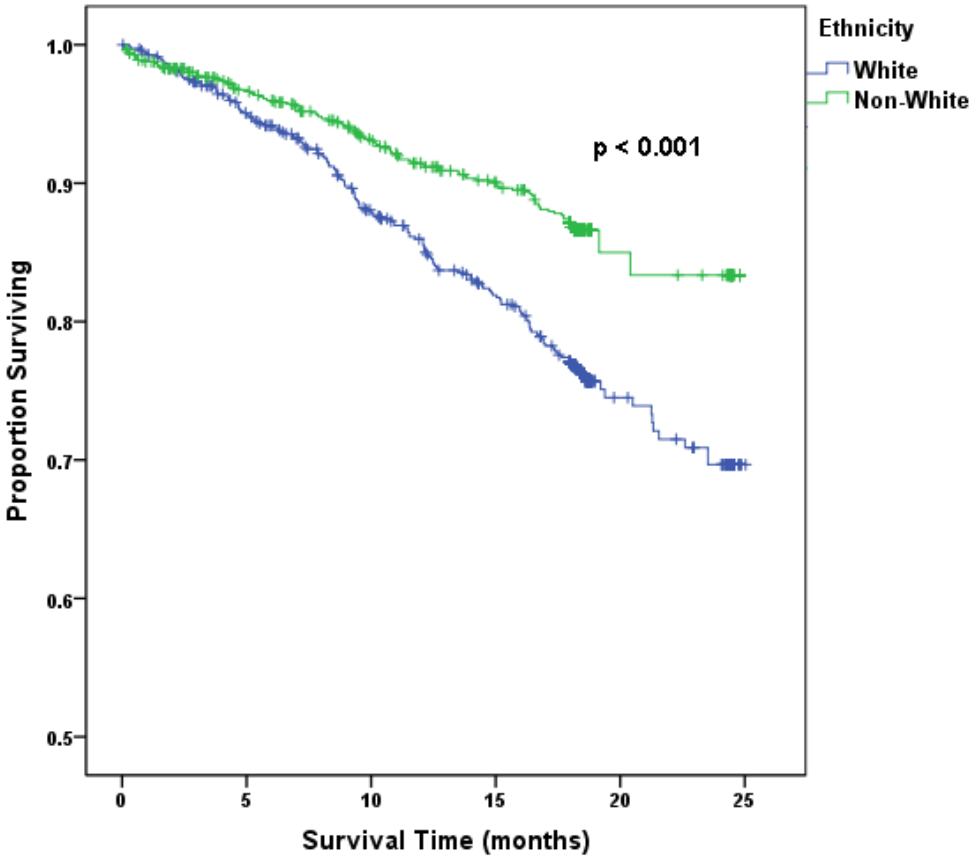


Figure 12-2: Kaplan-Meier plot of Ethnic difference in Survival

12.3.4 Survival based on Comorbidity

Kaplan-Meier analysis showed significantly higher survival rate in patients with low comorbidity (comorbidity score ≤ 3) compared to those with higher scores ($p < 0.001$, Log-rank test, see Figure 12-3).

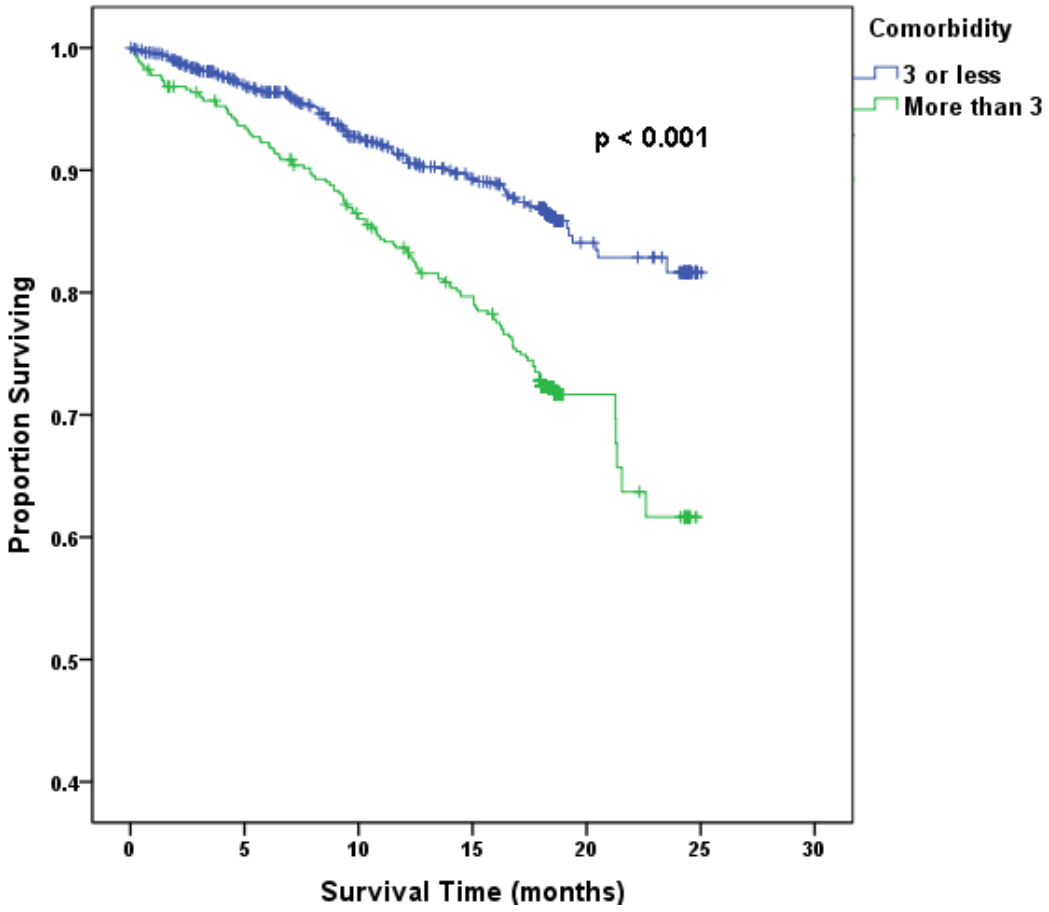


Figure 12-3: Kaplan-Meier plot of Survival based on Comorbidity

12.3.5 Determining the parameter that best predicts survival

Given the significant survival differences with regards to gender, ethnicity and comorbidity as shown above, these variables were included in the Cox regression models to explore the parameter that best predicts survival in this group of haemodialysis patients.

In order to allow comparison between these parameters, standardised measures of these variables – Kt/V, Kt/BSA, Kt/REE and Kt/TEE – were used in the Cox models which are shown in Table 12-3 through to Table 12-6.

Table 12-3: Cox Model of Survival using Standardised Kt/V

	p-value	Hazard Ratio	95% confidence interval for Hazard Ratio	
			Lower	Upper
Age	< 0.001	1.032	1.021	1.043
Male Gender	0.441	1.113	0.848	1.461
Non-White Ethnicity	0.004	0.684	0.528	0.884
Comorbidity Score	< 0.001	1.156	1.097	1.219
BMI	< 0.001	0.937	0.914	0.961
Mean Daily MET	0.022	0.157	0.032	0.761
Standardised Kt/V	< 0.001	0.721	0.626	0.831

Table 12-4: Cox Model of Survival using Standardised Kt/BSA

	p-value	Hazard Ratio	95% confidence interval for Hazard Ratio	
			Lower	Upper
Age	< 0.001	1.028	1.017	1.039
Male Gender	0.006	1.448	1.110	1.888
Non-White Ethnicity	0.003	0.675	0.522	0.874
Comorbidity Score	< 0.001	1.156	1.097	1.218
BMI	< 0.001	0.940	0.917	0.964
Mean Daily MET	0.025	0.163	0.034	0.797
Standardised Kt/BSA	< 0.001	0.708	0.617	0.812

Table 12-5: Cox Model of Survival using Standardised Kt/REE

	p-value	Hazard Ratio	95% confidence interval for Hazard Ratio	
			Lower	Upper
Age	< 0.001	1.037	1.026	1.048
Male Gender	0.062	1.286	0.988	1.674
Non-White Ethnicity	0.003	0.674	0.521	0.873
Comorbidity Score	< 0.001	1.156	1.097	1.218
BMI	< 0.001	0.942	0.919	0.965
Mean Daily MET	0.024	0.161	0.033	0.786
Standardised Kt/REE	< 0.001	0.717	0.627	0.820

Table 12-6: Cox Model of Survival using Standardised Kt/TEE

	p-value	Hazard Ratio	95% confidence interval for Hazard Ratio	
			Lower	Upper
Age	< 0.001	1.037	1.025	1.048
Male Gender	0.062	1.285	0.987	1.673
Non-White Ethnicity	0.003	0.673	0.520	0.871
Comorbidity Score	< 0.001	1.156	1.097	1.218
BMI	< 0.001	0.942	0.919	0.966
Mean Daily MET	< 0.001	0.032	0.006	0.174
Standardised Kt/TEE	< 0.001	0.697	0.600	0.810

In all the above models, Body Mass Index (BMI) was included as adjustment for body size and Mean Daily MET for level of physical activity. Age, ethnic origin, comorbidity, BMI and Mean Daily MET significantly predicted survival in all the models. Gender was a significant predictor in the model using Kt/BSA but was not so in other models. The hazard ratios for the standardised measures of dialysis doses represent the risk of death associated with a unitary increase in one standard deviation of the corresponding parameter. Standardised Kt/TEE had the lowest hazard ratio of death (0.697) in these models with Standardised Kt/V having the highest hazard ratio (0.721).

12.3.6 Determining the "optimum cut-off" point for the alternate parameters

The study data was analysed to identify the optimum cut-off point for dialysis doses expressed using the alternate parameters that would better predict the survival in the study cohort. A Cox regression model using quartiles of delivered Kt/BSA is shown in Table 12-7 along with the survival plot based on Kt/BSA quartiles (Figure 12-4).

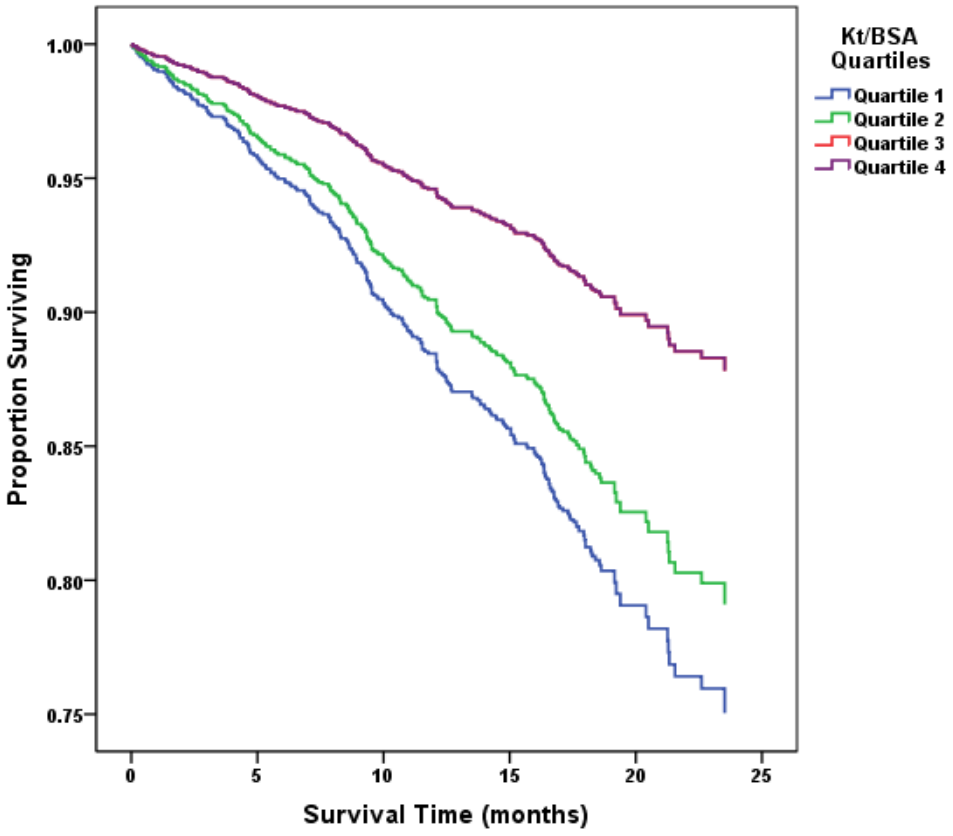


Figure 12-4: Survival Differences based on Kt/BSA Quartiles

Table 12-7: Survival model using Kt/BSA Quartiles

	p-value	Hazard Ratio	95% confidence interval for Hazard Ratio	
			Lower	Upper
Age	< 0.001	1.028	1.017	1.040
Male Gender	0.013	1.398	1.072	1.823
Asian Ethnicity	0.452	0.893	0.665	1.199
Black Ethnicity	< 0.001	0.444	0.302	0.651
Comorbidity Score	< 0.001	1.145	1.086	1.207
BMI	< 0.001	0.944	0.921	0.968
Mean Daily MET	0.010	0.120	0.024	0.605
Kt/BSA – Quartile 1	< 0.001	2.210	1.522	3.210
Kt/BSA – Quartile 2	0.002	1.805	1.239	2.628
Kt/BSA – Quartile 3	0.995	1.001	0.667	1.503

In this model, White ethnicity and the highest quartile of Kt/BSA (Quartile 4) were used as reference groups to compare with their respective counterparts in the other groups. Compared with the highest Kt/BSA quartile, the bottom 2 quartile groups had significantly higher risk of death but there was no difference in survival when compared to the quartile 3. This suggests that the median dose of delivered Kt/BSA would be the cut-off point where a significant impact on patient survival is seen. This is also clearly evident in the survival plot shown (Figure 12-4).

As Kt/TEE had the lowest hazard ratio for death in the overall Cox models discussed above, a similar model was constructed using Kt/TEE instead of Kt/BSA which is shown below (Table 12-8).

Table 12-8: Survival model using Kt/TEE Quartiles

	p-value	Hazard Ratio	95% confidence interval for Hazard Ratio	
			Lower	Upper
Age	< 0.001	1.036	1.025	1.048
Male Gender	0.054	1.298	0.995	1.692
Asian Ethnicity	0.489	0.901	0.670	1.212
Black Ethnicity	< 0.001	0.457	0.312	0.670
Comorbidity Score	< 0.001	1.143	1.084	1.206
BMI	< 0.001	0.945	0.922	0.969
Mean Daily MET	< 0.001	0.028	0.005	0.154
Kt/TEE – Quartile 1	< 0.001	2.408	1.659	3.493
Kt/TEE – Quartile 2	0.044	1.421	1.009	2.002
Kt/TEE – Quartile 3	0.503	1.125	0.798	1.585

Similar to the previous model, White ethnicity and highest quartile of Kt/TEE were used as reference groups. As with Kt/BSA, there was significantly higher risk of death pertaining to those in the lowest 2 quartiles of delivered Kt/TEE dose with no difference in survival between Kt/TEE quartiles 3 and 4. Again, this suggests the median delivered Kt/TEE dose to be the optimum cut-off point where survival differences are evident. A survival plot showing the differences in survival across these groups is shown in Figure 12-5. A model using Kt/REE also showed similar findings with the median value being the optimum cut-off point.

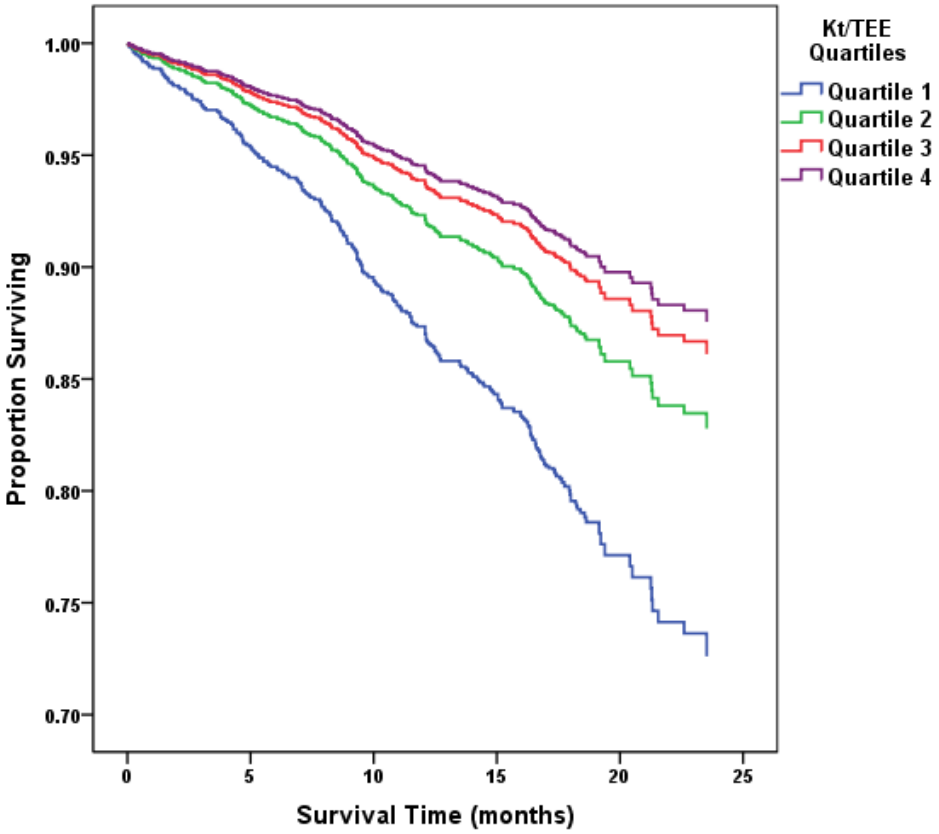


Figure 12-5: Survival Differences based on Kt/TEE Quartiles

12.3.7 Determining the Equivalent cut-off dose of Kt/V based on Kt/BSA

In order to provide equivalent dialysis doses across genders and patients of different body size, the minimum target Kt/V needs to be adjusted based on these factors. The study data was analysed to determine the equivalent cut-off dose of minimum target Kt/V based on Kt/BSA which will incorporate gender and body-size differences.

A plot of delivered Kt/BSA against delivered Kt/V based on gender-specific weight cut-offs is shown in Figure 12-6. Gender-specific median weight was used as the cut-off value for categorising into four groups (males and females, above and below median weight). The best fit line for each group was then populated. The relationship between Kt/BSA and Kt/V had a similar fit in females irrespective of their weight but some separation could be seen in males depending on weight. The horizontal reference line in the graph corresponds to median Kt/BSA as this was found to be the optimum threshold value for survival benefit. Equivalent Kt/V values for each of these groups were then interpreted from the graph as shown.

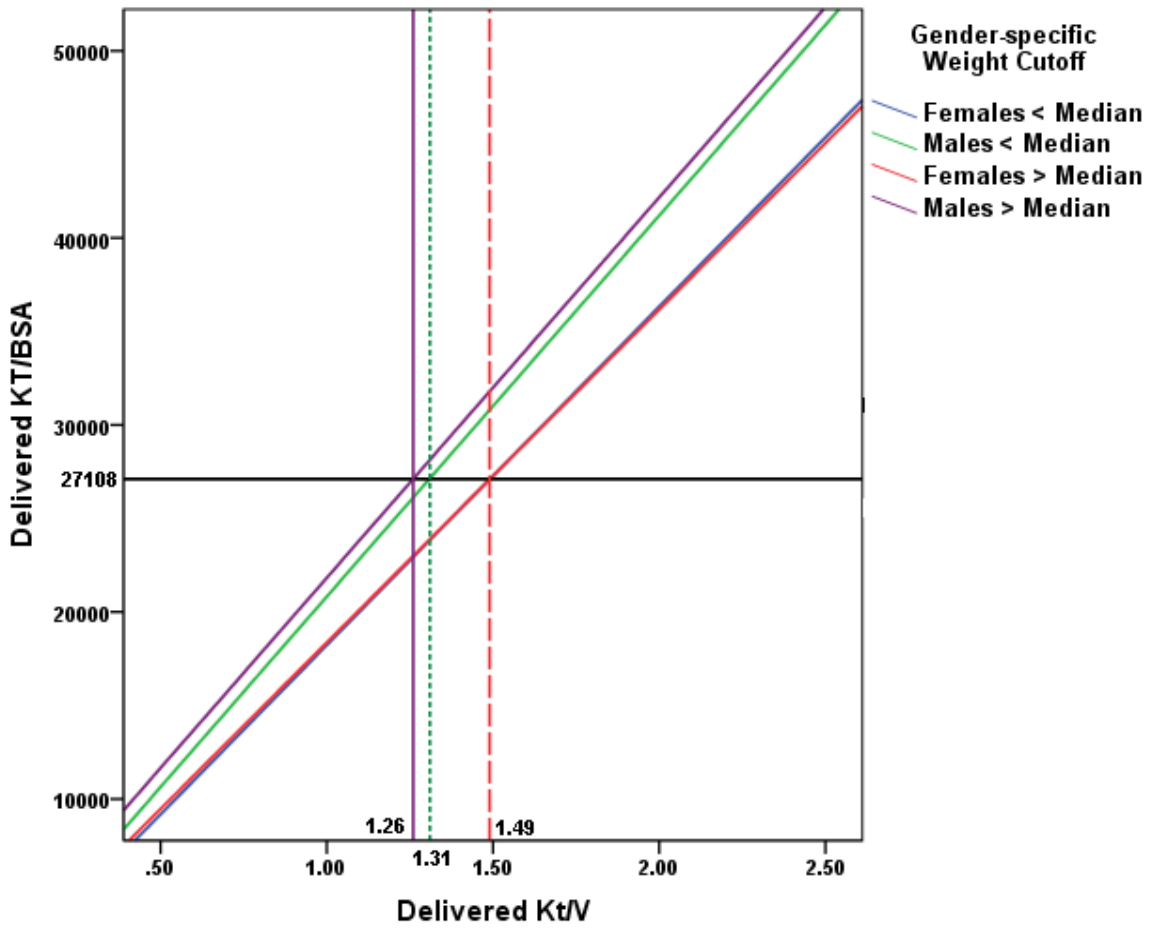


Figure 12-6: Equivalent Kt/V dose based on Kt/BSA

From the graph, the equivalent dose of Kt/V corresponding to the median Kt/BSA was 1.49 for females (irrespective of weight), 1.31 for smaller males (less than median body weight) and 1.26 for larger males (more than median weight). This equivalent dose of Kt/V incorporates gender- and body size-specific adjustments.

12.3.8 Activity-related adjustment of Kt/V dose

In order to find the magnitude of dose adjustment needed for different levels of physical activity, study participants were categorised into 4 activity categories based on the Mean Daily MET estimated from RPAQ. Those with daily MET of ≤ 1.2 were categorised as sedentary, 1.21 to 1.5 as Light Active, 1.51 to 2 as Active and > 2 as Highly Active.

Table 12-9 shows the mean delivered Kt/TEE doses for each of the activity categories. There were significant differences in the mean delivered doses across the groups as assessed by ANOVA ($p < 0.001$).

Table 12-9: Activity Categories and Delivered Kt/TEE doses

	Number of Patients	Mean	Std. Deviation
Sedentary (MET ≤ 1.2)	1100	29.13	4.68
Light Active (1.21 – 1.5)	346	25.65	4.11
Active (1.51 – 2)	52	20.06	3.42
Highly Active (> 2)	2	15.75	3.28

The relationship between the mean delivered Kt/V and delivered Kt/TEE is shown in Figure 12-7. The equivalent dose of Kt/V corresponding to median Kt/TEE, interpreted from the graph, was 1.37. The vertical broken lines in the graph represent the mean delivered Kt/TEE doses for each activity group discussed above. For each of these lines, the corresponding equivalent Kt/V dose was interpreted. The mean delivered Kt/V progressively decreased with increasing activity – 1.28 for Light Active, 1.09 for Active and 0.96 for Highly Active groups.

As median Kt/TEE was found to be the optimum threshold value to ensure better survival, the equivalent Kt/V dose of 1.37 is considered to be the target dose for all these groups. In order to achieve target Kt/TEE above median (28.0), the equivalent dose increment in Kt/V needed for each of these groups is as follows:

- Sedentary patients (median Kt/TEE – 28.6) = Nil
- Light Active patients (median Kt/TEE – 25.7) = 0.1
- Active patients (median Kt/TEE – 20.1) = 0.3
- Highly Active patients (median Kt/TEE – 15.8) = 0.4

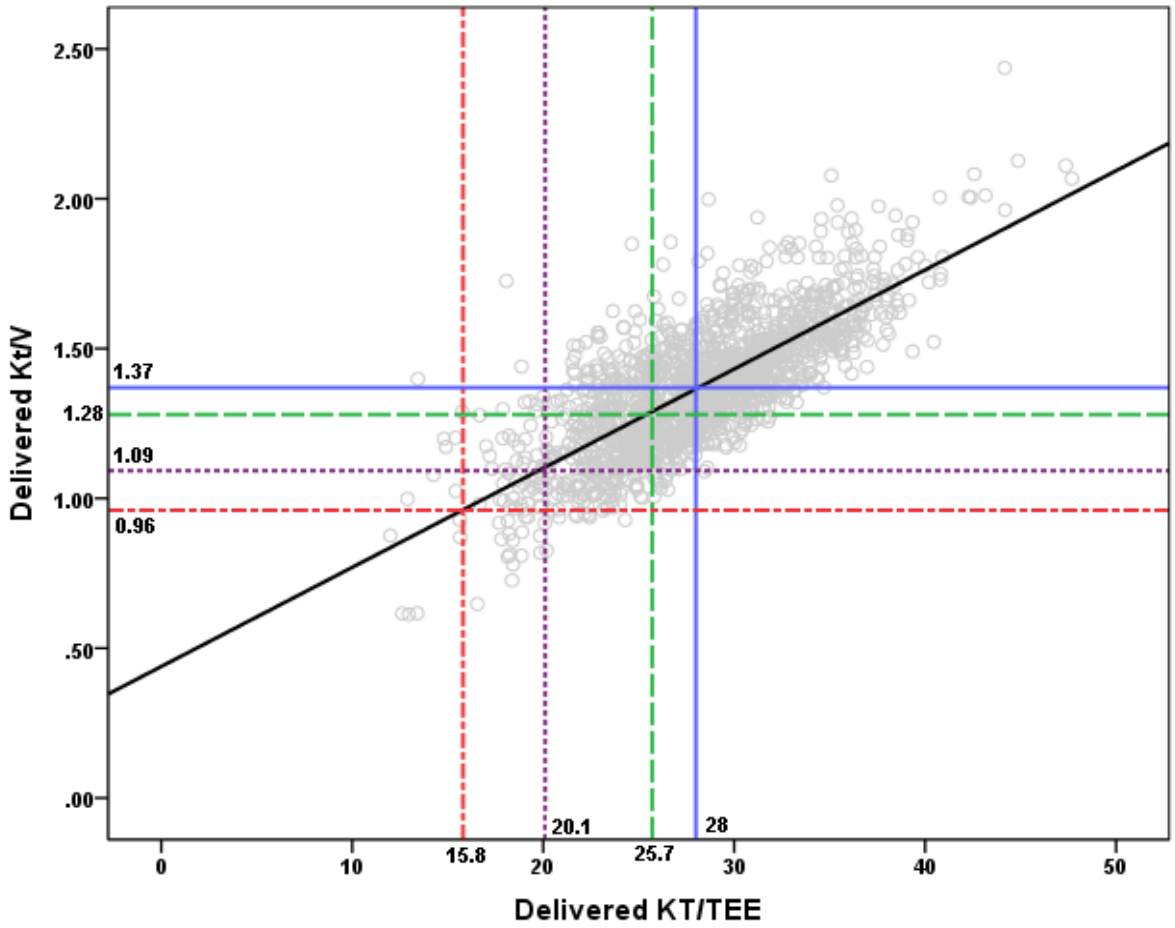


Figure 12-7: Equivalent Kt/V dose based on Activity Level

12.3.9 Comparison of Delivered and Recommended Kt/V doses

A recommended minimum target Kt/V dose was calculated for each individual patient based on the above gender, body-size and activity-related increments to the current minimum target Kt/V of 1.2. The frequency distribution of the recommended minimum target Kt/V is shown in Table 12-10. The minimum recommended Kt/V ranged from 1.26 to 1.89 based on gender, body-size and activity level with nearly 50% of them requiring a minimum dose of 1.41 or higher.

Table 12-10: Distribution of Recommended Minimum Target Kt/V

Recommended Minimum Kt/V	Number of Patients	% of cohort
1.26	311	20.7
1.31	317	21.1
1.36	119	7.9
1.41	117	7.8
1.49	472	31.5
1.56	25	1.7
1.59	110	7.3
1.61	20	1.3
1.71	1	0.1
1.79	7	0.5
1.89	1	0.1

The mean recommended and delivered Kt/V doses are shown in Table 12-11. The mean delivered Kt/V dose, both at baseline and throughout study period, was lower than the above recommended minimum target Kt/V.

Table 12-11: Recommended and Delivered Kt/V doses

	Mean	Std. Deviation
Recommended Minimum Kt/V	1.44	0.11
Delivered Kt/V at Baseline	1.34	0.26
Delivered Kt/V during the study	1.37	0.22

The mean delivered Kt/V for the entire study period was compared with the above recommended Kt/V for exploring the characteristics of patients who were under-dialysed. The proportion of patients who were under-dialysed based on gender alone and considering gender and body-size together are shown in Table 12-12 and Table 12-13. A higher proportion of females were under-dialysed compared to males (63.2% vs. 52.6%). The problem of under-dialysis was particularly high in women of greater than median weight with 79.7% of them being under-dialysed. This was significantly higher in comparison to smaller women (46.8%), smaller men (46.6%) and larger men (58.7%).

Table 12-12: Proportion of Patients Under-dialysed based on Gender

	Proportion of Patients Under-dialysed
Females	63.2%
Males	52.6%

Table 12-13: Proportion of Patients Under-dialysed based on Gender and Body Size

	Proportion of Patients Under-dialysed
Females > Median Weight	79.7%
Females < Median Weight	46.8%
Males > Median Weight	58.7%
Males < Median Weight	46.6%

12.3.10 Impact of Under-dialysis on Survival

The mortality risk associated with under-dialysis in comparison with the above recommended Kt/V was examined. Using Cox regression model, it was noted that patients who failed to achieve the average recommended minimum target Kt/V had around 76% increase in adjusted mortality risk (Table 12-14 and Figure 12-8).

Table 12-14: Adjusted Mortality Risk in Under-dialysed Patients

	p-value	Hazard Ratio	95% confidence interval for Hazard Ratio	
			Lower	Upper
Age	< 0.001	1.032	1.021	1.043
Male Gender	0.012	1.403	1.076	1.830
Asian Ethnicity	0.388	0.878	0.654	1.179
Black Ethnicity	< 0.001	0.451	0.308	0.662
Comorbidity Score	< 0.001	1.148	1.088	1.211
BMI	< 0.001	0.946	0.923	0.970
Mean Daily MET	0.001	0.069	0.014	0.351
Under-Dialysis	< 0.001	1.761	1.362	2.276

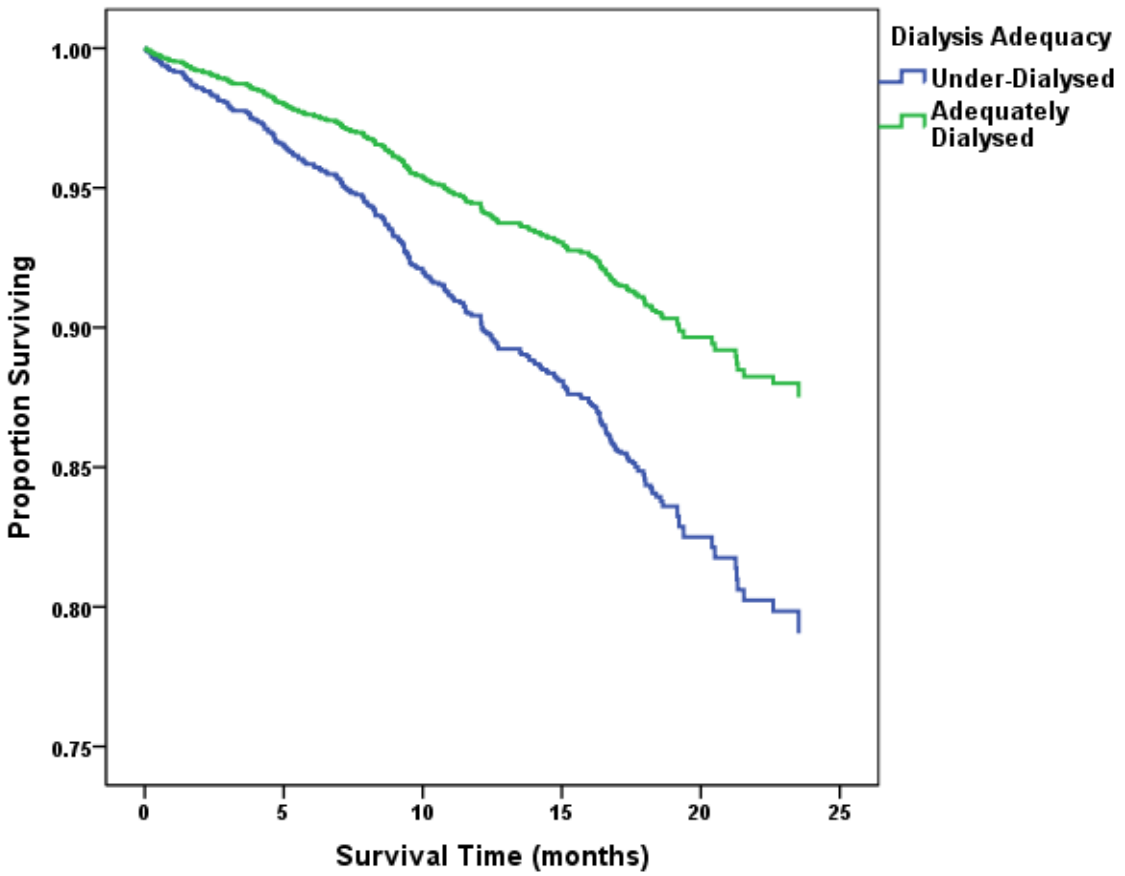


Figure 12-8: Adjusted Mortality Risk based on Dialysis Adequacy

12.4 Discussion

The primary hypothesis of this study was that one or more of the metabolic parameters predict survival better than Kt/V. Result from the study showed that body size and activity related adjustments to the dialysis dosing favourably impact on patient survival. The metabolic activity related parameters, especially BSA and TEE, might potentially be a better scaling parameter compared to Watson volume. The study results also showed that the mean recommended dose derived based on body size and physical activity level was significantly higher compared to the delivered dialysis dose and those under-dialysed as per this criteria has had unfavourable survival outcomes. These findings are in favour of rejecting the null hypothesis and accepting the experimental hypothesis that one or more of these parameters, when used for scaling dialysis dose, predict survival better than total body water.

In the current practice of using Kt/V for prescribing dialysis dose, the effect of using V as a denominator is to scale dialysis dose to body water volume which is linearly related to body weight. However Resting Energy Expenditure (REE) – and related parameters e.g. body surface area (BSA) - are related to body weight (W) by power functions of the form $REE = CW^b$, in which $b < 1$. Hence if such parameters, reflecting energy expenditure, were more appropriate dose scaling factors than V, then to achieve the same target dose expressed in these terms, smaller compared to larger individuals would require relatively larger dialysis doses expressed as Kt/V. Expressed another way, the relative concentration of metabolic wastes per unit of body weight may be higher in small individuals (Kotanko & Levin, 2007) and hence, the dialysis requirement per unit weight may be greater in these individuals.

The current guidelines for dialysis dose prescription recommend a minimum target single-pool Kt/V of 1.2 per session for both males and females ("Clinical Practice Guidelines for Hemodialysis Adequacy, Update 2006," 2006). Although, the guidelines suggest that the target may have to be higher in women than men, it falls short of stating gender-specific minimum targets for dialysis dosing. This study findings show that women are receiving generally higher dialysis doses than men in current clinical practice. The study results also show that women have better survival compared to men after adjustment for age, ethnicity and comorbidity. This is in line with the survival pattern seen in general population where women have reduced mortality risk compared to men. Previous reports have shown the loss of survival benefit in women receiving haemodialysis and as a result, have similar mortality risk as men ("UK Renal Registry - The Sixteenth Annual Report," 2013). It is plausible that the survival benefit for women shown in this study is due to higher delivered dialysis doses as this survival difference cease to exist when adjusted for delivered Kt/V. This argues for a case for gender-specific minimum haemodialysis dose with relatively higher doses for women compared to men.

Results from the study demonstrate that using BSA, REE or TEE for scaling dialysis dose will deliver higher dialysis doses to some patient groups who are at risk of under-dialysis with current practice. The standardised measures of dialysis dose using these parameters were compared and it was found that Kt/TEE had the lowest hazard ratio for death whilst Kt/V had the highest. Physiologically, using TEE for scaling dialysis would make sense as it encompasses the basal metabolic activity

and the physical activity component, thereby, serving as a good marker of total metabolic waste production. This is the first comparative study to date of these three metabolic parameters for dialysis dosing and the study data showed that TEE is the best parameter of them all with regards to predicting patient survival in haemodialysis population.

The major pitfall in using total body water (V) for scaling dialysis dose is that it does not adjust for the relative uraemic toxin concentration in individuals with different body sizes. This is the reasoning behind suggesting BSA as the scaling parameter instead of V. It is also important to account for the activity-related metabolic waste generation which is better captured by a parameter such as TEE. So, any novel alternative parameter to V for scaling dialysis dose should inherently adjust for body size and activity-related changes in the uraemic milieu.

Given the well-established practice of using Kt/V for dialysis dosing and the practical difficulties of converting to a novel scaling parameter, it is prudent to make specific adjustments to Kt/V itself depending on body size and activity level of the individual patient rather than adopting a completely different scaling parameter. With this in mind, an algorithm was developed to decide the minimum target Kt/V for an individual based on body size and activity level. Using Kt/BSA, the minimum target Kt/V values for women, smaller and larger men were identified. As shown in the previous chapter, female gender overrides any effect of weight in dosing based on these alternate scaling parameters and hence, women have a single minimum target irrespective of body size whereas in men, smaller men have higher minimum target level compared to larger men. To this minimum target values, an additional adjustment is made based on activity level to arrive at a recommended minimum target Kt/V value.

The study results showed that those under-dialysed based on the derived recommended minimum Kt/V had 76% higher risk of mortality compared to those who were adequately dialysed. This implies that adjusting dialysis dose for metabolic activity has a favourable impact on patient survival. This is the first study to have explored and suggested an algorithm to decide minimum target Kt/V based on metabolic activity.

12.5 Limitations

There are some limitations to this study. This is only a cross-sectional study with estimations of metabolic parameters carried out from a single anthropometric reading. However, anthropometric values from each subject were obtained through direct measurements and not derived from historic medical records. TEE was calculated from physical activity data collected through a recall questionnaire and hence, recall bias is a potential confounder in the accuracy of the data. Any questionnaire-based activity energy expenditure will only provide an estimate of TEE rather than an accurate value and the method employed here is no exception to this. It is worth noting that the algorithm for Kt/V adjustments for activity level is derived from the specific questionnaire that has been used in this study. Given the variable accuracy and precision of different activity questionnaires, it is likely that the results may vary if another physical activity questionnaire is employed and as such, this algorithm is only valid for use with RPAQ. Finally, the findings discussed are in relation to short term survival and hence, these cannot be extrapolated to long-term survival benefits.

12.6 Conclusion

In conclusion, the study results demonstrate that metabolic activity related parameters could potentially be used for scaling dialysis dose and one or more of these parameters may be better than the total body water currently used in clinical practice. The study has also shown that adjusting dialysis dose based on body size and habitual physical activity will have a positive impact on survival in haemodialysis patients. An algorithm has also been developed to make dialysis dose adjustments based on body size and reported physical activity. Further studies are needed to examine the validity and applicability of this algorithm for routine clinical practice.

Chapter 13

13. General Discussion

13.1 Overview

Kidneys perform a multitude of metabolic functions to maintain the internal milieu. Although the primary function is to excrete metabolic wastes from the body, kidneys play a very important role in maintaining body fluid volume, blood pressure regulation, acid-base and electrolyte balance, bone mineral metabolism and maintaining serum haemoglobin level. In end-stage renal disease, therefore, a wide range of metabolic abnormalities is seen relating to the native functions of the kidney. The spectrum of metabolic abnormalities and the associated clinical manifestations stretch beyond the effects of just uraemic solute accumulation. Anaemia and acidosis in advanced kidney disease is one such example. Similarly, derangement of bone mineral metabolism lead to higher serum phosphate levels resulting in increased vascular calcification and cardiovascular mortality risk.

The major adverse consequences of renal failure, though, are thought to be secondary to the effects of accumulated uraemic toxins in blood and tissues. Uraemic toxins are categorised according to their molecular size and protein-binding capacity. Many of these toxins have been shown to exert pathological effects on organ systems. For example, protein-bound toxins such as p-cresylsulphate and indoxyl sulphate have been shown to be associated with higher mortality risk in patients with kidney disease (Liabeuf et al., 2010; Barreto et al., 2009). Increased serum level of small molecules such as phosphate could lead to increased cardiovascular mortality through vascular calcification (Shanahan, Crouthamel, Kapustin, & Giachelli, 2011).

The synthesis and metabolic pathways of many of these uraemic toxins have not been mapped completely. However, many of these uraemic toxins are by-products of cellular metabolism and protein turnover in the body that have accumulated to toxic levels due to reduced metabolism and/or reduced excretion by the kidneys in renal failure. The goal of renal replacement therapy in end-stage renal disease is to

substitute for lost renal function. Although, some adverse consequences of renal failure such as fluid imbalance and acid-base and electrolyte disturbances could be managed with varying success through renal replacement therapy, the primary goal is removal of accumulated metabolic waste products. It is worth noting that achieving adequate solute removal through dialysis alone should not be considered as the optimal therapy. The definition of adequate dialysis should encompass the appropriate management of all the different pathological and clinical manifestations associated with kidney failure. As a result, judging minimal dialysis requirement is difficult. The current practice of deciding adequate dialysis based on urea clearance normalised to total body water may lead to some unintended consequences.

13.2 Dialysis Dosing and Patient Survival

Specific subgroups of haemodialysis patients seem to be at risk of reduced survival. For example, the survival benefit of women compared to men in the general population is attenuated in patients on renal replacement therapy i.e. there is no survival difference between women and men receiving renal replacement therapy ("UK Renal Registry - The Sixteenth Annual Report," 2013). There are also body size differences in survival – obese haemodialysis patients tend to have better survival compared to those with even ideal body weight (Port et al., 2002).

As discussed in chapter 2, some of these differences could be due, at least in part, to the practice of normalising dialysis dose based on total body water. A number of studies, discussed in detail in chapter 2, have been published suggesting that women and possibly small men are under-dialysed with current dosing practices. This has led to investigators suggesting alternate parameters such as body surface area, resting energy expenditure, high metabolic organ mass etc. to be considered as a factor for scaling dialysis dose instead of total body water. All these factors have been suggested to closely reflect metabolic waste production. However, the direct relationship of these parameters to a measure of uraemic toxin generation has not been studied to date. Though theoretical arguments have been presented in favour and against each of these alternate parameters for scaling dialysis dose (Daugirdas, Levin, et al., 2008), with the exception of body surface area, none of these factors have been examined in clinical studies with regards to their applicability and patient outcomes.

It is important to understand the factors that influence generation of these uraemic solutes and to identify ways to incorporate suitable measures of these factors in deciding minimum dialysis requirements. As uraemic toxins are metabolic waste products, it is plausible that measures of metabolic activity such as basal metabolic rate or total energy expenditure could be a suitable marker of uraemic toxin generation. One would presume that the dialysis dose be normalised based on these markers of uraemic toxin generation. The current practice, however, normalises dialysis dose based on total body water which is the volume in which the uraemic solutes are distributed. This is a 'one size fits all' method of treatment delivery and does not take into account individual variations in metabolic activity. There is also a knowledge gap here in that the impact of metabolic activity on uraemic solutes has not been studied in patients with advanced kidney disease.

The overarching aim of this thesis is of the form "optimal management of advanced kidney failure should take into account metabolic factors that influence the rate of uraemic toxin generation". In order to achieve this aim, a better understanding of the relationships between different measures of metabolic activity and uraemic toxin generation is needed. Studies in this thesis focus on two different aspects – firstly to examine the relationship between metabolic factors such as resting energy expenditure and total energy expenditure on metabolic waste production and secondly, to explore the impact of these factors on survival outcome in patients with advanced kidney disease. An important point of focus in many studies in this thesis is the physical activity level of patients with kidney disease. Physical activity is the most variable component of total energy expenditure and developing tools to quantify activity may facilitate better measurement of total metabolic needs on an individual basis.

13.3 Metabolic Factors Influencing Uraemic Toxin Generation

13.3.1 Basal Metabolic Rate

Basal metabolic rate is expressed as resting energy expenditure and is the sum total of the metabolic activities at rest. As mentioned above, it would be expected that resting energy expenditure is a good marker of uraemic toxin generation. The relationship between metabolic rate and urea generation rate was explored in this thesis. As shown in chapter 4, there was a significant positive correlation between

these two variables which was better than that observed between total body water and urea generation rate. This implies that higher levels of resting energy expenditure contribute to increased uraemic toxin generation and thereby, higher dialysis dose requirement. A study by Vilar et al (2014) showed that REE adjusted to body weight was higher in individuals with low body mass index compared to obese individuals (Vilar et al., 2014) suggesting that smaller individuals may require relatively higher dialysis dose compared to the larger individuals. This phenomenon might explain the poorer survival in women and small individuals as they would have received relatively lower dialysis doses in relation to their metabolic needs with current dialysis dosing.

The relationship between resting energy expenditure and whole body protein turnover was also explored. As shown in chapter 9, there is a significant relationship between whole body protein turnover and resting energy expenditure with the former contributing to about 55% of the basal metabolic rate. As many of the uraemic toxins are considered to be products of protein metabolism, the results from chapters 4 and 9 suggest a close relationship between resting energy expenditure and uraemic toxin production. And finally, the impact of declining renal function on resting energy expenditure was explored in chapter 6. The study data showed that there is no significant change in resting energy expenditure with decline in renal function from CKD stages 1 through to 5. This supports the previously proposed argument that metabolic rate is the driving force for GFR and not vice-versa.

The significant relationship between resting energy expenditure and uraemic toxin generation across these studies has been consistent. Combined with the lack of impact of renal function on metabolic rate, this strengthens the arguments in favour of resting energy expenditure being a better scaling parameter for dialysis dosing compared to total body water. The main strengths of these studies lie in the fact that resting energy expenditure was measured directly and was not estimated from predictive equations and that objective measures of uraemic toxin generation have been used. The studies carried out in this thesis have provided novel insights into the influence of metabolic rate on uraemic toxin generation.

13.3.2 Physical Activity

Physical activity is the most variable component of total energy expenditure and can be measured. Physical activity is being studied increasingly in patients with kidney disease. As a result, a multitude of physical and mental health benefits has been shown to be associated with active lifestyle in CKD patients. Many studies have also demonstrated benefits from intra-dialytic exercise programmes (Cheema, 2008). However, some questions regarding physical activity in this patient group are still not answered – are there any specific subgroups of patients who are at high risk of inactivity? What is the effect of increasing activity on metabolic waste generation? Should the dialysis dose be adjusted according to activity level?

Studies in this thesis have sought to answer these questions. A study of large cohort of haemodialysis patients in this thesis (chapter 10) has shown that physical activity is low in the overall dialysis population. But, there are specific subgroups of patients – females, elderly and those with high comorbidity – who are at increased risk of even lower activity than the rest of the group. This implies that exercise programmes in haemodialysis patients should make additional efforts to identify these subgroups of patients at higher risk and motivate them to lead a more active lifestyle.

A logical question that follows these efforts is whether higher physical activity contributes to increased metabolic waste generation. As shown in chapter 8, measured physical activity correlates with urea generation rate. Even after adjustment of demographic variables, increased levels of high-intensity activity remained a significant predictor of urea generation rate in regression models. This implies that higher physical activity levels may contribute to increased uraemic toxin generation. It would seem physiological to assume that physical activity will lead to increased metabolic waste production due to a number of potential changes associated with higher activity level. For example, increased activity may lead to changes in muscle protein turnover, increase in lean body mass and reduction in body fat (Park et al., 2014, 2011; Scott, Blizzard, Fell, & Jones, 2011). There may possibly be other associated changes such as increase in dietary protein intake and alterations in substrate metabolism secondary to changes in body composition. This aspect of physical activity has not been shown in clinical studies to date and this

thesis (chapter 8) has demonstrated the relationship between activity and uraemic toxin generation using objective measures.

Does this mean that physically active individuals need higher dialysis dose due to increased uraemic toxin generation? The answer to this has been explored in chapters 10 to 12. There is an interaction between physical activity and body size of individuals. The study data in Chapter 10 showed highly active individuals were heavier in body size compared to their sedentary counterparts. As has been the common theme in this thesis, it is likely that heavier individuals need relatively less dialysis dose with regards to their body size. However, they are likely to need a higher dose based on the activity level. These two effects would cancel out each other to certain extent. The direction in which the balance of dialysis dosing would tip in this patient subgroup depends on individual body size and activity level measures. The interaction between body size and physical activity means that dialysis dosing practice needs to focus on providing individualised doses based on metabolic needs and move away from a generalised approach. The combined results of these studies in this thesis have provided better understanding of physical activity behaviour and its impact on clinical practice in patients with kidney disease.

In addition to examining the above aspects of physical activity, two physical activity questionnaires have also been examined for their validity in CKD patients. Most of the existing physical activity questionnaires were derived from young, healthy active individuals. As a result, these questionnaires may not be suitable for elderly population and may not be useful in studying sedentary or low-intensity activity. Patients with kidney disease are likely to be older and perform only low levels of activity (Johansen et al., 2000). Hence, it is important to validate activity questionnaires in patients with kidney disease. The study results from chapter 7 showed the Recent Physical Activity Questionnaire (RPAQ) to perform better than the Stanford questionnaire. The activity-related energy expenditure derivation from the questionnaire was examined in detail and the best estimated measure had acceptable correlation with energy expenditure measured from doubly labelled water. This is the first study to date that has validated activity questionnaires in patients with kidney disease using doubly labelled water method. This validation study has thus demonstrated that RPAQ is a valid tool for estimating activity-related

energy expenditure in CKD patients which will facilitate easier activity data collection in routine clinical practice.

13.3.3 Total Energy Expenditure

If resting energy expenditure (REE) and physical activity impact on uraemic toxin generation on their own, does a combined measure of these – total energy expenditure (TEE) – have an even greater influence on metabolic waste generation? This question has been explored in two studies in this thesis (chapters 4 and 7). In one of these studies (chapter 4), TEE was estimated from measured REE and estimated physical activity from a questionnaire in haemodialysis patients. The study data showed a significant relationship between TEE and urea generation rate. The strength of the correlation between TEE and urea generation rate was stronger than that between REE or physical activity and urea generation rate. This suggests that TEE may be a better marker of uraemic toxin generation compared to the other two parameters.

In the study presented in chapter 6, TEE was measured by the gold standard method using doubly labelled water in patients with different levels of kidney function. The results of this study also showed a significant positive relationship between TEE and urea generation rate and the strength of the correlation was even stronger than that demonstrated in the previous study (chapter 4). Moreover, TEE along with fat-free mass was a significant determinant of urea generation rate in multivariate regression models as shown in chapter 4.

The gold-standard doubly labelled water technique has never been employed in CKD patients for TEE measurement and the reported study in this thesis is the first study to have directly measured TEE in this patient group using this method. These two studies have demonstrated a consistent relationship between total energy expenditure and urea generation rate suggesting that TEE has a significant impact on uraemic toxin generation in patients with kidney disease. Given the stronger correlation between these two variables, Total Energy Expenditure may possibly be a better marker of uraemic toxin generation compared to resting energy expenditure or physical activity-related energy expenditure alone.

13.3.4 Whole body protein turnover

The relationship between whole body protein turnover, REE and urea generation rate was explored in the study reported in chapter 9. The study showed that overall there was a negative protein balance both in pre-dialysis patients with advanced kidney disease and in chronic haemodialysis patients. The study also showed a significant correlation between protein breakdown and urea generation rate. In addition, urea generation rate increased with more negative net turnover balance. Haemodialysis patients in this study had higher protein turnover rate compared to pre-dialysis CKD patients. Although, there was no difference in net turnover balance between these groups in this study, the dietary protein intake was lower than recommended in haemodialysis patients which puts them at risk of worsening negative protein balance in the long-term. Increased negative balance may contribute to higher uraemic toxin generation. However, there is a close interaction between protein turnover and REE. It is unlikely that the contribution of whole body protein turnover to uraemic toxin generation is independent of REE as protein turnover is a subset of resting energy expenditure. The sample size of this study did not allow for such analysis to be performed in this thesis and whether protein turnover contributes to uraemic toxin generation independent of REE remains to be seen.

13.4 Practical Implications on Dialysis Dosing

Studies presented in this thesis have consistently suggested that measures of metabolic activity – resting energy expenditure (REE) and total energy expenditure (TEE) – impact on uraemic toxin generation. This argues in favour of these parameters to be used as a scaling factor for normalising dialysis dose instead of total body water. Body surface area (BSA) normalised dialysis dose has been shown to deliver higher dialysis doses to women and favourably impact on survival as well (Ramirez et al., 2012). However, REE and TEE have not been studied in this context till date.

The applicability of these parameters and the associated potential benefits of using these parameters for scaling dialysis dose have been explored in chapters 11 and 12. The study results showed that there is risk of under-dialysis to specific subgroups of patients with current practice of using total body water for scaling dialysis dose

and using the metabolic parameters, namely BSA, REE or TEE, will deliver relatively higher dialysis doses to these groups at risk. The study also showed that for any given Watson volume, women had higher level of any of these three metabolic parameters implying that use of these scaling parameters would deliver higher dialysis dose to women.

There has been no comparative study exploring the impact of all three parameters with regards to patient survival. This was examined in the study presented in chapter 12. The study data showed that all three parameters – BSA, REE and TEE – influence patient survival when used for normalising dialysis dose. TEE was found to be the best scaling parameter with the lowest hazard ratio for death compared to others including Watson volume (V). The study showed that women had significantly better survival compared to men even after adjustment for age, ethnicity and comorbidity which reflects the survival pattern in general population. The study also showed that women are receiving higher dialysis dose compared to men in current clinical practice and this may have contributed to the improved survival in this group. This is the only study that has explored the applicability of REE and TEE as dialysis scaling parameters with respect to patient survival and is the only comparative study of all three parameters for dialysis dosing till date.

What are the practical implications of this finding for dialysis dosing? The practice of prescribing and monitoring dialysis dose using Kt/V is ingrained in clinical practice worldwide. The best way forward to provide adequate dialysis based on individual's metabolic needs would be to define algorithms that can be used to make dose adjustments based on body size and physical activity. Is there any way of incorporating dose adjustments for body size and activity level to the current practice? To answer this, the equivalent doses of Kt/BSA and Kt/TEE that would provide survival benefit were explored. The study data was then used to develop algorithms to make adjustments to the current minimum Kt/V target. Recommended minimum target Kt/V for women, small and large men were derived and it was found that those who were under-dialysed based on this recommended target Kt/V had poorer survival outcomes.

These study results (chapters 11 and 12) have the potential to modify clinical practice to achieve better patient outcomes. These studies have strengthened the

notion that scaling dialysis dose should take into consideration individual differences in body size and metabolic activity. With further research to validate and refine, these algorithms developed for adjustment of Kt/V dose can potentially be applied to routine clinical practice. Dialysis dosing practice should move away from a 'one size fits all' therapy to a more practical and individualised form of therapy that would deliver the appropriate dose based on the individual's metabolic needs. The studies reported in this thesis are a step in that direction and with further research, this goal of individualising dialysis therapy can be a reality in the near future.

13.5 General Limitations of the Studies

The studies reported in this thesis have certain general limitations. Urea generation rate was used as a surrogate marker of uraemic toxin generation in these studies. There is no consensus on the ideal marker for uraemic toxin generation. The most recent publication of the European Uremic Toxin Work Group (EUTox) lists 88 uraemic retention solutes that have been identified in patients with end-stage renal disease (Duranton et al., 2012). Given the variations in the physiological properties, kinetics and metabolic pathways of these toxins, it is unlikely that a single molecule will be identified as an 'ideal' uraemic toxin to be studied. In addition, the kinetics of many of these toxins has not been clearly mapped at present. Since the current dialysis practice is based largely on urea clearance and that urea clearance has been shown to be associated with survival, using urea generation rate as a marker of uraemic toxin generation seems the best pragmatic solution to this problem. As such, results presented here relate to the factors influencing urea generation. The generation rate of other uraemic toxins may be influenced by a different set of variables.

The studies here have focused largely on haemodialysis and not on peritoneal dialysis. Patients receiving peritoneal dialysis (PD) may have different demographic and clinical characteristics compared to haemodialysis patient cohort. In addition, the activity level of PD patients may be different too. Measurement of dialysis dose in PD patients is also different to that for HD patients as many of these patients have significant residual renal function. As such, the results of the studies presented here should not be extrapolated directly to PD patients. It is likely that the general underlying principles of the relationship between metabolic rate and uraemic toxin

generation still holds true for PD patients although this needs to be tested in future clinical studies.

13.6 Future Directions

There are a number of topics that are discussed in this thesis where further research needs to be carried out. These potential areas for future studies are discussed below.

13.6.1 Developing a disease-specific physical activity questionnaire

Although the RPAQ has been shown to be a valid tool for activity data collection in patients with kidney disease, the sedentary and low intensity activities are not adequately captured in this questionnaire. As patients with end-stage kidney disease predominantly perform sedentary and light activity, a physical activity questionnaire that captures this level of activity will enable better estimation of total energy expenditure in routine clinical practice. In addition, activity questionnaires generally do not enquire about sleep disturbances. Sleep disturbances are common in patients with kidney disease and may lead to increased muscle activity contributing to total energy expenditure. Development of a new physical activity questionnaire should seek to integrate questions to capture sleep disturbances. Of course, the new questionnaire has to be validated against objective physical activity measures or against other validated activity questionnaires.

13.6.2 Validating the dialysis dosing algorithm in other dialysis cohorts

The dialysis dosing adjustment to Kt/V based on body size and activity level algorithm was developed from a single large cohort of patients in the study reported in chapter 12. This algorithm needs to be tested and validated in other cohorts of dialysis patients using survival outcomes based on this recommended dialysis dose. It is worth noting that the activity level adjustment for dialysis dose is specifically based on the data collected from RPAQ and has not been validated for use in conjunction with similar activity measures derived from other physical activity questionnaires. If this algorithm performs consistently across different dialysis cohorts, future research should focus on integrating this into routine clinical practice.

13.6.3 Monitoring of physical activity in haemodialysis patients

The dialysis dosing algorithm discussed in chapter 12 relies on obtaining the physical activity data from a questionnaire. Physical activity level is dependant on various factors and may vary time to time based on factors such as acute illness, personal circumstances etc. Increase in activity levels may necessitate an increase in dialysis dose. This implies that the physical activity level needs to be monitored regularly so that appropriate dialysis dose adjustments can be carried out. However, the frequency of monitoring needed cannot be decided based on current evidence. It is recommended that future studies measure physical activity longitudinally to examine the intra-individual variation in activity over a longer period. Such studies will enable decision-making on the appropriate frequency of activity monitoring for dialysis dose adjustments.

13.6.4 Impact of dialysis dosing alterations on protein turnover and nutrition

The study of whole body protein turnover (chapter 9) has demonstrated negative protein balance in haemodialysis patients which may lead to muscle wasting in the long term. Though there have been many molecular level disturbances that have been attributed as a cause for muscle wasting in renal failure, it is not known whether delivery of adequate dialysis dose will counteract the negative protein balance. The dialysis dosing algorithm (chapter 12) seeks to provide appropriate dialysis dose based on individual's metabolic needs. Future studies in this field could be designed to explore the impact of delivering dialysis dose based on this algorithm on whole body and muscle protein turnover.

13.7 Summary

In summary, the studies presented in this work have demonstrated the important influence of resting and total energy expenditure on uraemic toxin generation and the beneficial effect of using these parameters for scaling dialysis dose on patient survival. Moreover, it has been shown that physical activity levels impact on dialysis dose requirements. It is important to design further studies to explore appropriate methods to incorporate measures of energy expenditure in management of patients with kidney disease.

References

Abahji, T. N., Nill, L., Ide, N., Keller, C., Hoffmann, U., & Weiss, N. (2007). Acute hyperhomocysteinemia induces microvascular and macrovascular endothelial dysfunction. *Archives of Medical Research, 38*(4), 411-416.

Adey, D., Kumar, R., McCarthy, J. T., & Nair, K. S. (2000). Reduced synthesis of muscle proteins in chronic renal failure. *American Journal of Physiology Endocrinology and Metabolism, 278*(2), E219-225.

Ainsworth, B. E., Haskell, W. L., Leon, A. S., Jacobs, D. R., Montoye, H. J., Sallis, J. F., & Paffenbarger, R. S. (1993). Compendium of physical activities: classification of energy costs of human physical activities. *Medicine and Science in Sports and Exercise, 25*(1), 71-80.

Ainsworth, B. E., Haskell, W. L., Whitt, M. C., Irwin, M. L., & et al. (2000). Compendium of physical activities: an update of activity codes and MET intensities. *Medicine and Science in Sports and Exercise, 32*(9), S498-S504.

Ainsworth, Barbara E., Haskell, William L., Herrmann, Stephen D., Meckes, Nathanael, Bassett, David R., Jr., Tudor-Locke, Catrine, . . . Leon, Arthur S. (2011). 2011 Compendium of Physical Activities: A Second Update of Codes and MET Values. *Medicine and Science in Sports and Exercise, 43*(8), 1575-1581.

Aizawa, T., Hiramatsu, K., Ohtsuka, H., Kobayashi, M., Koizumi, Y., Miyamoto, T., . . . Yamada, T. (1986). An elevation of BUN/creatinine ratio in patients with hyperthyroidism. *Hormone and Metabolic Research, 18*(11), 771-774.

Albu, J., Shur, M., Curi, M., Murphy, L., Heymsfield, S. B., & Pi-Sunyer, F. X. (1997). Resting metabolic rate in obese, premenopausal black women. *American Journal of Clinical Nutrition, 66*(3), 531-538.

Anand, S., Johansen, K. L., Grimes, B., Kaysen, G. A., Dalrymple, L. S., Kutner, N. G., & Chertow, G. M. (2013). Physical activity and self-reported symptoms of insomnia, restless legs syndrome, and depression: the comprehensive dialysis study. *Hemodial Int, 17*(1), 50-58.

Arnold, W. C., & Holliday, M. A. (1979). Tissue resistance to insulin stimulation of amino acid uptake in acutely uremic rats. *Kidney International, 16*(2), 124-129.

Au-Yeung, K. K., Yip, J. C., Siow, Y. L., & O, K. (2006). Folic acid inhibits homocysteine-induced superoxide anion production and nuclear factor kappa B activation in macrophages. *Canadian Journal of Physiology and Pharmacology, 84*(1), 141-147.

Avesani, Carla Maria, Draibe, Sergio Antonio, Kamimura, Maria Ayako, Dalboni, Maria Aparecida, Colugnati, Fernando Antonio Basile, & Cuppari, Lilian. (2004). Decreased resting energy expenditure in non-dialysed chronic kidney disease patients. *Nephrology Dialysis Transplantation, 19*(12), 3091-3097.

Avesani, Carla Maria, Trolonge, Stanislas, Deleaval, Patrik, Baria, Flavia, Mafra, Denise, Faxen-Irving, Gerd, . . . Fouque, Denis. (2012). Physical activity and energy expenditure in haemodialysis patients: an international survey. *Nephrology Dialysis Transplantation, 27*(6), 2430-2434.

Baria, Flavia, Kamimura, Maria Ayako, Avesani, Carla Maria, Lindholm, Bengt, Stenvinkel, Peter, Draibe, Sergio Antonio, & Cuppari, Lilian. (2011). Activity-related Energy Expenditure of Patients Undergoing Hemodialysis. *Journal of Renal Nutrition, 21*(3), 226-234.

Barreto, F. C., Barreto, D. V., Liabeuf, S., Meert, N., Glorieux, G., Temmar, M., . . . (EUTox), European Uremic Toxin Work Group. (2009). Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. *Clinical Journal of the American Society of Nephrology, 4*(10), 1551-1558.

Beddhu, Srinivasan, Pappas, Lisa M., Ramkumar, Nirupama, & Samore, Matthew. (2003). Effects of Body Size and Body Composition on Survival in Hemodialysis Patients. *Journal of the American Society of Nephrology, 14*(9), 2366-2372.

Bellach, B. (1993). Remarks on the use of Pearson's correlation coefficient and other association measures in assessing validity and reliability of dietary assessment methods. *European Journal of Clinical Nutrition, 47 Suppl 2*, S42-45.

Bergström, J. (1995). Nutrition and mortality in hemodialysis. *Journal of the American Society of Nephrology, 6*(5), 1329-1341.

Berkelhammer, C. H., Baker, J. P., Leiter, L. A., Uldall, P. R., Whittall, R., Slater, A., & Wolman, S. L. (1987). Whole-body protein turnover in adult hemodialysis patients as measured by 13C-leucine. *American Journal of Clinical Nutrition, 46*(5), 778-783.

Besson, Herve, Brage, S., Jakes, Rupert W., Ekelund, Ulf, & Wareham, Nicholas J. (2010). Estimating physical activity energy expenditure, sedentary time, and physical activity intensity by self-report in adults. *American Journal of Clinical Nutrition, 91*(1), 106-114.

Blair, Steven N., Haskell, William L., Ho, Ping, Paffenbarger, Ralph S., Vranizan, Karen M., Farquhar, John W., & Wood, Peter D. (1985). Assessment of Habitual Physical Activity by a Seven-day Recall in a Community Survey and Controlled Experiments. *American Journal of Epidemiology, 122*(5), 794-804.

Bland, Martin J., & Altman, Douglas G. (1986). Statistical Methods for Assessing Agreement Between Two Methods of Clinical Measurement. *The Lancet, 327*(8476), 307-310.

Bloembergen, W. E., Stannard, D. C., Port, F. K., Wolfe, R. A., Pugh, J. A., Jones, C. A., . . . Held, P. J. (1996). Relationship of dose of hemodialysis and cause-specific mortality. *Kidney International, 50*(2), 557-565.

Boirie, Y., Beaufrère, B., & Ritz, P. (2001). Energetic cost of protein turnover in healthy elderly humans. *International Journal of Obesity and Related Metabolic Disorders, 25*(5), 601-605.

Bonnefoy, Marc, Normand, Sylvie, Pachiaudi, Christiane, Lacour, Jean Ren ©, Laville, Martine, & Kostka, Tomasz. (2001). Simultaneous Validation of Ten Physical Activity Questionnaires in Older Men: A Doubly Labeled Water Study. *Journal of the American Geriatrics Society, 49*(1), 28-35.

Bonomini, V., Feletti, C., Scolari, M. P., & Stefoni, S. (1985). Benefits of early initiation of dialysis. *Kidney International. Supplement, 17*, S57-59.

Borah, Michael F., Schoenfeld, Patricia Y., Gotch, Frank A., Sargent, John A., Wolfson, Marsha, & Humphreys, Michael H. (1978). Nitrogen balance during intermittent dialysis therapy of uremia. *Kidney International, 14*(5), 491-500.

Boyce, Mary L., Robergs, Robert A., Avasthi, Pratap S., Roldan, Carlos, Foster, Angelique, Montner, Paul, . . . Nelson, Chris. (1997). Exercise training by individuals with predialysis renal failure: Cardiorespiratory endurance, hypertension, and renal function. *American Journal of Kidney Diseases, 30*(2), 180-192.

Brändström, E., Grzegorczyk, A., Jacobsson, L., Friberg, P., Lindahl, A., & Aurell, M. (1998). GFR measurement with iohexol and 51Cr-EDTA. A comparison of the two favoured GFR markers in Europe. *Nephrology Dialysis Transplantation, 13*(5), 1176-1182.

Brenner, B. M., Cooper, M. E., de Zeeuw, D., Keane, W. F., Mitch, W. E., Parving, H. H., . . . Investigators, RENAAL Study. (2001). Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *New England Journal of Medicine, 345*(12), 861-869.

Brown, D. R., Yore, M. M., Ham, S. A., & Macera, C. A. (2005). Physical activity among adults >or=50 yr with and without disabilities, BRFSS 2001. *Medicine and Science in Sports and Exercise, 37*(4), 620-629.

Brundin, T., & Wahren, J. (1994). Renal oxygen consumption, thermogenesis, and amino acid utilization during i.v. infusion of amino acids in man. *American Journal of Physiology, 267*(5 Pt 1), E648-655.

Burrowes, J. D., Larive, B., Cockram, D. B., Dwyer, J., Kusek, J. W., McLeroy, S., . . . Rocco, M. V. (2003). Effects of dietary intake, appetite, and eating habits on dialysis and non-dialysis treatment days in hemodialysis patients: cross-sectional results from the HEMO study. *Journal of Renal Nutrition, 13*(3), 191-198.

Calle, E. E., Thun, M. J., Petrelli, J. M., Rodriguez, C., & Heath, C. W. (1999). Body-mass index and mortality in a prospective cohort of U.S. adults. *New England Journal of Medicine, 341*(15), 1097-1105.

Cappy, Carol Stine, Jablonka, John, & Schroeder, Edward T. (1999). The effects of exercise during hemodialysis on physical performance and nutrition assessment. *Journal of Renal Nutrition, 9*(2), 63-70.

Carrero, J. J., Chmielewski, M., Axelsson, J., Snaedal, S., Heimbürger, O., Bárány, P., . . . Qureshi, A. R. (2008). Muscle atrophy, inflammation and clinical outcome in incident and prevalent dialysis patients. *Clinical Nutrition, 27*(4), 557-564.

Carrero, J. J., Qureshi, A. R., Axelsson, J., Avesani, C. M., Suliman, M. E., Kato, S., . . . Stenvinkel, P. (2007). Comparison of nutritional and inflammatory markers in dialysis patients with reduced appetite. *American Journal of Clinical Nutrition, 85*(3), 695-701.

Casino, F. G., & Lopez, T. (1996). The equivalent renal urea clearance: a new parameter to assess dialysis dose. *Nephrology Dialysis Transplantation, 11*(8), 1574-1581.

Castaneda, Carmen, Gordon, Patricia L., Parker, Russell C., Uhlin, Katherine Leigh, Roubenoff, Ronenn, & Levey, Andrew S. (2004). Resistance training to reduce the malnutrition-inflammation complex syndrome of chronic kidney disease. *American Journal of Kidney Diseases, 43*(4), 607-616.

Castaneda, Carmen, Gordon, Patricia L., Uhlin, Katherine Leigh, Levey, Andrew S., Kehayias, Joseph J., Dwyer, Johanna T., . . . Singh, Maria Fiatarone. (2001). Resistance Training To Counteract the Catabolism of a Low-Protein Diet in Patients with Chronic Renal Insufficiency. *Annals of Internal Medicine, 135*(11), 965-976.

Castellino, P., Solini, A., Luzi, L., Barr, J. G., Smith, D. J., Petrides, A., . . . DeFronzo, R. A. (1992). Glucose and amino acid metabolism in chronic renal failure: effect of insulin and amino acids. *American Journal of Physiology, 262*(2 Pt 2), F168-176.

Castenfors, J. (1967). Renal function during exercise. With special reference to exercise proteinuria and the release of renin. *Acta Physiologica Scandinavica. Supplementum, 293*, 1-44.

Cavanaugh, Kerri L., Merkin, Sharon Stein, Plantinga, Laura C., Fink, Nancy E., Sadler, John H., & Powe, Neil R. (2008). Accuracy of Patients' Reports of Comorbid

Disease and Their Association With Mortality in ESRD. *American Journal of Kidney Diseases, 52*(1), 118-127.

Chandna, S. M., Schulz, J., Lawrence, C., Greenwood, R. N., & Farrington, K. (1999). Is there a rationale for rationing chronic dialysis? A hospital based cohort study of factors affecting survival and morbidity. *BMJ, 318*(7178), 217-223.

Charlson, M. E., Pompei, P., Ales, K. L., & MacKenzie, C. R. (1987). A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *Journal of Chronic Diseases, 40*(5), 373-383.

Charra, B., Calemard, E., Ruffet, M., Chazot, C., Terrat, J. C., Vanel, T., & Laurent, G. (1992). Survival as an index of adequacy of dialysis. *Kidney International, 41*(5), 1286-1291.

Cheema, B. S. B., & Fiatarone Singh, M. A. (2005). Exercise Training in Patients Receiving Maintenance Hemodialysis: A Systematic Review of Clinical Trials. *American Journal of Nephrology, 25*(4), 352-364.

Cheema, Birinder Singh Bobby. (2008). Tackling the survival issue in end-stage renal disease: Time to get physical on haemodialysis. *Nephrology, 13*(7), 560-569.

Cheema, Bobby, Abas, Haifa, Smith, Benjamin, O'Sullivan, Anthony, Chan, Maria, Patwardhan, Aditi, . . . Singh, Maria Fiatarone. (2007). Progressive Exercise for Anabolism in Kidney Disease (PEAK): A Randomized, Controlled Trial of Resistance Training during Hemodialysis. *Journal of the American Society of Nephrology, 18*(5), 1594-1601.

Chertow, G. M., Owen, W. F., Lazarus, J. M., Lew, N. L., & Lowrie, E. G. (1999). Exploring the reverse J-shaped curve between urea reduction ratio and mortality. *Kidney International, 56*(5), 1872-1878.

Cheung, A. K., Levin, N. W., Greene, T., Agodoa, L., Bailey, J., Beck, G., . . . Eknoyan, G. (2003). Effects of high-flux hemodialysis on clinical outcomes: results of the HEMO study. *Journal of the American Society of Nephrology, 14*(12), 3251-3263.

Chiarelli, F., Verrotti, A., Mohn, A., & Morgese, G. (1997). The importance of microalbuminuria as an indicator of incipient diabetic nephropathy: therapeutic implications. *Annals of Medicine, 29*(5), 439-445.

Clinical Practice Guidelines for Hemodialysis Adequacy, Update 2006. (2006). *American Journal of Kidney Diseases, 48*, S2-S90.

Cockcroft, D. W., & Gault, M. H. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron, 16*(1), 31-41.

Cohen, Jacob. (1960). A Coefficient of Agreement for Nominal Scales. *Educational and Psychological Measurement, 20*(1), 37-46.

Collins, A. J., Ma, J. Z., Xia, A., & Ebben, J. (1998). Trends in anemia treatment with erythropoietin usage and patient outcomes. *American Journal of Kidney Diseases, 32*(6, Supplement 4), S133-S141.

Conway, J. M., Seale, J. L., Jacobs, D. R., Irwin, M. L., & Ainsworth, B. E. (2002). Comparison of energy expenditure estimates from doubly labeled water, a physical activity questionnaire, and physical activity records. *American Journal of Clinical Nutrition, 75*(3), 519-525.

Cooper, J. A., Watras, A. C., O'Brien, M. J., Luke, A., Dobratz, J. R., Earthman, C. P., & Schoeller, D. A. (2009). Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *Journal of the American Dietetic Association, 109*(1), 128-132.

Cunningham, J. J. (1990). Calculation of energy expenditure from indirect calorimetry: assessment of the Weir equation. *Nutrition, 6*(3), 222-223.

Cupisti, A., Capitanini, A., Betti, G., D'Alessandro, C., & Barsotti, G. (2011). Assessment of habitual physical activity and energy expenditure in dialysis patients and relationships to nutritional parameters. *Clinical Nephrology, 75*(3), 218-225.

D'Hooge, R., Van de Vijver, G., Van Bogaert, P. P., Marescau, B., Vanholder, R., & De Deyn, P. P. (2003). Involvement of voltage- and ligand-gated Ca2+ channels in the neuroexcitatory and synergistic effects of putative uremic neurotoxins. *Kidney International, 63*(5), 1764-1775.

Daugirdas, J. T. (1993). Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. *Journal of the American Society of Nephrology, 4*(5), 1205-1213.

Daugirdas, J. T., Greene, T., Chertow, G. M., & Depner, T. A. (2010). Can rescaling dose of dialysis to body surface area in the HEMO study explain the different responses to dose in women versus men? *Clinical Journal of the American Society of Nephrology, 5*(9), 1628-1636.

Daugirdas, J. T., Levin, N. W., Kotanko, P., Depner, T. A., Kuhlmann, M. K., Chertow, G. M., & Rocco, M. V. (2008). Comparison of proposed alternative methods for rescaling dialysis dose: resting energy expenditure, high metabolic rate organ mass, liver size, and body surface area. *Seminars in Dialysis, 21*(5), 377-384.

Daugirdas, J. T., Meyer, K., Greene, T., Butler, R. S., & Poggio, E. D. (2009). Scaling of measured glomerular filtration rate in kidney donor candidates by anthropometric estimates of body surface area, body water, metabolic rate, or liver size. *Clinical Journal of the American Society of Nephrology, 4*(10), 1575-1583.

Daugirdas, John T., Depner, Thomas A., Greene, Tom, Kuhlmann, Martin K., Levin, Nathan W., Chertow, Glenn M., & Rocco, Michael V. (2008). Surface-Area-Normalized Kt/V: A Method of Rescaling Dialysis Dose to Body Surface Area - Implications for Different-Size Patients by Gender. *Seminars in Dialysis, 21*(5), 415-421.

Daugirdas, John T., Depner, Thomas A., Greene, Tom, & Silisteanu, Paul. (2009). Solute-Solver: A Web-Based Tool for Modeling Urea Kinetics for a Broad Range of Hemodialysis Schedules in Multiple Patients. *American Journal of Kidney Diseases, 54*(5), 798-809.

Davies, C. E., Mackinnon, J., & Platts, M. M. (1952). Renal circulation and cardiac output in low-output heart failure and in myxoedema. *British Medical Journal, 2*(4784), 595-597.

de Boer, J. O., van Es, A. J., Voorrips, L. E., Blokstra, F., & Vogt, J. E. (1988). Energy metabolism and requirements in different ethnic groups. *European Journal of Clinical Nutrition, 42*(12), 983-997.

Depner, Thomas A. (2001). Uremic toxicity: urea and beyond. *Seminars in Dialysis, 14*(4), 246-251.

Depner, Thomas A., Daugirdas, J., Greene, T., Allon, M., Beck, G., Chumlea, C., . . . Eknoyan, G. (2004). Dialysis dose and the effect of gender and body size on outcome in the HEMO Study. *Kidney International, 65*(4), 1386-1394.

Deurenberg, P., Deurenberg-Yap, M., & Guricci, S. (2002). Asians are different from Caucasians and from each other in their body mass index/body fat per cent relationship. *Obes Rev, 3*(3), 141-146.

Dou, L., Bertrand, E., Cerini, C., Faure, V., Sampol, J., Vanholder, R., . . . Brunet, P. (2004). The uremic solutes p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. *Kidney International, 65*(2), 442-451.

Dou, L., Jourde-Chiche, N., Faure, V., Cerini, C., Berland, Y., Dignat-George, F., & Brunet, P. (2007). The uremic solute indoxyl sulfate induces oxidative stress in endothelial cells. *J Thromb Haemost, 5*(6), 1302-1308.

Duranton, Flore, Cohen, Gerald, De Smet, Rita, Rodriguez, Mariano, Jankowski, Joachim, Vanholder, Raymond, . . . Group, on behalf of the European Uremic Toxin Work. (2012). Normal and Pathologic Concentrations of Uremic Toxins. *Journal of the American Society of Nephrology, 23*(7), 1258-1270.

The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. (1993). *New England Journal of Medicine, 329*(14), 977-986.

Eknoyan, Garabed, Beck, Gerald J., Cheung, Alfred K., Daugirdas, John T., Greene, Tom, Kusek, John W., . . . Toto, Robert. (2002). Effect of Dialysis Dose and Membrane Flux in Maintenance Hemodialysis. *New England Journal of Medicine, 347*(25), 2010-2019.

Eloot, Sunny, Torremans, An, De Smet, Rita, Marescau, Bart, De Deyn, Peter Paul, Verdonck, Pascal, & Vanholder, Raymond. (2007). Complex Compartmental Behavior of Small Water-Soluble Uremic Retention Solutes: Evaluation by Direct Measurements in Plasma and Erythrocytes. *American Journal of Kidney Diseases, 50*(2), 279-288.

Eloot, Sunny, Torremans, An, De Smet, Rita, Marescau, Bart, De Wachter, Dirk, De Deyn, Peter Paul, . . . Vanholder, Raymond. (2005). Kinetic behavior of urea is different from that of other water-soluble compounds: The case of the guanidino compounds. *Kidney International, 67*(4), 1566-1575.

Esliger, Dale W., Rowlands, Ann V., Hurst, Tina L., Catt, Michael, Murray, Peter, & Eston, Roger G. (2011). Validation of the GENEA Accelerometer. *Medicine and Science in Sports and Exercise, 43*(6), 1085-1093.

Finkelstein, J., Joshi, A., & Hise, M. K. (2006). Association of physical activity and renal function in subjects with and without metabolic syndrome: a review of the Third National Health and Nutrition Examination Survey (NHANES III). *American journal of kidney diseases : the official journal of the National Kidney Foundation, 48*(3), 372-382.

Fleischmann, E., Teal, N., Dudley, J., May, W., Bower, J. D., & Salahudeen, A. K. (1999). Influence of excess weight on mortality and hospital stay in 1346 hemodialysis patients. *Kidney International, 55*(4), 1560-1567.

Foley, Robert N., Parfrey, Patrick S., Morgan, Janet, Barré, Paul E., Campbell, Patricia, Cartier, Pierre, . . . Wells, George A. (2000). Effect of hemoglobin levels in hemodialysis patients with asymptomatic cardiomyopathy. *Kidney International, 58*(3), 1325-1335.

Ford, R. V., Owens, J. C., Curd, G. W., Moyer, J. H., & Spurr, C. L. (1961). Kidney function in various thyroid states. *Journal of Clinical Endocrinology and Metabolism, 21*, 548-553.

Foster, G. D., Knox, L. S., Dempsey, D. T., & Mullen, J. L. (1987). Caloric requirements in total parenteral nutrition. *Journal of the American College of Nutrition, 6*(3), 231-253.

Foster, G. D., Wadden, T. A., & Vogt, R. A. (1997). Resting energy expenditure in obese African American and Caucasian women. *Obesity Research, 5*(1), 1-8.

Fürstenberg, A., & Davenport, A. (2011a). Assessment of Body Composition in Peritoneal Dialysis Patients Using Bioelectrical Impedance and Dual-Energy X-Ray Absorptiometry. *American Journal of Nephrology, 33*(2), 150-156.

Fürstenberg, Antje, & Davenport, Andrew. (2011b). Comparison of Multifrequency Bioelectrical Impedance Analysis and Dual-Energy X-ray Absorptiometry Assessments in Outpatient Hemodialysis Patients. *American Journal of Kidney Diseases, 57*(1), 123-129.

Gallagher, Dympna, Albu, Jeanine, He, Qing, Heshka, Stanley, Boxt, Lawrence, Krasnow, Norman, & Elia, Marinos. (2006). Small organs with a high metabolic rate explain lower resting energy expenditure in African American than in white adults. *American Journal of Clinical Nutrition, 83*(5), 1062-1067.

Gallagher, Dympna, Belmonte, Daniel, Deurenberg, Paul, Wang, Zimian, Krasnow, Norman, Pi-Sunyer, F. Xavier, & Heymsfield, Steven B. (1998). Organ-tissue mass measurement allows modeling of REE and metabolically active tissue mass. *American Journal of Physiology Endocrinology and Metabolism, 275*(2), E249-E258.

Garibotto, G., Pastorino, N., & Dertenois, L. (2003). Protein and Amino Acid Metabolism in Renal Disease and in Renal Failure. In J. D. Kopple & S. G. Massry (Eds.), *Nutritional Management of Renal Disease* (pp. 1-23). USA: William and Wilkins.

Glorieux, G., Helling, R., Henle, T., Brunet, P., Deppisch, R., Lameire, N., & Vanholder, R. (2004). In vitro evidence for immune activating effect of specific AGE structures retained in uremia. *Kidney International, 66*(5), 1873-1880.

Glorieux, G. L., Dhondt, A. W., Jacobs, P., Van Langeraert, J., Lameire, N. H., De Deyn, P. P., & Vanholder, R. C. (2004). In vitro study of the potential role of guanidines in leukocyte functions related to atherogenesis and infection. *Kidney International, 65*(6), 2184-2192.

Goldwasser, P., Mittman, N., Antignani, A., Burrell, D., Michel, M. A., Collier, J., & Avram, M. M. (1993). Predictors of mortality in hemodialysis patients. *Journal of the American Society of Nephrology, 3*(9), 1613-1622.

Goodkin, D. A., Bragg-Gresham, J. L., Koenig, K. G., Wolfe, R. A., Akiba, T., Andreucci, V. E., . . . Young, E. W. (2003). Association of comorbid conditions and mortality in hemodialysis patients in Europe, Japan, and the United States: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Journal of the American Society of Nephrology, 14*(12), 3270-3277.

Goodship, T. H., Mitch, W. E., Hoerr, R. A., Wagner, D. A., Steinman, T. I., & Young, V. R. (1990). Adaptation to low-protein diets in renal failure: leucine turnover and nitrogen balance. *Journal of the American Society of Nephrology, 1*(1), 66-75.

Gotch, F. A. (1998). The current place of urea kinetic modelling with respect to different dialysis modalities. *Nephrology Dialysis Transplantation, 13 Suppl 6*, 10-14.

Gotch, F. A. (2000). Kt/V is the best dialysis dose parameter. *Blood Purification, 18*(4), 276-285.

Gotch, F. A., & Sargent, J. A. (1985). A mechanistic analysis of the National Cooperative Dialysis Study (NCDS). *Kidney International, 28*(3), 526-534.

Grassmann, A., Gioberge, S., Moeller, S., & Brown, G. (2005). ESRD patients in 2004: global overview of patient numbers, treatment modalities and associated trends. *Nephrology Dialysis Transplantation, 20*(12), 2587-2593.

Grimby, Gunnar. (1965). Renal clearances during prolonged supine exercise at different loads. *Journal of Applied Physiology, 20*(6), 1294-1298.

Gulyassy, P. F., Aviram, A., & Peters, J. H. (1970). Evaluation of amino acid and protein requirements in chronic uremia. *Archives of Internal Medicine, 126*(5), 855-859.

Hakim, R. M. (1990). Assessing the adequacy of dialysis. *Kidney International, 37*(2), 822-832.

Hakim, R. M., & Levin, N. (1993). Malnutrition in hemodialysis patients. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 21*(2), 125-137.

Harris, J. A., & Benedict, F. G. (1918). A Biometric Study of Human Basal Metabolism. *Proceedings of the National Academy of Sciences of the United States of America, 4*(12), 370-373.

Harter, H. R., Karl, I. E., Klahr, S., & Kipnis, D. M. (1979). Effects of reduced renal mass and dietary protein intake on amino acid release and glucose uptake by rat muscle in vitro. *Journal of Clinical Investigation, 64*(2), 513-523.

Hawkins, Marquis S., Sevick, Mary Ann, Richardson, Caroline R., Fried, Linda F., Arena, Vincent C., & Kriska, Andrea M. (2011). Association between Physical Activity and Kidney Function: National Health and Nutrition Examination Survey. *Medicine and Science in Sports and Exercise, 43*(8), 1457-1464.

Haycock, George B., Schwartz, George J., & Wisotsky, David H. (1978). Geometric method for measuring body surface area: A height-weight formula validated in infants, children, and adults. *Journal of Pediatrics, 93*(1), 62-66.

Heinemann, A., Wischhusen, F., Püschel, K., & Rogiers, X. (1999). Standard liver volume in the Caucasian population. *Liver Transplantation and Surgery, 5*(5), 366-368.

Heymsfield, Steven B., Gallagher, Dympna, Kotler, Donald P., Wang, ZiMian, Allison, David B., & Heshka, Stanley. (2002). Body-size dependence of resting energy expenditure can be attributed to nonenergetic homogeneity of fat-free mass. *American Journal of Physiology Endocrinology and Metabolism, 282*(1), E132-E138.

Hoek, F. J., Korevaar, J. C., Dekker, F. W., Boeschoten, E. W., & Krediet, R. T. (2007). Estimation of residual glomerular filtration rate in dialysis patients from the plasma cystatin C level. *Nephrology Dialysis Transplantation, 22*(6), 1633-1638.

Holliday, M. A., Chantler, C., MacDonnell, R., & Keitges, J. (1977). Effect of uremia on nutritionally-induced variations in protein metabolism. *Kidney International, 11*(4), 236-245.

Huang, Cindy X., Tighiouart, Hocine, Beddhu, Srinivasan, Cheung, Alfred K., Dwyer, Johanna T., Eknoyan, Garabed, . . . Sarnak, Mark J. (2010). Both low muscle mass and low fat are associated with higher all-cause mortality in hemodialysis patients. *Kidney International, 77*(7), 624-629.

Hunsicker, L. G., Adler, S., Caggiula, A., England, B. K., Greene, T., Kusek, J. W., . . . Teschan, P. E. (1997). Predictors of the progression of renal disease in the Modification of Diet in Renal Disease Study. *Kidney International, 51*(6), 1908-1919.

Ikizler, T. A. (2007). Protein and Energy Intake in Advanced Chronic Kidney Disease: How Much is Too Much? *Seminars in Dialysis, 20*(1), 5-11.

Ikizler, T. A., & Hakim, R. M. (1996). Nutrition in end-stage renal disease. *Kidney International, 50*(2), 343-357.

Ikizler, T. A., Wingard, R. L., Harvell, J., Shyr, Y., & Hakim, R. M. (1999). Association of morbidity with markers of nutrition and inflammation in chronic hemodialysis patients: a prospective study. *Kidney International, 55*(5), 1945-1951.

Ikizler, T. A., Wingard, R. L., Sun, M., Harvell, J., Parker, R. A., & Hakim, R. M. (1996). Increased energy expenditure in hemodialysis patients. *Journal of the American Society of Nephrology, 7*(12), 2646-2653.

Ikizler, T. Alp, Pupim, Lara B., Brouillette, John R., Levenhagen, Deanna K., Farmer, Kali, Hakim, Raymond M., & Flakoll, Paul J. (2002). Hemodialysis stimulates muscle and whole body protein loss and alters substrate oxidation. *American Journal of Physiology Endocrinology and Metabolism, 282*(1), E107-E116.

Inker, Lesley A., Coresh, Josef, Levey, Andrew S., Tonelli, Marcello, & Muntner, Paul. (2011). Estimated GFR, Albuminuria, and Complications of Chronic Kidney Disease. *Journal of the American Society of Nephrology, 22*(12), 2322-2331.

Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. (1998). *Lancet, 352*(9131), 837-853.

Iseki, K., Kawazoe, N., & Fukiyama, K. (1993). Serum albumin is a strong predictor of death in chronic dialysis patients. *Kidney International, 44*(1), 115-119.

Izuhara, Y., Nangaku, M., Inagi, R., Tominaga, N., Aizawa, T., Kurokawa, K., . . . Miyata, T. (2005). Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. *Journal of the American Society of Nephrology, 16*(12), 3631-3641.

Jafar, T. H., Stark, P. C., Schmid, C. H., Landa, M., Maschio, G., de Jong, P. E., . . . Group, AIPRD Study. (2003). Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: a patient-level meta-analysis. *Annals of Internal Medicine, 139*(4), 244-252.

Jafar, T. H., Stark, P. C., Schmid, C. H., Landa, M., Maschio, G., Marcantoni, C., . . . Disease, AIPRD Study Group. Angiotensin-Converting Enzymne Inhibition and Progression of Renal. (2001). Proteinuria as a modifiable risk factor for the progression of non-diabetic renal disease. *Kidney International, 60*(3), 1131-1140.

Jain, Poorva, Cockwell, Paul, Little, Jane, Ferring, Martin, Nicholas, Johann, Richards, Nick, . . . Smith, Steve. (2009). Survival and transplantation in end-stage renal disease: a prospective study of a multiethnic population. *Nephrology Dialysis Transplantation, 24*(12), 3840-3846.

Johansen, K. L. (2005). Exercise and chronic kidney disease: current recommendations. *Sports Medicine, 35*(6), 485-499.

Johansen, Kirsten L., Chertow, Glenn M., Kutner, Nancy G., Dalrymple, Lorien S., Grimes, Barbara A., & Kaysen, George A. (2010). Low level of self-reported physical activity in ambulatory patients new to dialysis. *Kidney International, 78*(11), 1164-1170.

Johansen, Kirsten L., Chertow, Glenn M., Ng, Alexander V., Mulligan, Kathleen, Carey, Susan, Schoenfeld, Patricia Y., & Kent-Braun, Jane A. (2000). Physical activity levels in patients on hemodialysis and healthy sedentary controls. *Kidney International, 57*(6), 2564-2570.

Johansen, Kirsten L., Painter, Patricia, Kent-Braun, Jane A., Ng, Alexander V., Carey, Susan, Da Silva, Makani, & Chertow, Glenn M. (2001). Validation of

questionnaires to estimate physical activity and functioning in end-stage renal disease. *Kidney International, 59*(3), 1121-1127.

Johansen, Kirsten L., Young, Belinda, Kaysen, George A., & Chertow, Glenn M. (2004). Association of body size with outcomes among patients beginning dialysis. *American Journal of Clinical Nutrition, 80*(2), 324-332.

Johnson, W. J., Hagge, W. W., Wagoner, R. D., Dinapoli, R. P., & Rosevear, J. W. (1972). Effects of urea loading in patients with far-advanced renal failure. *Mayo Clinic Proceedings, 47*(1), 21-29.

Jones, C. A., McQuillan, G. M., Kusek, J. W., Eberhardt, M. S., Herman, W. H., Coresh, J., . . . Agodoa, L. Y. (1998). Serum creatinine levels in the US population: third National Health and Nutrition Examination Survey. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 32*(6), 992-999.

Jones, Michael R., Kopple, Joel D., & Swendseid, Marian E. (1978). Phenylalanine metabolism in uremic and normal man. *Kidney International, 14*(2), 169-179.

Jonker, J. T., de Mol, P., de Vries, S. T., Widya, R. L., Hammer, S., van Schinkel, L. D., . . . Lamb, H. J. (2013). Exercise and type 2 diabetes mellitus: changes in tissue-specific fat distribution and cardiac function. *Radiology, 269*(2), 434-442.

K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. (2002). *American journal of kidney diseases : the official journal of the National Kidney Foundation, 39*(2 Suppl 1), S1-266.

Kachadorian, W. A., & Johnson, R. E. (1970). Renal responses to various rates of exercise. *Journal of Applied Physiology, 28*(6), 748-752.

Kalantar-Zadeh, K., Block, G., McAllister, C. J., Humphreys, M. H., & Kopple, J. D. (2004). Appetite and inflammation, nutrition, anemia, and clinical outcome in hemodialysis patients. *American Journal of Clinical Nutrition, 80*(2), 299-307.

Kalantar-Zadeh, K., Kuwae, N., Wu, D. Y., Shantouf, R. S., Fouque, D., Anker, S. D., . . . Kopple, J. D. (2006). Associations of body fat and its changes over time with quality of life and prospective mortality in hemodialysis patients. *American Journal of Clinical Nutrition, 83*(2), 202-210.

Kambham, N., Markowitz, G. S., Valeri, A. M., Lin, J., & D'Agati, V. D. (2001). Obesity-related glomerulopathy: an emerging epidemic. *Kidney International, 59*(4), 1498-1509.

Kanda, E., Ai, M., Yoshida, M., Kuriyama, R., & Shiigai, T. (2013). High serum bicarbonate level within the normal range prevents the progression of chronic kidney disease in elderly chronic kidney disease patients. *BMC Nephrol, 14*, 4.

Katz, Jeffrey N., Chang, Lily C., Sangha, Oliver, Fossel, Anne H., & Bates, David W. (1996). Can Comorbidity Be Measured by Questionnaire Rather than Medical Record Review? *Medical Care, 34*(1), 73-84.

KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. (2013). *Kidney International, 3 (Supplement)*(1), 1-150.

Keane, W. F., & Eknoyan, G. (1999). Proteinuria, albuminuria, risk, assessment, detection, elimination (PARADE): a position paper of the National Kidney Foundation. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 33*(5), 1004-1010.

Khan, I. H. (1998). Comorbidity: the major challenge for survival and quality of life in end-stage renal disease. *Nephrology Dialysis Transplantation, 13 Suppl 1*, 76-79.

Khan, I. H., Catto, G. R., Edward, N., Fleming, L. W., Henderson, I. S., & MacLeod, A. M. (1993). Influence of coexisting disease on survival on renal-replacement therapy. *Lancet, 341*(8842), 415-418.

Klahr, S., Levey, A. S., Beck, G. J., Caggiula, A. W., Hunsicker, L., Kusek, J. W., & Striker, G. (1994). The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. *New England Journal of Medicine, 330*(13), 877-884.

Koivula, R. W., Tornberg, A. B., & Franks, P. W. (2013). Exercise and diabetes-related cardiovascular disease: systematic review of published evidence from observational studies and clinical trials. *Curr Diab Rep, 13*(3), 372-380.

Kong, Chiew H., Tattersall, James E., Greenwood, Roger N., & Farrington, Ken. (1999). The effect of exercise during haemodialysis on solute removal. *Nephrology Dialysis Transplantation, 14*(12), 2927-2931.

Kopple, J. D. (1994). Effect of nutrition on morbidity and mortality in maintenance dialysis patients. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 24*(6), 1002-1009.

Kopple, Joel D. (1978). Abnormal amino acid and protein metabolism in uremia. *Kidney International, 14*(4), 340-348.

Kopple, Joel D. (2007). Phenylalanine and Tyrosine Metabolism in Chronic Kidney Failure. *Journal of Nutrition, 137*(6), 1586S-1590S.

Kotanko, P., & Levin, N. W. (2007). The impact of visceral mass on survival in chronic hemodialysis patients. *International Journal of Artificial Organs, 30*(11), 993-999.

Kotanko, P., Thijssen, S., Kitzler, T., Wystrychowski, G., Sarkar, S. R., Zhu, F., . . . Levin, N. W. (2007). Size matters: body composition and outcomes in maintenance hemodialysis patients. *Blood Purification, 25*(1), 27-30.

Kouidi, E., Grekas, D., Deligiannis, A., & Tourkantonis, A. (2004). Outcomes of long-term exercise training in dialysis patients: comparison of two training programs. *Clinical Nephrology, 61 Suppl 1*, S31-38.

KREBS, H. A. (1950). Body size and tissue respiration. *Biochimica et Biophysica Acta, 4*(1-3), 249-269.

Kronborg, J., Solbu, M., Njølstad, I., Toft, I., Eriksen, B. O., & Jenssen, T. (2008). Predictors of change in estimated GFR: a population-based 7-year follow-up from the Tromso study. *Nephrology Dialysis Transplantation, 23*(9), 2818-2826.

Küchle, C., Fricke, H., Held, E., & Schiffl, H. (1996). High-flux hemodialysis postpones clinical manifestation of dialysis-related amyloidosis. *American Journal of Nephrology, 16*(6), 484-488.

Kuhlmann, U., Schwickardi, M., Trebst, R., & Lange, H. (2001). Resting metabolic rate in chronic renal failure. *Journal of Renal Nutrition, 11*(4), 202-206.

Kushner, R. F., Racette, S. B., Neil, K., & Schoeller, D. A. (1995). Measurement of physical activity among black and white obese women. *Obesity Research, 3 Suppl 2*, 261s-265s.

Laffel, L. M., McGill, J. B., & Gans, D. J. (1995). The beneficial effect of angiotensin-converting enzyme inhibition with captopril on diabetic nephropathy in normotensive IDDM patients with microalbuminuria. North American Microalbuminuria Study Group. *American Journal of Medicine, 99*(5), 497-504.

Lawrence, M., Thongprasert, K., & Durnin, J. V. (1988). Between-group differences in basal metabolic rates: an analysis of data collected in Scotland, the Gambia and Thailand. *European Journal of Clinical Nutrition, 42*(10), 877-891.

Leavey, Sean F., McCullough, Keith, Hecking, Erwin, Goodkin, David, Port, Friedrich K., & Young, Eric W. (2001). Body mass index and mortality in 'healthier' as compared with 'sicker' haemodialysis patients: results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Nephrology Dialysis Transplantation, 16*(12), 2386-2394.

Leavey, Sean F., Strawderman, R. L., Jones, C. A., Port, F. K., & Held, P. J. (1998). Simple nutritional indicators as independent predictors of mortality in hemodialysis patients. *American Journal of Kidney Diseases, 31*(6), 997-1006.

Lecker, S. H., & Mitch, W. E. (2011). Proteolysis by the ubiquitin-proteasome system and kidney disease. *Journal of the American Society of Nephrology, 22*(5), 821-824.

Leikis, Murray J., McKenna, Michael J., Petersen, Aaron C., Kent, Annette B., Murphy, Kate T., Leppik, James A., . . . McMahon, Lawrence P. (2006). Exercise Performance Falls over Time in Patients with Chronic Kidney Disease Despite Maintenance of Hemoglobin Concentration. *Clinical Journal of the American Society of Nephrology, 1*(3), 488-495.

Lemon, P. W., Dolny, D. G., & Yarasheski, K. E. (1997). Moderate physical activity can increase dietary protein needs. *Canadian Journal of Applied Physiology, 22*(5), 494-503.

Levey, A. S. (1990). Measurement of renal function in chronic renal disease. *Kidney International, 38*(1), 167-184.

Levey, A. S., Bosch, J. P., Lewis, J. B., Greene, T., Rogers, N., & Roth, D. (1999). A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Annals of Internal Medicine, 130*(6), 461-470.

Levin, A., Bakris, G. L., Molitch, M., Smulders, M., Tian, J., Williams, L. A., & Andress, D. L. (2006). Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: Results of the study to evaluate early kidney disease. *Kidney International, 71*(1), 31-38.

Lewis, E. J., Hunsicker, L. G., Bain, R. P., & Rohde, R. D. (1993). The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *New England Journal of Medicine, 329*(20), 1456-1462.

Lewis, E. J., Hunsicker, L. G., Clarke, W. R., Berl, T., Pohl, M. A., Lewis, J. B., . . . Group, Collaborative Study. (2001). Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *New England Journal of Medicine, 345*(12), 851-860.

Lewis, E. J., & Lewis, J. B. (2003). Treatment of diabetic nephropathy with angiotensin II receptor antagonist. *Clin Exp Nephrol, 7*(1), 1-8.

Liabeuf, S., Barreto, D. V., Barreto, F. C., Meert, N., Glorieux, G., Schepers, E., . . . (EUTox), European Uraemic Toxin Work Group. (2010). Free p-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease. *Nephrology Dialysis Transplantation, 25*(4), 1183-1191.

Lim, V. S., Bier, D. M., Flanigan, M. J., & Sum-Ping, S. T. (1993). The effect of hemodialysis on protein metabolism. A leucine kinetic study. *Journal of Clinical Investigation, 91*(6), 2429-2436.

Lim, V. S., Yarasheski, K. E., & Flanigan, M. J. (1998). The effect of uraemia, acidosis, and dialysis treatment on protein metabolism: a longitudinal leucine kinetic study. *Nephrology Dialysis Transplantation, 13*(7), 1723-1730.

Lim, Victoria S., & Kopple, Joel D. (2000). Protein metabolism in patients with chronic renal failure: Role of uremia and dialysis. *Kidney International, 58*(1), 1-10.

Lin, J., Knight, E. L., Hogan, M. L., & Singh, A. K. (2003). A comparison of prediction equations for estimating glomerular filtration rate in adults without kidney disease. *Journal of the American Society of Nephrology, 14*(10), 2573-2580.

Lindsay, R. M., Huang, S. H., & Filler, G. (2010). Cystatin C measurements in the assessment of residual renal function, dialysis adequacy, and beyond. *Peritoneal Dialysis International, 30*(4), 437-439.

Linet, Martha S., Harlow, Sioƀán D., McLaughlin, Joseph K., & McCaffrey, Lee D. (1989). A comparison of interview data and medical records for previous medical conditions and surgery. *Journal of Clinical Epidemiology, 42*(12), 1207-1213.

Lippi, G., Banfi, G., Luca Salvagno, G., Montagnana, M., Franchini, M., & Cesare Guidi, G. (2008). Comparison of creatinine-based estimations of glomerular filtration rate in endurance athletes at rest. *Clinical Chemistry and Laboratory Medicine, 46*(2), 235-239.

Lippi, G., Banfi, G., Salvagno, G. L., Franchini, M., & Guidi, G. C. (2008). Glomerular filtration rate in endurance athletes. *Clinical Journal of Sport Medicine, 18*(3), 286-288.

Locatelli, F., Hannedouche, T., Jacobson, S., La Greca, G., Loureiro, A., Martin-Malo, A., . . . Vanholder, R. (1999). The effect of membrane permeability on ESRD: design of a prospective randomised multicentre trial. *J Nephrol, 12*(2), 85-88.

Locatelli, F., Martin-Malo, A., Hannedouche, T., Loureiro, A., Papadimitriou, M., Wizemann, V., . . . Group, Membrane Permeability Outcome (MPO) Study. (2009). Effect of membrane permeability on survival of hemodialysis patients. *Journal of the American Society of Nephrology, 20*(3), 645-654.

Lowrie, E. G. (2000). The normalized treatment ratio (Kt/V) is not the best dialysis dose parameter. *Blood Purification, 18*(4), 286-294.

Lowrie, E. G., Chertow, G. M., Lew, N. L., Lazarus, J. M., & Owen, W. F. (1999). The urea [clearance x dialysis time] product (Kt) as an outcome-based measure of hemodialysis dose. *Kidney International, 56*(2), 729-737.

Lowrie, E. G., Laird, N. M., Parker, T. F., & Sargent, J. A. (1981). Effect of the hemodialysis prescription of patient morbidity: report from the National Cooperative Dialysis Study. *New England Journal of Medicine, 305*(20), 1176-1181.

Lowrie, E. G., Li, Z., Ofsthun, N., & Lazarus, J. M. (2004). Measurement of dialyzer clearance, dialysis time, and body size: death risk relationships among patients. *Kidney International, 66*(5), 2077-2084.

Lowrie, Edmund G., Li, Zhensheng, Ofsthun, Norma, & Lazarus, J. Michael. (2005). The online measurement of hemodialysis dose (Kt): Clinical outcome as a function of body surface area. *Kidney International, 68*(3), 1344-1354.

Luck, J., Peabody, J. W., Dresselhaus, T. R., Lee, M., & Glassman, P. (2000). How well does chart abstraction measure quality? A prospective comparison of standardized patients with the medical record. *American Journal of Medicine, 108*(8), 642-649.

Mackay, Eaton M., & Sherrill, James W. (1943). Influence of Thyroid Activity upon Renal Function. *Journal of Clinical Endocrinology, 3*(8), 462-465.

Maduell, F., Vera, M., Arias, M., Serra, N., Blasco, M., Bergadá, E., . . . Campistol, J. M. (2008). Influence of the ionic dialysance monitor on Kt measurement in hemodialysis. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 52*(1), 85-92.

Majchrzak, K. M., Pupim, L. B., Sundell, M., & Ikizler, T. A. (2007). Body composition and physical activity in end-stage renal disease. *Journal of Renal Nutrition, 17*(3), 196-204.

Majchrzak, Karen M., Pupim, Lara B., Chen, Kong, Martin, Cathi J., Gaffney, Sheila, Greene, Jane H., & Ikizler, T. Alp. (2005). Physical activity patterns in chronic hemodialysis patients: Comparison of dialysis and nondialysis days. *Journal of Renal Nutrition, 15*(2), 217-224.

Marieb, Elaine N., & Hoehn, Katja N. (2014). The Urinary System *Human Anatomy & Physiology* (Ninth ed., pp. 1023 - 1060). Harlow, UK: Pearson Education Limited.

Marshall, M. R., Byrne, B. G., Kerr, P. G., & McDonald, S. P. (2006). Associations of hemodialysis dose and session length with mortality risk in Australian and New Zealand patients. *Kidney International, 69*(7), 1229-1236.

Matsuzawa, R., Matsunaga, A., Wang, G., Kutsuna, T., Ishii, A., Abe, Y., . . . Takahira, N. (2012). Habitual physical activity measured by accelerometer and survival in maintenance hemodialysis patients. *Clinical Journal of the American Society of Nephrology, 7*(12), 2010-2016.

Matthews, D. E., Schwarz, H. P., Yang, R. D., Motil, K. J., Young, V. R., & Bier, D. M. (1982). Relationship of plasma leucine and alpha-ketoisocaproate during a L-[1-13C]leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment. *Metabolism: Clinical and Experimental, 31*(11), 1105-1112.

McClellan, W. M., Soucie, J. M., & Flanders, W. D. (1998). Mortality in end-stage renal disease is associated with facility-to-facility differences in adequacy of hemodialysis. *Journal of the American Society of Nephrology, 9*(10), 1940-1947.

Measurement of renal function. European Best Practice Guidelines for Haemodialysis. (2002). *Nephrology Dialysis Transplantation, 17*(suppl 7), 7-15.

Merkus, M. P., Jager, K. J., Dekker, F. W., de Haan, R. J., Boeschoten, E. W., & Krediet, R. T. (2000). Predictors of poor outcome in chronic dialysis patients: The Netherlands Cooperative Study on the Adequacy of Dialysis. The NECOSAD Study Group. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 35*(1), 69-79.

Mifflin, M. D., St Jeor, S. T., Hill, L. A., Scott, B. J., Daugherty, S. A., & Koh, Y. O. (1990). A new predictive equation for resting energy expenditure in healthy individuals. *American Journal of Clinical Nutrition, 51*(2), 241-247.

Mogensen, C. E. (1987). Microalbuminuria as a predictor of clinical diabetic nephropathy. *Kidney International, 31*(2), 673-689.

Monteon, Francisco J., Laidlaw, Stewart A., Shaib, John K., & Kopple, Joel D. (1986). Energy expenditure in patients with chronic renal failure. *Kidney International, 30*(5), 741-747.

Montoye, H J. (1997). *Measuring physical activity and energy expenditure. Human Kinetics, Champaign, IL, 1996.* (Vol. 41): WILEY-VCH Verlag GmbH.

Morton, A. R., & Singer, M. A. (2007). The problem with Kt/V: dialysis dose should be normalized to metabolic rate not volume. *Seminars in Dialysis, 20*(1), 12-15.

Müller, M. J., Bosy-Westphal, A., Kutzner, D., & Heller, M. (2002). Metabolically active components of fat-free mass and resting energy expenditure in humans: recent lessons from imaging technologies. *Obesity Reviews, 3*(2), 113-122.

Mustata, S., Groeneveld, S., Davidson, W., Ford, G., Kiland, K., & Manns, B. (2011). Effects of exercise training on physical impairment, arterial stiffness and health-related quality of life in patients with chronic kidney disease: a pilot study. *International Urology and Nephrology, 43*(4), 1133-1141.

Myers, Jonathan. (2003). Exercise and Cardiovascular Health. *Circulation, 107*(1), e2-e5.

Nair, K. S., & Halliday, D. (1985). Energy and Protein Metabolism in Diabetes and Obesity. In J. S. Garrow & D. Halliday (Eds.), *Substrate and Energy Metabolism in Man* (pp. 195-202). London: John Libbey & Co Ltd.

Naylor, H. L., Jackson, H., Walker, G. H., Macafee, S., Magee, K., Hooper, L., . . . Association, British Dietetic. (2013). British Dietetic Association evidence-based guidelines for the protein requirements of adults undergoing maintenance haemodialysis or peritoneal dialysis. *J Hum Nutr Diet, 26*(4), 315-328.

Neilson, Heather K., Robson, Paula J., Friedenreich, Christine M., & Csizmadi, Ilona. (2008). Estimating activity energy expenditure: how valid are physical activity questionnaires? *American Journal of Clinical Nutrition, 87*(2), 279-291.

Newman, D. J., Mattock, M. B., Dawnay, A. B., Kerry, S., McGuire, A., Yaqoob, M., . . . Hawke, C. (2005). Systematic review on urine albumin testing for early detection of diabetic complications. *Health Technology Assessment, 9*(30), iii-vi, xiii-163.

Neyra, R., Chen, K. Y., Sun, M., Shyr, Y., Hakim, R. M., & Ikizler, T. A. (2003). Increased resting energy expenditure in patients with end-stage renal disease. *J Parenter Enteral Nutr, 27*(1), 36-42.

O'Hare, A. M., Tawney, K., Bacchetti, P., & Johansen, K. L. (2003). Decreased survival among sedentary patients undergoing dialysis: results from the dialysis morbidity and mortality study wave 2. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 41*(2), 447-454.

Olsson, B., Aulie, A., Sveen, K., & Andrew, E. (1983). Human pharmacokinetics of iohexol. A new nonionic contrast medium. *Investigative Radiology, 18*(2), 177-182.

Owen, W. F., Lew, N. L., Liu, Y., Lowrie, E. G., & Lazarus, J. M. (1993). The urea reduction ratio and serum albumin concentration as predictors of mortality in patients undergoing hemodialysis. *New England Journal of Medicine, 329*(14), 1001-1006.

Painter, Patricia. (2005). Physical functioning in end-stage renal disease patients: Update 2005. *Hemodialysis International, 9*(3), 218-235.

Painter, Patricia, Carlson, Laurie, Carey, Susan, Paul, Steven M., & Myll, Jeffrey. (2000). Physical functioning and health-related quality-of-life changes with exercise training in hemodialysis patients. *American Journal of Kidney Diseases, 35*(3), 482-492.

Panesar, A., & Agarwal, R. (2003). Resting energy expenditure in chronic kidney disease: relationship with glomerular filtration rate. *Clinical Nephrology, 59*(5), 360-366.

Park, J., Ishikawa-Takata, K., Tanaka, S., Hikihara, Y., Ohkawara, K., Watanabe, S., . . . Tabata, I. (2011). Relation of body composition to daily physical activity in free-living Japanese adult women. *British Journal of Nutrition, 106*(7), 1117-1127.

Park, J., Ishikawa-Takata, K., Tanaka, S., Hikihara, Y., Ohkawara, K., Watanabe, S., . . . Tabata, I. (2014). The relationship of body composition to daily physical activity in free-living Japanese adult men. *British Journal of Nutrition, 111*(1), 182-188.

Peabody, J. W., Luck, J., Glassman, P., Dresselhaus, T. R., & Lee, M. (2000). Comparison of vignettes, standardized patients, and chart abstraction: a prospective validation study of 3 methods for measuring quality. *JAMA, 283*(13), 1715-1722.

Penne, E. L., van der Weerd, N. C., Grooteman, M. P., Mazairac, A. H., van den Dorpel, M. A., Nubé, M. J., . . . investigators, CONTRAST. (2011). Role of residual renal function in phosphate control and anemia management in chronic hemodialysis patients. *Clinical Journal of the American Society of Nephrology, 6*(2), 281-289.

Perrone, R. D., Steinman, T. I., Beck, G. J., Skibinski, C. I., Royal, H. D., Lawlor, M., & Hunsicker, L. G. (1990). Utility of radioisotopic filtration markers in chronic renal insufficiency: simultaneous comparison of 125I-iothalamate, 169Yb-DTPA, 99mTc-DTPA, and inulin. The Modification of Diet in Renal Disease Study. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 16*(3), 224-235.

Poggio, E. D., Wang, X., Greene, T., Van Lente, F., & Hall, P. M. (2005). Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. *Journal of the American Society of Nephrology, 16*(2), 459-466.

Ponda, M. P., Quan, Z., Melamed, M. L., Raff, A., Meyer, T. W., & Hostetter, T. H. (2010). Methylamine clearance by haemodialysis is low. *Nephrology Dialysis Transplantation, 25*(5), 1608-1613.

Poortmans, J. R. (1984). Exercise and renal function. *Sports Medicine, 1*(2), 125-153.

Poortmans, J. R., & Labilloy, D. (1988). The influence of work intensity on postexercise proteinuria. *Eur J Appl Physiol Occup Physiol, 57*(2), 260-263.

Poortmans, J. R., Rampaer, L., & Wolfs, J. C. (1989). Renal protein excretion after exercise in man. *Eur J Appl Physiol Occup Physiol, 58*(5), 476-480.

Port, F. K., Ashby, V. B., Dhingra, R. K., Roys, E. C., & Wolfe, R. A. (2002). Dialysis dose and body mass index are strongly associated with survival in hemodialysis patients. *Journal of the American Society of Nephrology, 13*(4), 1061-1066.

Port, F. K., Wolfe, R. A., Hulbert-Shearon, T. E., McCullough, K. P., Ashby, V. B., & Held, P. J. (2004). High dialysis dose is associated with lower mortality among women but not among men. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 43*(6), 1014-1023.

Prentice, AM. (1990). The doubly-labelled water method for measuring energy expenditure: technical recommendations for use in humans - a consensus report by the IDECG Working Group.

Raj, Dominic S. C., Zager, Philip, Shah, Vallbh O., Dominic, Elizabeth A., Adeniyi, Oladipo, Blandon, Pedro, . . . Ferrando, Arny. (2004). Protein turnover and amino acid transport kinetics in end-stage renal disease. *American Journal of Physiology Endocrinology and Metabolism, 286*(1), E136-E143.

Ramirez, S. P., Kapke, A., Port, F. K., Wolfe, R. A., Saran, R., Pearson, J., . . . Daugirdas, J. T. (2012). Dialysis dose scaled to body surface area and size-adjusted, sex-specific patient mortality. *Clinical Journal of the American Society of Nephrology, 7*(12), 1977-1987.

Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). (1997). *Lancet, 349*(9069), 1857-1863.

Raphael, K. L., Zhang, Y., Wei, G., Greene, T., Cheung, A. K., Beddhu, S., & El Nahas, M. (2013). Serum bicarbonate and mortality in adults in NHANES III. *Nephrology Dialysis Transplantation, 28*(5), 1207-1213.

Rashid, G., Benchetrit, S., Fishman, D., & Bernheim, J. (2004). Effect of advanced glycation end-products on gene expression and synthesis of TNF-alpha and endothelial nitric oxide synthase by endothelial cells. *Kidney International, 66*(3), 1099-1106.

Rattanasompattikul, M., Feroze, U., Molnar, M. Z., Dukkipati, R., Kovesdy, C. P., Nissenson, A. R., . . . Kalantar-Zadeh, K. (2012). Charlson comorbidity score is a strong predictor of mortality in hemodialysis patients. *International Urology and Nephrology, 44*(6), 1813-1823.

Ravussin, E., & Bogardus, C. (1989). Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. *American Journal of Clinical Nutrition, 49*(5 Suppl), 968-975.

Robinson-Cohen, C., Katz, R., Mozaffarian, D., Dalrymple, L. S., de Boer, I., Sarnak, M., . . . Kestenbaum, B. (2009). Physical activity and rapid decline in kidney function among older adults. *Archives of Internal Medicine, 169*(22), 2116-2123.

Robinson-Cohen, Cassianne, Littman, Alyson J., Duncan, Glen E., Roshanravan, Baback, Ikizler, T. Alp, Himmelfarb, Jonathan, & Kestenbaum, Bryan R. (2013). Assessment of Physical Activity in Chronic Kidney Disease. *Journal of Renal Nutrition, 23*(2), 123-131.

Roderick, Paul, Byrne, Catherine, Casula, Anna, Steenkamp, Retha, Ansell, David, Burden, Richard, . . . Feest, Terry. (2009). Survival of patients from South Asian and Black populations starting renal replacement therapy in England and Wales. *Nephrology Dialysis Transplantation, 24*(12), 3774-3782.

Ruggenenti, Piero, Perna, Annalisa, Mosconi, Lidia, Pisoni, Roberto, & Remuzzi, Giuseppe. (1998). Urinary protein excretion rate is the best independent predictor of ESRF in non-diabetic proteinuric chronic nephropathies. *Kidney International, 53*(5), 1209-1216.

Sallis, James F., Haskell, William L., Wood, Peter D., Fortmann, Stephen P., Rogers, Todd, Blair, Steven N., & Paffenbarger, Ralph S. (1985). Physical Activity Assessment Methodology in the Five-City Project. *American Journal of Epidemiology, 121*(1), 91-106.

Sangha, O., Stucki, G., Liang, M. H., Fossel, A. H., & Katz, J. N. (2003). The Self-Administered Comorbidity Questionnaire: a new method to assess comorbidity for clinical and health services research. *Arthritis and Rheumatism, 49*(2), 156-163.

Sarkar, S. R., Kuhlmann, M. K., Kotanko, P., Zhu, F., Heymsfield, S. B., Wang, J., . . . Levin, N. W. (2006). Metabolic consequences of body size and body composition in hemodialysis patients. *Kidney International, 70*(10), 1832-1839.

Schepers, E., Meert, N., Glorieux, G., Goeman, J., Van der Eycken, J., & Vanholder, R. (2007). P-cresylsulphate, the main in vivo metabolite of p-cresol, activates leucocyte free radical production. *Nephrology Dialysis Transplantation, 22*(2), 592-596.

Schmidt, M. E., & Steindorf, K. (2006). Statistical methods for the validation of questionnaires--discrepancy between theory and practice. *Methods of Information in Medicine, 45*(4), 409-413.

Schneeweiss, B., Graninger, W., Stockenhuber, F., Druml, W., Ferenci, P., Eichinger, S., . . . Lenz, K. (1990). Energy metabolism in acute and chronic renal failure. *American Journal of Clinical Nutrition, 52*(4), 596-601.

Schofield, W. N. (1985). Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition. Clinical Nutrition, 39 Suppl 1*, 5-41.

Schwimmer, J. A., Markowitz, G. S., Valeri, A. M., Imbriano, L. J., Alvis, R., & D'Agati, V. D. (2003). Secondary focal segmental glomerulosclerosis in non-obese patients with increased muscle mass. *Clinical Nephrology, 60*(4), 233-241.

Scott, D., Blizzard, L., Fell, J., & Jones, G. (2011). Prospective associations between ambulatory activity, body composition and muscle function in older adults. *Scandinavian Journal of Medicine and Science in Sports, 21*(6), e168-175.

Shanahan, C. M., Crouthamel, M. H., Kapustin, A., & Giachelli, C. M. (2011). Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. *Circulation Research, 109*(6), 697-711.

Shinzato, T., Nakai, S., Akiba, T., Yamazaki, C., Sasaki, R., Kitaoka, T., . . . Maeda, K. (1997). Survival in long-term haemodialysis patients: results from the annual survey of the Japanese Society for Dialysis Therapy. *Nephrology Dialysis Transplantation, 12*(5), 884-888.

Singer, Michael A. (2001). Of mice and men and elephants: Metabolic rate sets glomerular filtration rate. *American Journal of Kidney Diseases, 37*(1), 164-178.

Singer, Michael A., & Morton, A. Ross. (2000). Mouse to elephant: Biological scaling and Kt/v. *American Journal of Kidney Diseases, 35*(2), 306-309.

Spalding, E. M., Chandna, S. M., Davenport, A., & Farrington, K. (2008). Kt/V underestimates the hemodialysis dose in women and small men. *Kidney International, 74*(3), 348-355.

Speakman, J. R., Nair, K. S., & Goran, M. I. (1993). Revised equations for calculating CO2 production from doubly labeled water in humans. *American Journal of Physiology Endocrinology and Metabolism, 264*(6), E912-917.

Speakman, John R. (1997). *Doubly Labelled Water: Theory and Practice*. London: Chapman & Hall.

Sridharan, Sivakumar, Vilar, Enric, Berdeprado, Jocelyn, & Farrington, Ken. (2013). Energy metabolism, body composition, and urea generation rate in hemodialysis patients. *Hemodialysis International, 17*(4), 502-509.

Stack, Austin G., Molony, Donald A., Rives, Terry, Tyson, Jon, & Murthy, Bhamidipati V. R. (2005). Association of physical activity with mortality in the US dialysis population. *American Journal of Kidney Diseases, 45*(4), 690-701.

Stack, Austin G., & Murthy, Bhamidipati. (2008). Exercise and Limitations in Physical Activity Levels among New Dialysis Patients in the United States: An Epidemiologic Study. *Annals of Epidemiology, 18*(12), 880-888.

Stevens, L. A., Coresh, J., Greene, T., & Levey, A. S. (2006). Assessing kidney function--measured and estimated glomerular filtration rate. *New England Journal of Medicine, 354*(23), 2473-2483.

Suh, M. R., Jung, H. H., Kim, S. B., Park, J. S., & Yang, W. S. (2002). Effects of regular exercise on anxiety, depression, and quality of life in maintenance hemodialysis patients. *Renal Failure, 24*(3), 337-345.

Tarnopolsky, M. A., Atkinson, S. A., MacDougall, J. D., Chesley, A., Phillips, S., & Schwarcz, H. P. (1992). Evaluation of protein requirements for trained strength athletes. *Journal of Applied Physiology, 73*(5), 1986-1995.

Taylor, C. B., Coffey, T., Berra, K., Iaffaldano, R., Casey, K., & Haskell, W. L. (1984). Seven-day activity and self-report compared to a direct measure of physical activity. *American Journal of Epidemiology, 120*(6), 818-824.

Tentori, Francesca, Elder, Stacey J., Thumma, Jyothi, Pisoni, Ronald L., Bommer, Juergen, Fissell, Rachel B., . . . Robinson, Bruce M. (2010). Physical exercise among participants in the Dialysis Outcomes and Practice Patterns Study (DOPPS): correlates and associated outcomes. *Nephrology Dialysis Transplantation, 25*(9), 3050-3062.

Teschan, P. E., Ginn, H. E., Bourne, J. R., & Ward, J. W. (1977). Neurobehavioral probes for adequacy of dialysis. *Transactions - American Society for Artificial Internal Organs, 23*, 556-559.

Thompson, G. N., Pacy, P. J., Merritt, H., Ford, G. C., Read, M. A., Cheng, K. N., & Halliday, D. (1989). Rapid measurement of whole body and forearm protein turnover using a [2H5]phenylalanine model. *American Journal of Physiology Endocrinology and Metabolism, 256*(5), E631-E639.

Thong, Melissa S. Y., Kaptein, Adrian A., Benyamini, Yael, Krediet, Raymond T., Boeschoten, Elisabeth W., & Dekker, Friedo W. (2008). Association Between a Self-Rated Health Question and Mortality in Young and Old Dialysis Patients: A Cohort Study. *American Journal of Kidney Diseases, 52*(1), 111-117.

Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. (1998). *BMJ, 317*(7160), 703-713.

Tizianello, A., De Ferrari, G., Garibotto, G., Gurreri, G., & Robaudo, C. (1980). Renal metabolism of amino acids and ammonia in subjects with normal renal function and in patients with chronic renal insufficiency. *Journal of Clinical Investigation, 65*(5), 1162-1173.

Tizianello, A., Deferrari, G., Garibotto, G., Robaudo, C., Acquarone, N., & Ghiggeri, G. M. (1982). Renal ammoniagenesis in an early stage of metabolic acidosis in man. *Journal of Clinical Investigation, 69*(1), 240-250.

Tjiong, H. L., Swart, R., Rietveld, T., Wattimena, J. L., Hop, W. C., Fieren, M. W., & van den Berg, J. W. (2008). Whole-body protein turnover in peritoneal dialysis patients: a comparison of the [15N]glycine end product and the [13C]leucine precursor methods. *Nephrology Dialysis Transplantation, 23*(8), 2660-2665.

Traynor, J., Mactier, R., Geddes, C. C., & Fox, J. G. (2006). How to measure renal function in clinical practice. *BMJ, 333*(7571), 733-737.

. UK Renal Registry - The Sixteenth Annual Report. (2013).

Vallance, P., Leone, A., Calver, A., Collier, J., & Moncada, S. (1992). Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet, 339*(8793), 572-575.

van Manen, J. G., Korevaar, J. C., Dekker, F. W., Boeschoten, E. W., Bossuyt, P. M., & Krediet, R. T. (2002). How to adjust for comorbidity in survival studies in ESRD patients: a comparison of different indices. *American journal of kidney diseases : the official journal of the National Kidney Foundation, 40*(1), 82-89.

van Olden, R. W., Krediet, R. T., Struijk, D. G., & Arisz, L. (1996). Measurement of residual renal function in patients treated with continuous ambulatory peritoneal dialysis. *Journal of the American Society of Nephrology, 7*(5), 745-750.

van Vilsteren, Marieke C. B. A., de Greef, Mathieu H. G., & Huisman, Roel M. (2005). The effects of a low-to-moderate intensity pre-conditioning exercise programme linked with exercise counselling for sedentary haemodialysis patients in The Netherlands: results of a randomized clinical trial. *Nephrology Dialysis Transplantation, 20*(1), 141-146.

van Ypersele de Strihou, C., Jadoul, M., Malghem, J., Maldague, B., & Jamart, J. (1991). Effect of dialysis membrane and patient's age on signs of dialysis-related amyloidosis. The Working Party on Dialysis Amyloidosis. *Kidney International, 39*(5), 1012-1019.

Vanholder, R., Argiles, A., Baurmeister, U., Brunet, P., Clark, W., Cohen, G., . . . Wratten, M. L. (2001). Uremic toxicity: present state of the art. *International Journal of Artificial Organs, 24*(10), 695-725.

Vanholder, R., De Smet, R., Waterloos, M. A., Van Landschoot, N., Vogeleere, P., Hoste, E., & Ringoir, S. (1995). Mechanisms of uremic inhibition of phagocyte reactive species production: characterization of the role of p-cresol. *Kidney International, 47*(2), 510-517.

Vanholder, Raymond, Baurmeister, Ulrich, Brunet, Philippe, Cohen, Gerald, Glorieux, Griet, Jankowski, Joachim, & for the European Uremic Toxin Work, Group. (2008). A Bench to Bedside View of Uremic Toxins. *Journal of the American Society of Nephrology, 19*(5), 863-870.

Vanholder, Raymond, Glorieux, Griet, & Lameire, Norbert. (2002). The Other Side of the Coin: Impact of Toxin Generation and Nutrition on the Uremic Syndrome. *Seminars in Dialysis, 15*(5), 311-314.

Viana, J. L., Kosmadakis, G. C., Watson, E. L., Bevington, A., Feehally, J., Bishop, N. C., & Smith, A. C. (2014). Evidence for Anti-Inflammatory Effects of Exercise in CKD. *Journal of the American Society of Nephrology*.

Vilar, Enric, Machado, Ashwini, Garrett, Andrew, Kozarski, Robert, Wellsted, David, & Farrington, Ken. (2014). Disease-Specific Predictive Formulas for Energy Expenditure in the Dialysis Population. *Journal of Renal Nutrition, 24*(4), 243-251.

Vilar, Enric, Wellsted, D., Chandna, S. M., Greenwood, R. N., & Farrington, K. (2009). Residual renal function improves outcome in incremental haemodialysis despite reduced dialysis dose. *Nephrology Dialysis Transplantation, 24*(8), 2502-2510.

Wang, J., Thornton, J. C., Burastero, S., Shen, J., Tanenbaum, S., Heymsfield, S. B., & Pierson, R. N. (1996). Comparisons for body mass index and body fat percent among Puerto Ricans, blacks, whites and Asians living in the New York City area. *Obesity Research, 4*(4), 377-384.

Wang, J., Thornton, J. C., Russell, M., Burastero, S., Heymsfield, S., & Pierson, R. N. (1994). Asians have lower body mass index (BMI) but higher percent body fat than do whites: comparisons of anthropometric measurements. *American Journal of Clinical Nutrition, 60*(1), 23-28.

Wang, Z., Heshka, S., Gallagher, D., Boozer, C. N., Kotler, D. P., & Heymsfield, S. B. (2000). Resting energy expenditure-fat-free mass relationship: new insights provided by body composition modeling. *American Journal of Physiology Endocrinology and Metabolism, 279*(3), E539-545.

Washburn, R. A., Smith, K. W., Jette, A. M., & Janney, C. A. (1993). The Physical Activity Scale for the Elderly (PASE): development and evaluation. *Journal of Clinical Epidemiology, 46*(2), 153-162.

Washburn, Richard A., Jette, Alan M., & Janney, Carol A. (1990). Using Age-Neutral Physical Activity Questionnaires in Research with the Elderly. *Journal of Aging and Health, 2*(3), 341-356.

Waterlow, J. C. (2006). *Protein Turnover*: CAB International.

Watson, P. E., Watson, I. D., & Batt, R. D. (1980). Total body water volumes for adult males and females estimated from simple anthropometric measurements. *American Journal of Clinical Nutrition, 33*(1), 27-39.

Weinsier, R. L., Schutz, Y., & Bracco, D. (1992). Reexamination of the relationship of resting metabolic rate to fat-free mass and to the metabolically active components of fat-free mass in humans. *American Journal of Clinical Nutrition, 55*(4), 790-794.

Weir, J. B. de V. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *Journal of Physiology, 109*(1-2), 1-9.

Welch, W. A., Bassett, D. R., Thompson, D. L., Freedson, P. S., Staudenmayer, J. W., John, D., . . . Fitzhugh, E. C. (2013). Classification accuracy of the wrist-worn gravity estimator of normal everyday activity accelerometer. *Medicine and Science in Sports and Exercise, 45*(10), 2012-2019.

Welle, S. (1990). Two-point vs multipoint sample collection for the analysis of energy expenditure by use of the doubly labeled water method. *American Journal of Clinical Nutrition, 52*(6), 1134-1138.

Welle, S., & Nair, K. S. (1990). Relationship of resting metabolic rate to body composition and protein turnover. *American Journal of Physiology Endocrinology and Metabolism, 258*(6), E990-E998.

Weyer, Christian, Snitker, S., Bogardus, Clifton, & Ravussin, Eric. (1999). Energy metabolism in African Americans: potential risk factors for obesity. *American Journal of Clinical Nutrition, 70*(1), 13-20.

White, C. R., & Seymour, R. S. (2005). Allometric scaling of mammalian metabolism. *Journal of Experimental Biology, 208*(Pt 9), 1611-1619.

Williams, E., Klesges, R. C., Hanson, C. L., & Eck, L. H. (1989). A prospective study of the reliability and convergent validity of three physical activity measures in a field research trial. *Journal of Clinical Epidemiology, 42*(12), 1161-1170.

Wlodarek, D., Glabska, D., & Rojek-Trebicka, J. (2011). Physical activity of predialysis patients with chronic kidney disease measured using SenseWear Armband. *Journal of Sports Medicine and Physical Fitness, 51*(4), 639-646.

Wolfe, Robert A., Ashby, Valarie B., Daugirdas, John T., Agodoa, Lawrence Y. C., Jones, Camille A., & Port, Friedrich K. (2000). Body size, dose of hemodialysis, and mortality. *American Journal of Kidney Diseases, 35*(1), 80-88.

Workeneh, B. T., & Mitch, W. E. (2010). Review of muscle wasting associated with chronic kidney disease. *American Journal of Clinical Nutrition, 91*(4), 1128S-1132S.

Wouters-Adriaens, Mirjam P. E., & Westerterp, Klaas R. (2008). Low Resting Energy Expenditure in Asians Can Be Attributed to Body Composition. *Obesity, 16*(10), 2212-2216.

Wright, J. T., Bakris, G., Greene, T., Agodoa, L. Y., Appel, L. J., Charleston, J., . . . Group, African American Study of Kidney Disease and Hypertension Study. (2002). Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA, 288*(19), 2421-2431.

Yoshizumi, T., Gondolesi, G. E., Bodian, C. A., Jeon, H., Schwartz, M. E., Fishbein, T. M., . . . Emre, S. (2003). A simple new formula to assess liver weight. *Transplantation Proceedings, 35*(4), 1415-1420.

Zamojska, Sylwia, Szklarek, Magdalena, Niewodniczy, Maciej, & Nowicki, Michal. (2006). Correlates of habitual physical activity in chronic haemodialysis patients. *Nephrology Dialysis Transplantation, 21*(5), 1323-1327.

Zhang, S., Rowlands, A. V., Murray, P., & Hurst, T. L. (2012). Physical activity classification using the GENEA wrist-worn accelerometer. *Medicine and Science in Sports and Exercise, 44*(4), 742-748.

Appendix

A Ethical Approval of research studies

B Recent Physical Activity Questionnaire (RPAQ)

C Stanford 7-day Recall Questionnaire

D Self-report Comorbidity Questionnaire

E Portability analysis of doubly labelled water isotope sample

F Certificate of analysis for leucine and sodium bicarbonate stable isotopes

Appendix A

GIG CYMRU NHS WALES
Bwrdd Iechyd Prifysgol
Betsi Cadwaladr
University Health Board

North Wales REC (Central and East)
G1/G2 Croesnewydd Hall
Croesnewydd Road
Wrexham Technology Park
Wrexham
LL13 7YP

Telephone: 01978 726377

23 February 2012

Dr Sivakumar Sridharan
Renal Research Fellow
East and North Hertfordshire NHS Trust
Renal Research Lab L84, Lister Hospital,
Corey's Mill Lane,
Stevenage,
SG1 4AB

Dear Dr Sridharan

Study title:	**Alternative Methods of Scaling Dialysis Dose: Impact on Survival**
REC reference:	**12/WA/0060**

The Proportionate Review Sub-committee of the North Wales REC (Central and East) reviewed the above application on 23 February 2012.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

The Committee suggest that consideration be given to the use of your personal mobile phone for research purposes. A designated 'pay as you go' mobile number or work related contact details would be a more effective solution for inclusion in the participant documentation.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation.

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Investigator CV – S Sridharan		14 February 2012
Investigator CV - D M Wellsted		13 February 2012
Participant Consent Form	1.1	13 February 2012
Participant Information Sheet	1.1	13 February 2012
Protocol	1.1	13 February 2012
Questionnaire: Co-morbidity questionnaire		
Questionnaire: Physical Activity Questionnaire		
REC application 98376/292746/1/265	1.1	14 February 2012
Referees or other scientific critique report		29 December 2011
Summary/Synopsis	1.1	13 February 2012

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/WA/0060	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

T.a. Biggs

p.p **Professor Alex Carson**
Chair

Email: Tracy.Biggs@wales.nhs.uk

NHS
Health Research Authority
NRES Committee East of England - Essex
East of England Rec Office
Victoria House
Capital Park
Fulbourn
Cambridge
CB21 5XB

Telephone: 01223 597656
Facsimile: 01223 597645

26 April 2013

Dr Sivakumar Sridharan
Renal Research Fellow
East and North Hertfordshire NHS Trust
Renal Research Lab L84, Lister Hospital,
Corey's Mill Lane,
Stevenage,
SG1 4AB

Dear Dr Sridharan

Study title: **Influence of Physical Activity on Urea Generation Rate**
REC reference: **13/EE/0053**
IRAS project ID: **124319**

Thank you for your letter of 24 March 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Miss April Saunders, nrescommittee.eastofengland-essex@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

1. Only the submitted and approved questionnaire versions are used.
2. The protocol wording is modified to reflect that the consent forms are not the sole record linking identity to study number, as link files will also be used. An amended protocol with suggested modification is attached.
3. The Royal Free section of the protocol should be amended to clearly state that the research team will only approach a patient after they normal care team has obtained consent for that to happen. Suggested amendment made in the attached protocol.
4. 'Baseline anthropometric measurements' are limited to those specified in the protocol, as the use of the term 'such as' is unacceptably open-ended. Suggested amendment made in the attached protocol.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Investigator CV		20 January 2013
Other: CV of supervisors Ken Farrington and Justin Roberts		20 January 2013
Other: Letter from funder		09 January 2013
Other: Accelerometer picture		
Other: Accelerometer picture		
Other: Flow Chart of study Procedure	1.1	20 January 2013
Other: Peer review - Dr Justin Roberts		19 March 2013
Other: Peer review - Ken Farrington		
Other: Confirmation of grant - British Kidney Patient Association		09 January 2013
Participant Consent Form	1.1	20 January 2013
Participant Information Sheet: East and North Hertfordshire	1.2	22 March 2013
Participant Information Sheet: Royal Free Hampstead	1.2	22 March 2013
Protocol	1.2	22 March 2013
Questionnaire: MOS Sleep Scale	validated	
Questionnaire: RPAQ	validated	
Questionnaire: SF36		21 March 2013
REC application		30 January 2013
Response to Request for Further Information	Sivakumar Sridharan	24 March 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

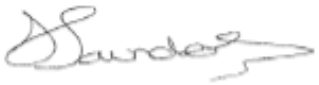
Further information is available at National Research Ethics Service website > After Review

13/EE/0053	Please quote this number on all correspondence

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely



pp

Dr Alan Lamont
Chair

Email:nrescommittee.eastofengland-essex@nhs.net

NHS
Health Research Authority

NRES Committee East of England - Essex
East of England Rec Office
Victoria House
Capital Park
Fulbourn
Cambridge
CB21 5XB

Telephone: 020 797 22587
Facsimile: 020 797 22592

20 May 2013

Dr Sivakumar Sridharan
Renal Research Fellow
East and North Hertfordshire NHS Trust
Renal Research Lab L84, Lister Hospital,
Corey's Mill Lane,
Stevenage,
SG1 4AB

Dear Dr Sridharan

Study title: **Influence of Physical Activity on Urea Generation Rate**
REC reference: **13/EE/0053**
IRAS project ID: **124319**

Thank you for your letter of 26 April 2013. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 26 April 2013

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Protocol	1.3	26 April 2013

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Investigator CV		20 January 2013
Other: CV of supervisors Ken Farrington and Justin Roberts		20 January 2013
Other: Letter from funder		09 January 2013
Other: Accelerometer picture		

Other: Accelerometer picture		
Other: Flow Chart of study Procedure	1.1	20 January 2013
Other: Peer review - Dr Justin Roberts		19 March 2013
Other: Peer review - Ken Farrington		
Other: Confirmation of grant - British Kidney Patient Association		09 January 2013
Participant Consent Form	1.1	20 January 2013
Participant Information Sheet: East and North Hertfordshire	1.2	22 March 2013
Participant Information Sheet: Royal Free Hampstead	1.2	22 March 2013
Protocol	1.3	26 April 2013
Questionnaire: MOS Sleep Scale	validated	
Questionnaire: RPAQ	validated	
Questionnaire: SF36		21 March 2013
REC application		30 January 2013
Response to Request for Further Information	Sivakumar Sridharan	24 March 2013

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

13/EE/0053 **Please quote this number on all correspondence**

Yours sincerely

pp

Mr Atul Patel
Committee Co-ordinator

E-mail: nrescommittee.eastofengland-essex@nhs.net

NHS
Health Research Authority
NRES Committee East of England - Cambridge East
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 01158839697

27 June 2013

Dr Sivakumar Sridharan
Renal Research Lab L84, Lister Hospital,
Corey's Mill Lane,
Stevenage,
SG1 4AB

Dear Dr Sridharan,

Study title:	**Protein Turnover and Resting Energy Expenditure in Renal Failure**
REC reference:	**13/EE/0158**
IRAS project ID:	**108969**

Thank you for your letter of 25 June 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Rachel Nelson, .

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Investigator CV	Sivakumar Sridharan	29 April 2013
Investigator CV	Ken Farrington	29 April 2013
Investigator CV	Justin Roberts	29 April 2013
Participant Consent Form	1.2	25 June 2013
Participant Information Sheet: Part 1	1.2	26 June 2013
Protocol	1.1	19 March 2013
Questionnaire: Recent Physical Activity		29 April 2013
REC application		03 May 2013
Response to Request for Further Information		25 June 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/EE/0158	Please quote this number on all correspondence

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely,

pp

Dr Daryl Rees
Chair

Email: NRESCommittee.EastofEngland-CambridgeEast@nhs.net

NRES Committee East of England - Hatfield
Room 002, TEDCO Business Centre
Rolling Mill Road
Jarrow
Tyne and Wear
NE32 3DT

Telephone: 0191 428 3387

27 November 2013

Dr Jonathan P Wong
Renal Research L84
Lister Hospital
Corey's Mill Lane
SG1 4AB

Dear Dr Wong

Study title:	**Measures of inflammation, activity-related and total energy expenditure in chronic kidney disease**
REC reference:	**13/EE/0388**
IRAS project ID:	**139951**

The Research Ethics Committee reviewed the above application at the meeting held on 13 November 2013. Thank you for attending to discuss the application.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager Miss Sarah Grimshaw, nrescommittee.eastofengland-hatfield@nhs.net.

Ethical opinion

The Chair, Professor Barry Hunt, welcomed you to the meeting and thanked you for attending.

The Committee requested clarification as to how participants would be consented.

You replied that patients were seen routinely at outpatient clinics. Potential participants would be identified from the clinic lists and would be approached whilst waiting for their appointments; at this time they would be told about the study and be given a Participant Information Sheet.

It was noted that the researchers would be recruiting patients in stages 1-5 of the disease, and that they would be grouped into stages 1-3 and 4-5.

You confirmed that that was correct and that this would be so you would analyse the data of participants with mild and severe renal failure.

Members noted that the researchers would recruit 40 participants and questioned whether the sample size would be adequate in order to ensure the validity and reliability of the questionnaires.

You responded that this was an extension of a previous study but that this did not have enough numbers to be able to validate the data accrued.

The REC therefore queried whether this study had the same design as the previous study.

You confirmed that it did. The previous study had used the techniques with doubly-labelled water, but did not include the physical activity interventions or the questionnaires.

It was noted that two similar but different questionnaires had been submitted – the RPAQ and the CKD-RPAQ – but there was confusion as to whether both would be used.

You clarified that the RPAQ had been used to design the CKD-RPAQ and that the RPAQ would not be used.

The Committee noted that page 6 of the protocol stated "To ensure safety we will be analysing a sample of the doubly labelled water batch used in the Pharmacy Quality Control laboratory to ensure it is safe and meets standards for water potability", but was unsure how using doubly-labelled water would be unsafe.

You replied that it was not unsafe as it was present in tap water in low doses and was not radioactive.

Members requested further clarification was to why the water would be tested by the Pharmacy.

You clarified that it would be tested to ensure that it was pure and that there were no bacteria.

You left the room.

The Committee discussed the responses.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publicly accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
GP/Consultant Information Sheets	1.1	14 October 2013
Investigator CV	Jonathan Wong	14 October 2013
Investigator CV	Enric Hall	14 October 2013
Investigator CV	Ken Farrington	14 October 2013
Investigator CV	Sivakumar Sridharan	14 October 2013
Investigator CV	Jocelyn Berdeprado	14 October 2013
Investigator CV	Ashwini Machado	14 October 2013
Participant Consent Form	1.1	14 October 2013
Participant Information Sheet	1.1	14 October 2013
Protocol	1.3	14 October 2013
Questionnaire: RPAQ		
Questionnaire: Stanford Questionnaire		
Questionnaire: CKD-PAQ	1.1	14 October 2013
REC application	IRAS Version 3.5, 139951/514474/1/828	14 October 2013

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/EE/0388	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

pp
Professor Barry Hunt
Chair

Email: nrescommittee.eastofengland-hatfield@nhs.net

Appendix B

Recent Physical Activity Questionnaire

MRC | Epidemiology Unit

Participant study No.

RPAQ

Recent Physical Activity Questionnaire

This questionnaire is designed to find out about your physical activity in your everyday life in the last 4 weeks

This questionnaire is divided into 3 sections
Please try to answer every question.

- **Section A** asks about your physical activity patterns in and around the house.
- **Section B** is about travel to work and your activity at work.
- **Section C** asks about recreations that you may have engaged in during the last 4 weeks.

Your answers will be treated as strictly confidential and will be used only for medical research

Section A Home Activities

Getting about

Which form of transport have you used **most often** in the last 4 weeks apart from your journey to and from work? (Please tick (✓) one box only)

Usual mode of travel			
Car/ motor vehicle	Walk	Public transport	Cycle

TV, DVD or Video Viewing (Please put a tick (✓) on every line)

Hours of TV, DVD or Video watched per day	Average over the last 4 weeks					
	None	Less than 1 hour a day	1 to 2 hours a day	2 to 3 hours a day	3 to 4 hours a day	More than 4 hours a day
On a weekday before 6 pm						
On a weekday after 6 pm						
On a weekend day before 6 pm						
On a weekend day after 6 pm						

Computer use at home *but not at work* (e.g. internet, email, Playstation, Xbox etc)

(Please put a tick (✓) on every line)

Hours of computer use per day	Average over the last 4 weeks					
	None	Less than 1 hour a day	1 to 2 hours a day	2 to 3 hours a day	3 to 4 hours a day	More than 4 hours a day
On a weekday before 6 pm						
On a weekday after 6 pm						
On a weekend day before 6 pm						
On a weekend day after 6 pm						

Stair climbing at home (Please put a tick (✓) on every line)

Number of times you climbed a flight of stairs (approx 10 steps) each day at home	Average over the last 4 weeks					
	None	1 to 5 times a day	6 to 10 times a day	11 to 15 times a day	15 to 20 times a day	More than 20 times a day
On a weekday						
On a weekend day						

Section B Activity at work

Please answer this section to describe if you have been in paid employment at any time **during the last 4 weeks** or you have done regular, organised voluntary work.

Have you been in employment during the last 4 weeks? *Yes* ☐ *No* ☐

During the last 4 weeks how many hours work did you do per week?

	4 weeks ago	3 weeks ago	2 weeks ago	1 week ago
Work hours (excluding travel)				

Type of work

We would like to know the type and amount of physical activity involved in your work. **Please tick** (✓) the option that **best** corresponds with your occupation(s) in the last 4 weeks from the following four possibilities:

Please tick only one of the following

1. Sedentary occupation
You spend most of your time sitting (such as in an office) ☐

2. Standing occupation
You spend most of your time standing or walking. However, your work does not require intense physical effort (e.g. shop assistant, hairdresser, guard) ☐

3. Manual work
This involves some physical effort including handling of heavy objects and use of tools (e.g. plumber, electrician, carpenter) ☐

4. Heavy manual work
This implies very vigorous physical activity including handling of very heavy objects (e.g. dock worker, miner, bricklayer, construction worker) ☐

Section B Activity at work

Travel to and from work in the last 4 weeks

What is the approximate distance from your home to your work?

			Miles	**or**				Kilometers

How many times a week did you travel from home to your main work? *Count outward journeys only*

Please tick (✓) one box **only** per line

How did you normally travel to work?	Always	Usually	Occasionally	Never or rarely
By car/motor vehicle				
By works or public transport				
By bicycle				
Walking				

What is the postcode for your main place of work during the last 4 weeks?

Postcode

If not known please give your work address

Work address - _____

_____

_____

What is the postcode for your home address?

Postcode

Section C Recreation

The following questions ask about how you spent your leisure time.

Please indicate how often you did each activity on average over the last 4 weeks

Please indicate the average length of time that you spent doing the activity on each occasion.

Example

If you went walking for pleasure for 40 minutes once a week.
If you had done weeding or pruning every fortnight and took 1 hour and 10 minutes on each occasion.
You would complete the table below as follows:

Please give an answer for the NUMBER OF TIMES you did the following activities in the past 4 weeks and the AVERAGE TIME you spent on each activity.

Please complete EACH line

	Number of times you did the activity in the last 4 weeks							Average time per episode	
	None	Once in the last 4 weeks	2 to 3 times in the last 4 weeks	Once a week	2 to 3 times a week	4 to 5 times a week	Every day	Hours	Minutes
Weeding and pruning			✓					*1*	*10*
Walking for pleasure				✓					*40*

Now complete the table on pages 7 and 8

Please give an answer for the average time you spent on each activity and the number of times you did that activity in the past 4 weeks

Please complete each line

	Number of times you did the activity in the last 4 weeks							Average time per episode	
	None	Once in the last 4 weeks	2 to 3 times in the last 4 weeks	Once a week	2 to 3 times a week	4 to 5 times a week	Every day	Hours	Minutes
Swimming – competitive									
Swimming leisurely									
Backpacking or mountain climbing									
Walking for pleasure (not as a means of transport)									
Racing or rough terrain cycling									
Cycling for pleasure (not as a means of transport)									
Mowing the lawn									
Watering the lawn or garden									
Digging, shovelling or chopping wood									
Weeding or pruning									
DIY e.g., carpentry, home or car maintenance									
High impact aerobics or step aerobics									
Other types of aerobics									
Exercise with weights									
Conditioning exercises e.g., using a bike or rowing machine									
Floor exercises e.g., stretching, bending, yoga									

Please complete each line

	Number of times you did the activity in the last 4 weeks							Average time per episode	
	None	Once in the last 4 weeks	2 to 3 times in the last 4 weeks	Once a week	2 to 3 times a week	4 to 5 times a week	Every day	Hours	Minutes
Dancing e.g., ballroom or disco									
Competitive running									
Jogging									
Bowling – indoor, lawn or 10-pin									
Tennis or badminton									
Squash									
Table tennis									
Golf									
Football, rugby or hockey									
Cricket									
Rowing									
Netball, volleyball or basketball									
Fishing									
Horse-riding									
Snooker, billiards or Darts									
Musical instrument playing or singing									
Ice Skating									
Sailing, wind-surfing or boating									
Martial arts, boxing or wrestling									
Other activities – please list below									

Thank you

Appendix C

Stanford 7-day Recall Questionnaire

ID #: **Date:**

SEVEN-DAY PHYSICAL ACTIVITY RECALL

Information from this questionnaire will be used to estimate the number of calories you burn through physical activity.

(1) On the average, how many hours did you sleep each night during the last five weekday nights? _____ (0 if not applicable)

(2) On the average, how many hours did you sleep each night during the last weekend? _____ (0 if not applicable)

(3) How many hours did you spend during the last 5 weekdays doing these moderate activities or others like them? _____ (0 if not applicable)

(4) How many hours did you spend last weekend doing these moderate activities? _____ (0 if not applicable)

(5) How many hours did you spend during the last 5 weekdays doing these hard activities or others like them? _____ (0 if not applicable)

(6) How many hours did you spend last weekend doing these hard activities? _____ (0 if not applicable)

(7) How many hours did you spend during the last 5 weekdays doing these very hard activities or others like them? _____ (0 if not applicable)

(8) How many hours did you spend last weekend doing these very hard activities? _____ (0 if not applicable)

(9) Were you employed outside the home during the last 7 days? If no, put zeros for questions 9-13. If yes, how many days? _____ (0 if not applicable)

(10) How many hours per day? _____ (0 if not applicable)

(11) How many of these hours per day were spent doing moderate activities? _____ (0 if not applicable)

(12) How many of these hours per day were spent doing hard activities? _____ (0 if not applicable)

(13) How many of these hours per day were spent doing very hard activities? _____ (0 if not applicable)

(14) Compared to your physical activity over the past 3 months, was last week's physical activity more, less or about the same?

1-More 2-Less 3-About the same

Appendix D

Self-report Comorbidity Questionnaire

SELF-REPORT COMORBIDITY QUESTIONNAIRE

The following is a list of common problems. Please indicate if you currently have the problem in the first column. If you do not have the problem, skip to the next problem.

If you do have the problem, please indicate in the second column if you receive medications or some other type of treatment for the problem.

In the third column, please indicate if the problem limits any of your activities.

Finally, indicate all medical conditions that are not listed, under "other medical problems" at the end of this page.

PROBLEM	Do you have the problem? (Please tick)	Do you receive treatment for it? (Please tick)	Does it limit your activities? (Please tick)
Heart disease such as angina or poor heart function			
Previous heart attacks			
Diabetes			
Cancer			
Lung disease			
Liver disease			
Arthritis			
Depression			
Other medical conditions (please write in)			

Appendix E

Potability Analysis of Doubly Labelled Water Isotope Sample

Quality Control Department - Total Viable Count

Level 2, Lister Hospital, Stevenage, Hertfordshire, SG1 4AB
Tele: 01438 314333 ext. 5417 or 01438 781013 (direct)

Customer: Lister
Unit: Renal Unit
Date : 27/01/2014
Read by: SH
QC No: 1509

Type	Batch No	Expiry	Temp	Days
R2A	14/004	13-Apr-14	22.00	7.0

Loc'n	Tested?	Tested by	Media	Vol	CFU/ plate	CFU/ ml	Identifications	Warning level	Action level
H2O/DEUT	✔	AD	R2A	5.0	9	1.8	N0		
			R2A	5.0	5	1.0	N0		

Prepared by Dr. Jon Wong

H2O = LVH0077X
Deut = STBD4355V

No limits - monitoring only

Comments:

Signed: *Microbiologist*
Date: 4-2-14

Principal staff Pharmacist
Date: 5/2/14

Appendix F

Certificate of Analysis for Leucine and Sodium bicarbonate Stable Isotopes

ISOTEC
A MEMBER OF THE SIGMA-ALDRICH FAMILY

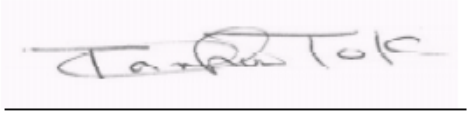
CERTIFICATE OF ANALYSIS

DATE RELEASED 12/22/2005

L-Leucine-1-^{13}C, S & P tested

ISOTEC NUMBER T83-11039 **ALDRICH NUMBER** 661538 **BATCH NUMBER** ST1786

METHODS	SPECIFICATIONS	TEST RESULTS
% Water	<= 0.5%	0.12%
Chiral HPLC	Minimum 99% chemical purity Maximum 1% D-form	99.9% Meets requirement
^{1}H-NMR (FT)	Structure and purity verification	Meets requirement
^{13}C-NMR	Structure and purity verification	Meets requirement
Isotopic Enrich.	Minimum 99 atom% ^{13}C based upon labelled precursor	99.5 atom% ^{13}C
Appearance	White powder	White powder
Sterility	Meets Test Requirements of Membrane Sterility Test based upon USP <71>	Meets requirement (Please see next page)
Pyrogens	<0.5 EU/ml at 10 mg/ml based upon a two step kinetic assay	Meets requirement (Please see next page)
	Sterility & Pyrogenicity Test Date (Month/Year)	May 2005



C.T. Tan, Ph. D.
Analytical & Quality Control
Manager

3858 Benner Road, Miamisburg, Ohio 45342 U.S.A. • FAX (937) 859-4878
Sales (800) 448-9760 • www.sigmaaldrich.com/chemistry/stable-isotopes-isotec.html

ISOTEC
A MEMBER OF THE SIGMA-ALDRICH FAMILY

CERTIFICATE OF ANALYSIS

DATE RELEASED 12/22/2005

L-Leucine-1-^{13}C, S & P tested

ISOTEC NUMBER T83-11039 **ALDRICH NUMBER** 661538 **BATCH NUMBER** ST1786

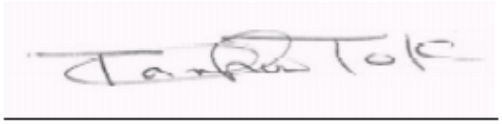
Sterility & Pyrogen Testing Results:

Pyrogenicity Test: The assay met all the kinetic-QCL testing validation requirements per the FDA established guidelines for Limulus Amebocyte Lysate (LAL) testing. The test result indicated that bacterial endotoxins were not present in the test solution (10 mg/ml) at the level of 0.5 EU/ml.

Sterility Test: Membrane Filtration Method, cultured in two (2) media: The test sample meets the requirements of the Sterility Test.

Note: All the compounds that Isotec offers are manufactured and tested to the highest purity in the industry. Researchers intending to use these products in applications involving humans do so at their own responsibility and must comply with all applicable regulations. Isotec will provide supporting information to assist medical research groups in obtaining regulatory approval in their countries.

Isotec can test products for sterility and pyrogenicity . The testing is done in bulk form, before subdivision packaging . This bulk test does not guarantee that the subdivision or repackaged aliquot is sterile and pyrogen free when it is received or used by the customer, and does not imply suitability for any particular purpose. If the product must be sterile and pyrogen free for the intended application, Isotec recommends that the product be tested prior to actual use.



C.T. Tan, Ph. D.
Analytical & Quality Control
Manager

3858 Benner Road, Miamisburg, Ohio 45342 U.S.A. • FAX (937) 859-4878
Sales (800) 448-9760 • www.sigmaaldrich.com/chemistry/stable-isotopes-isotec.html

V5.15RS

ISOTEC
A MEMBER OF THE SIGMA-ALDRICH FAMILY

CERTIFICATE OF ANALYSIS

DATE RELEASED 08/10/2012

Sodium Bicarbonate-^{13}C, S&P tested

ISOTEC NUMBER T83-00058 **ALDRICH NUMBER** 660930 **BATCH NUMBER** EB1513

METHODS	SPECIFICATIONS	TEST RESULTS
Titration	Minimum 99% chemical purity	99%
Mass. Spect.	Minimum 98 atom% ^{13}C	99.0 atom% ^{13}C
Appearance	White powder	White powder
Sterility	Meets Test Requirement of Membrane Sterility Test based upon USP <71>	Meets requirement (Please see next page)
Pyrogens	<0.5 EU/ml at 10 mg/ml based upon USP <85> Bacterial Endotoxins Test	Meets requirement (Please see next page)
	Sterility & Pyrogenicity Test Date (Month/Year)	July 2012

C.T. Tan, Ph. D.
Analytical & Quality Control
Manager

3858 Benner Road, Miamisburg, Ohio 45342 U.S.A. • FAX (937) 859-4878
Sales (800) 448-9760 • www.sigmaaldrich.com/chemistry/stable-isotopes-isotec.html

ISOTEC
A MEMBER OF THE SIGMA-ALDRICH FAMILY

CERTIFICATE OF ANALYSIS

DATE RELEASED 08/10/2012

Sodium Bicarbonate-^{13}C, S&P tested

ISOTEC NUMBER T83-00058 **ALDRICH NUMBER** 660930 **BATCH NUMBER** EB1513

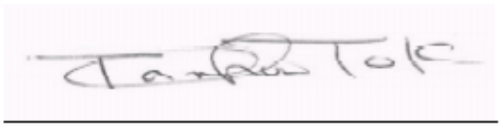
Sterility & Pyrogen Testing Results:

Pyrogenicity Test: The assay met all the kinetic-QCL testing validation requirements per the FDA established guidelines for Limulus Amebocyte Lysate (LAL) testing. The test result indicated that bacterial endotoxins were not present in the test solution (10mg/ml) at the level of 0.5 EU/ml.

Sterility Test: Membrane Filtration Method, cultured in two (2) media: The test sample meets the requirements of the Sterility Test.

Note: All the compounds that Isotec offers are manufactured and tested to the highest purity in the industry. Researchers intending to use these products in applications involving humans do so at their own responsibility and must comply with all applicable regulations. Isotec will provide supporting information to assist medical research groups in obtaining regulatory approval in their countries.

Isotec can test products for sterility and pyrogenicity . The testing is done in bulk form, before subdivision packaging . This bulk test does not guarantee that the subdivision or repackaged aliquot is sterile and pyrogen free when it is received or used by the customer, and does not imply suitability for any particular purpose. If the product must be sterile and pyrogen free for the intended application, Isotec recommends that the product be tested prior to actual use.



C.T. Tan, Ph. D.
Analytical & Quality Control
Manager

3858 Benner Road, Miamisburg, Ohio 45342 U.S.A. • FAX (937) 859-4878
Sales (800) 448-9760 • www.sigmaaldrich.com/chemistry/stable-isotopes-isotec.html

V5.15RS