EFFECTS OF BODY COMPOSITION ON CLINICAL AND QUALITY OF LIFE OUTCOMES IN KIDNEY TRANSPLANT RECIPIENTS

by

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Abstract of Thesis

Sarcopenic obesity is common among kidney transplant recipients. Fluid volume status has not been well-investigated following kidney transplantation. This thesis aimed to explore the effects of body composition, including fat mass, muscle mass and fluid volume status, on post-transplantation morbidity and fatigue. These are potential contributing factors to long-term patient- and graft- survival, as well as quality of life.

Firstly, the associations between adiposity with inflammation, hepcidin and haemoglobin levels were investigated. Secondly, the effects of hypervolemia on blood pressure and levels of N-terminal fragment of pro-hormone B-type natriuretic peptide (NT-proBNP) were explored. Thirdly, the role of muscle mass and fat mass on all domains of fatigue were studied. Finally, the mechanistic aetiology of physical fatigue was examined by evaluation of muscle mass, muscular and cardiovascular functions, and fatigue perception.

This thesis concluded that while adiposity displays significant independent association with inflammation, its role in determining hepcidin and haemoglobin levels remains uncertain. Reduced muscle mass may be correlated with physical fatigue, but independent contribution of fat mass in fatigue remains undefined. Hypervolemia is associated with raised blood pressure and elevated levels of NT-proBNP. The findings from this thesis set the scene for future interventional research and therapeutic strategies.

Dedication

This thesis is dedicated to the loving memory of my best, my dearest friend, Elaine. You left me beautiful memories, you've always been there to help me, guiding me through difficult times, though you're no longer here, you'll forever be in my heart.

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List of Abbreviations

CHAPTER 1

QoL …..……	Quality of Life
BMI ……...	Body Mass Index
DEXA ……	Dual Energy X-ray Absorptiometry
2-C …….	Two-Compartment
CKD ……..	Chronic Kidney Disease
3-C …….	Three-Compartment
4-C …….	Four-Compartment
KTRs …….	Kidney Transplant Recipients
BCM ……..	Body Composition Monitor
CVD ……..	Cardiovascular Disease
ALERT ……..	Assessment of LEscol in Renal Transplantation
IL-6 ……	Interleukin-6
TNF-α ……	Tumor Necrosis Factor-α
hsCRP ……	high-sensitivity C-Reactive Protein
CRP ……...	C-Reactive Protein
VCAM ……...	Vascular Cell Adhesion Molecule
NT-proBNP ...	N-terminal fragment of Pro-hormone of B-Type Natriuretic Peptide
pro-BNP ……	Pro-hormone B-Type Natriuretic Peptide

CHAPTER 2

KTRs …….	Kidney Transplant Recipients
CAPRIT …….	Correction of Anaemia and Progression of Renal Insufficiency in Transplant Patients Study
CKD ……..	Chronic Kidney Disease
BMI ……...	Body Mass Index
NODAT …….	New Onset Diabetes After Transplantation

TSAT ……. Transferrin Saturation
hsCRP …… high sensitivity C-Reactive Protein
eGFR ……. Estimated Glomerular Filtration Rate
MDRD ……... Modification of Diet in Renal Disease
MMF ……. Mycophenolate Mofetil
ACEI ……. Angiotensin Converting Enzyme Inhibitors
ARB ……... Angiotensin Receptor Blockers
BCM …….. Body Composition Monitor
FTI ……. Fat Tissue Index
DEXA …… Dual Energy X-ray Absoptiometry
ALERT …….. Assessment of LEscol in Renal Transplantation
CRP ……... C-Reactive Protein

CHAPTER 3

KTRs ……. Kidney Transplant Recipients
NT-proBNP ... N-terminal fragment of Pro-hormone of B-Type Natriuretic Peptide
ESRD ……. End Stage Renal Disease
BNP ……... B-Type Natriuretic Peptide
pro-BNP …… Pro-hormone of B-Type Natriuretic Peptide
CKD …….. Chronic Kidney Disease
Pre-DM …….. Pre-existing Diabetes Mellitus
NODAT ……. New Onset Diabetes After Transplantation
ACEI ……. Angiotensin-Converting-Enzyme Inhibitor
ARB …….. Angiotensin-Receptor Blocker
BAB ……... Beta-Adrenergic Blocker
CCB ……... Calcium-Channel Blocker
AAB …….. Alpha-Adrenergic Blocker
hsCRP …… high-sensitivity C-Reactive Protein
eGRF ……. Estimated Glomerular Filtration Rate
ACR ……... Albumin : Creatinine Ratio
BCM …….. Body Composition Monitor

%VE …….. Percentage Volume Expansion

LTI ……. Lean Tissue Index

FTI ……. Fat Tissue Index

KDIGO …….. Kidney Disease: Improving Global Outcomes

ERBP ……. European Renal Best Practice

UKRA ……... United Kingdom Renal Association

CHAPTER 4

KTRs ……. Kidney Transplant Recipients

QoL ……... Quality of Life

hsCRP …… high-sensitivity C-Reactive Protein

eGRF ……. Estimated Glomerular Filtration Rate

LTI ……. Lean Tissue Index

BMI ……... Body Mass Index

MFI-20 …….. Multi-Dimensional Fatigue Inventory-20

CFS …… Chronic Fatigue Syndrome

SF-36 ……. Medical Outcomes Study Short Form-36

ICED ……. Index of Coexisting Disease

HEMO ……... Haemodialysis

Pre-DM …….. Diabetes Pre-transplantation

NODAT ……. New Onset Diabetes After Transplantation

BCM …….. Body Composition Monitor

FTI ……. Fat Tissue Index

DEXA …… Dual Energy X-ray Absorptiometry

HADS …… Hospital Anxiety and Depression Scale

PSQI …….. Pittsburgh Sleep Quality Index

MS ……. Multiple Sclerosis

TREAT …….. Trial to Reduce cardiovascular Events with Aranesp Therapy

CHAPTER 5

KTRs …….	Kidney Transplant Recipients
QoL ……...	Quality of Life
RPE ……...	Rating of Perceived Exertion
MFI-20 ……..	Multi-Dimensional Fatigue Inventory-20
SF-36 …….	Medical Outcomes Short Form-36
DEXA ……	Dual Energy X-Ray Absorptiometry
LTM ……..	Lean Tissue Mass
LLTM ……	Lower Limb Lean Tissue Mass
FM …….	Fat Mass
Ht^2 ……..	Height Squared
CMJ ……...	Counter Movement Jump
BM …….	Total Body Mass
VO_2max …….	Estimated Maximal Oxygen Consumption
O_2 pulse …….	Oxygen Pulse
VO_2…….	Oxygen Consumption
HR …….	Heart Rate
RPE_{index}……..	Rating of Perceived Exertion Index
ICED …….	Index of Co-Existing Disease
HEMO ……...	Haemodialysis
Pre-DM ……..	Presence of Diabetes Pre-Transplantation
NODAT …….	New Onset Diabetes After Transplantation
hsCRP ……	high sensitivity C-Reactive Protein
eGFR …….	Estimated Glomerular Filtration Rate
HADS ……	Hospital Anxiety and Depression Scale
PSQI ……..	Pittsburgh Sleep Quality Index

CHAPTER 6

QoL ……...	Quality of Life
KTRs …….	Kidney Transplant Recipients
NT-proBNP ...	N-Terminal fragment of Pro-hormone B-Type Natriuretic Peptide
ESRD …….	End Stage Renal Disease
BMI ……...	Body Mass Index
CKD ……..	Chronic Kidney Disease
DASH ……	Dietary Approach to Stop Hypertension
eGFR …….	Estimated Glomerular Filtration Rate
NODAT …….	New Onset Diabetes After Transplantation

Conference Presentations by the Candidate

Invited Oral Presentation:

Chan W (2012) *Assessing Nutritional Status in Kidney Transplant Recipients*, The British Renal Society Annual Conference, Manchester, UK.

Oral Presentation with Published Abstract:

Chan W, Ward D, Kaur O, Tselepis C, Jones D, Bosch J, Borrows R (2012) *Is Obesity Associated with Anaemia in Kidney Transplant Recipients?*, 16th International Congress on Nutrition and Metabolism in Renal Disease, 1st World Renal Nutrition Week, Hawaii, USA.

Published Abstract: Chan W, Ward D, Kaur O, Tselepis C, Jones D, Bosch J, Borrows R. Is Obesity Associated with Anaemia in Kidney Transplant Recipients? Kidney Research and Clinical Practice. 2012; 31(2): A22.

Oral & Poster Presentations with Published Abstract:

Chan W, Ward D, Kaur O, Tselepis C, Jones D, Bosch J, Borrows R (2012) *Anaemia in Renal Transplant Recipients: Is There a Role for Hepcidin?*, 24th International Congress of The Transplantation Society, Berlin, Germany.

Published Abstract: Chan W, Ward D, Kaur O, Tselepis C, Jones D, Bosch J, Borrows R. Anaemia in Renal Transplant Recipients: Is There a Role for Hepcidin?: 2036. Transplantation. 2012; 94(10S): 833.

Oral Presentation:

Chan W, Phillips A, Jones D, Kaur O, McTernan P, Inston N, Moore S, McClean A, Harper L, Borrows R, Bosch J (2014) *Inflammation is an Independent Predictor of Depressive Symptoms and Fatigue in Stable Kidney Transplant Recipients*, 13th International Congress of Behavioral Medicine, Groningen, The Netherlands.

Oral Presentation:

Chan W, Bosch J, Jones D, McTernan P, Inston N, Moore S, Kaur O, Phillips A, Borrows R (2014) *Hypervolemia and Blood Pressure in Prevalent Kidney Transplant Recipients*, 17th International Congress on Nutrition and Metabolism in Renal Disease, Wurzburg, Germany.

Oral Presentation:

Chan W, Jones D, Bosch J, McPhee, Crabtree N, McTernan P, Kaur O, Inston N, Moore S, McClean A, Harper L, Phillips A, Borrows R (2014) *The Mechanisms of Physical Fatigue in Prevalent Kidney Transplant Recipients*, Joint Conference of The British Renal Society & The Renal Association (UK Kidney Week 2014), Glasgow, UK.

Oral Presentation:

Chan W, Phillips A, McTernan P, Jones D, Kaur O, Inston N, Moore S, McClean A, Harper L, Borrows R, Bosch J (2014) *Inflammatory Activity is an Independent Predictor of Depression and Fatigue in Stable Kidney Transplant Recipients*, Joint Conference of The British Renal Society & The Renal Association (UK Kidney Week 2014), Glasgow, UK.

Oral Presentation:

Chan W, Bosch J, Jones D, McTernan P, Inston N, Moore S, Kaur O, Phillips A, Borrows R (2014) *Hypervolemia and Blood Pressure in Prevalent Kidney Transplant Recipients*, Joint Conference of The British Renal Society & The Renal Association (UK Kidney Week 2014), Glasgow, UK.

Poster Presentation with Best Poster Prize:

Chan W, Kaur O, Phillips A, Borrows R (2013) *Nutritional Status, Body Composition & Energy Expenditure in Kidney Transplant Recipients*, The British Renal Society Annual Conference, Manchester, UK.

Late Breaking Poster Presentation:

Chan W, Jones D, Crabtree N, Bosch J, Phillips A, Kaur O, Inston N, Moore S, McClean A, Harper L, Borrows R (2013) *Fatigue in Kidney Transplant Recipients: Is It Psychology or Physiology?*, 37th Congress of The International Union of Physiological Sciences, Birmingham, UK.

Poster Presentation with Published Abstract:

Chan W, Jones D, Phillips A, Bosch J, Okdeep K, McClean A, Harper L, Borrows R (2014) *Cardiorespiratory Fitness in Kidney Transplant Recipients*, World Transplant Congress 2014, San Francisco, California, USA.

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Published Abstract: Chan W, Jones D, Bosch JA, Crabtree N, Kaur O, McClean A, Harper L, Phillips AC, Borrows R. The Mechanisms of Physical Fatigue in Kidney Transplant Recipients. *American Journal of Transplantation*. 2014; 14(S3): 841.

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Poster Presentation:

Chan W, Kaur O, Phillips A, Borrows R (2014) *Nutritional Status, Body Composition, Energy Intake and Energy Expenditure in Kidney Transplant Recipients*, 17th International Congress on Nutrition and Metabolism in Renal Disease, Wurzburg, Germany.

Poster Presentation:

Chan W, Jones D, Bosch J, McTernan P, Inston N, Moore S, Kaur O, McClean A, Harper L, Phillips A, Borrows R (2014) *The Prevalence, Predictors & Impact of Reduced Cardiorespiratory Fitness in Kidney Transplant Recipients*, Joint Conference of The British Renal Society & The Renal Association (UK Kidney Week 2014), Glasgow, UK.

Poster Presentation:

Chan W, Kaur O, Phillips A, Borrows R (2013) *Fluid Status and Predictors of Fluid Overload in Kidney Transplantation*, The British Renal Society Annual Conferences, Manchester, UK.

Poster Presentation:

Chan W, Ward D, Kaur O, Tselepis C, Jones D, Bosch J, Borrows R (2012) *Anaemia in Renal Transplant Recipients: Is There a Role for Hepcidin?*, The Renal Association Conference, Newcastle, UK.

Poster Presentation:

Chan W, Jones D, Bosch J, Borrows R (2012) *Evaluation of Nutritional Risk Profile in Kidney Transplant Recipients: Going Beyond BMI*, The British Renal Society Annual Conference, Manchester, UK.

Other Oral and Poster Presentations during Candidacy

Invited Oral Presentation:

Chan W, Bosch JA, Jones D, Kaur O, Inston N, Moore S, McClean A, McTernan PG, Harper L, Phillips AC, Borrows R (2013) *Cardiovascular Fitness, Perceived Exertion, Muscle Function & Lean Tissue Mass in Relation to Fatigue in Kidney Transplant Recipients,* Queen Elizabeth Hospital Birmingham Transplantation Research Showcase, Birmingham, UK.

Invited Oral Presentation:

Chan W (2010) *Kidney Transplantation: Dietitians' Perspective*, Annual Meeting of West Midlands Renal Nutrition Group, Birmingham, UK.

Invited Oral Presentation:

Chan W (2010) *Lanthanum Carbonate is an Effective Hypophosphataemic Agent for Haemodialysis Patients Intolerant of Other Phosphate Binders*, Annual Meeting of East Anglia Renal Nutrition Group, Cambridge, UK.

Oral Presentation:

Chand S, Shabir S, Chan W, Briggs D, McCaughan J, McKnight AJ, Maxwell AP, Borrows R (2014) *Candidate Gene SNP Association Could Offer New Insights in Post Renal Transplant Hyperglycaemia Development*, Joint Conference of The British Renal Society & The Renal Association (UK Kidney Week 2014), Glasgow, UK.

Poster Presentation:

Chand S, Shabir S, Chan W, McCaughan J, McKnight AJ, Maxwell AP, Borrows R (2014) *β Cell Glucotoxic-associated SNPs in impaired Glucose Tolerance and NODAT*, Joint Conference of The British Renal Society & The Renal Association (UK Kidney Week 2014), Glasgow, UK.

Poster Presentation:

Chand S, Shabir S, Chan W, Cramb R, Borrows R (2014) *Total 25-Hydroxyvitamin D Levels and Post Transplant Hyperglycaemia,* Joint Conference of The British Renal Society & The Renal Association (UK Kidney Week 2014), Glasgow, UK.

Poster Presentation:

Chan W and Borrows R (2011) *Serial Assessment of Nutritional and Inflammatory Status in Kidney Transplant Recipients*, Queen Elizabeth Hospital Birmingham Annual Research Showcase, Birmingham, UK.

Poster Presentation:

Chan W & Borrows R (2010) *Serial Assessment of Nutritional and Inflammatory Status in Kidney Transplant Recipients*, West Midlands Strategic Health Authority Nursing, Midwifery and Allied Health Professionals Research Training Award Ceremony, Birmingham, UK.

List of Publications by the Candidate

Published Journal Articles

Contributing to Chapter 1:

Chan W, Bosch JA, Jones D, McTernan PG, Phillips AC, Borrows R. Obesity in Kidney Transplantation. *Journal of Renal Nutrition: The Official Journal of the Council on Renal Nutrition of the National Kidney Foundation.* 2014; 24(1): 1-12.

Chapter 2:

Chan W, Ward DG, McClean A, Bosch JA, Jones D, Kaur O, Drayson M, Whitelegg A, Iqbal T, McTernan PG, Tselepis C, Borrows R. The Role of Hepcidin-25 in Kidney Transplantation. *Transplantation.* 2013; 95(11): 1390-5.

Chapter 3:

Chan W, Bosch JA, Jones D, McTernan PG, Inston N, Moore S, Kaur O, Phillips AC, Borrows R. Hypervolemia and Blood Pressure in Prevalent Kidney Transplant Recipients. *Transplantation.* 2014; 98(3): 320-7.

Chapter 4:

Chan W, Bosch JA, Jones D, Kaur O, Inston N, Moore S, McClean A, McTernan PG, Harper L, Phillips AC, Borrows R. Predictors and Consequences of Fatigue in Prevalent Kidney Transplant Recipients. *Transplantation.* 2013; 96(11): 987-94.

Journal Articles undergoing Peer Review

Chapter 5:

Chan W, Bosch JA, Jones D, McTernan PG, Inston N, Moore S, Kaur O, Phillips AC, Borrows R. Cardiovascular, Muscular and Perceptual Contributions to Physical Fatigue in Prevalent Kidney Transplant Recipients. [Undergoing Peer Review by *Kidney International*]

Other Journal Articles undergoing Peer Review

Chan W, Bosch JA, Jones D, Kaur O, Inston N, Moore S, McClean A, McTernan PG, Harper L, Phillips AC, Borrows R. Cardiorespiratory Fitness in Kidney Transplant Recipients. [Undergoing Peer Review by *American Journal of Kidney Disease*]

Other Published Journal Articles

Chan WLW, Rounsley K, Chapman E, Collings K, Dale C, De Waal S, Patel V, Tanner J, Turner E, Moore J, Borrows R. Lanthanum Carbonate Is an Effective Hypophosphatemic Agent for Hemodialysis Patients Intolerant of Other Phosphate Binders. *Journal of Renal Nutrition: The Official Journal of the Council on Renal Nutrition of the National Kidney Foundation.* 2010; 20(4): 270-7.

Moore J, Shabir S, Chand S, Bentall A, McClean A, Chan W, Jham S, Benavente D, Sharif A, Ball S, Cockwell P, Borrows R. Assessing and Comparing Rival Definitions of Delayed Renal Allograft Function for Predicting Subsequent Graft Failure. *Transplantation.* 2010; 90(10): 1113-6.

Grants, Awards, Prizes & Bursaries awarded to the Candidate

2014

Renal Nutrition Group of The British Dietetic Association Research Bursary for Conference Presentation

2013

British Renal Society Best Poster Prize

2009

West Midlands Strategic Health Authority PhD Research Training Fellowship

2008

British Renal Society Research Grant

External Academic Engagement during Candidacy

2014

Judge of The UK National Science and Engineering Competition

2014 – Present

Editorial Board Member of International Journal of Nutrition

2013 – Present

Postgraduate Education Lead of The Department of Nutrition & Dietetics at The Queen Elizabeth Hospital Birmingham

2011 – Present

Member of Kidney Research UK Research Grant External Referee Panel

2012 – 2014

Journal Reviewer for Kidney International; Journal of Renal Nutrition; Patient Preference and Adherence; International Journal of Nephrology and Renovascular Disease; Peritoneal Dialysis International

2012 – 2013

Member of UK Expert Reviewing Panel for Practice-based Evidence in Nutrition (PEN) Knowledge Pathway in "Nutrition in Kidney Transplantation", International Collaboration between UK, Canada, Australia and New Zealand

2012 – 2013

Subjective Global Assessment Trainer for The European QUALity "EQUAL" Study: A European Pilot Study on When to Start Dialysis in the Elderly

GENERAL INTRODUCTION

Chapter 1

Effects of Body Composition on Clinical and Quality of Life Outcomes in Kidney Transplant Recipients

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Chan W, Bosch JA, Jones D, McTernan PG, Phillips AC, Borrows R. Obesity in Kidney Transplantation. *Journal of Renal Nutrition*: The Official Journal of the Council on Renal Nutrition of the National Kidney Foundation. 2014; 24(1): 1-12.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Body Composition

Body composition describes the quantities of adipose, skeletal and muscle tissues, as well as the amount of fluid in human body[1,2]. Different body composition parameters, specifically the amount of muscle mass, the quantity and distribution of fat mass, and fluid volume status are now increasingly considered valuable in clinical practice to advance our understanding of their effects on health and quality of life (QoL) outcomes[1,3-10].

Body composition can be assessed using several different methods. The most common assessment techniques in clinical practice are anthropometry, including weight, height, body mass index (BMI), waist circumference, waist-hip ratio, skinfold thickness, arm muscle measurements, and bio-impedance analysis. These techniques are inexpensive, safe, non-invasive, with minimal requirement for training and subject compliance. More sophisticated techniques are also available, mostly used in research settings, examples include near-infrared interactance, dual-energy x-ray absorptiometry (DEXA), densitometry (underwater weighing and air displacement plethysmography), isotope dilution, magnetic resonance imaging, computed tomography imaging, and whole body potassium scanning. These techniques are costly, cumbersome, time-consuming, require specific equipment and expertise knowledge.

The purpose of body composition assessment is to compartmentalise the body's constituents. BMI is frequently used in clinical practice and is often considered as a surrogate measure of adiposity due to its positive correlation with body fat[1]. However, it only provides a simple estimation of fat mass based on weight and height squared, lacking sensitivity and specificity to dissect the differences between varying compartments of body composition (fat mass, muscle mass and fluid volume)[1]. Therefore, it does not truly reflect adiposity as excess body weight may be attributable to increased muscle mass and/or volume overload[11]. In addition, it may be affected by gender, age, genetics, activity level, and ethnicity[12].

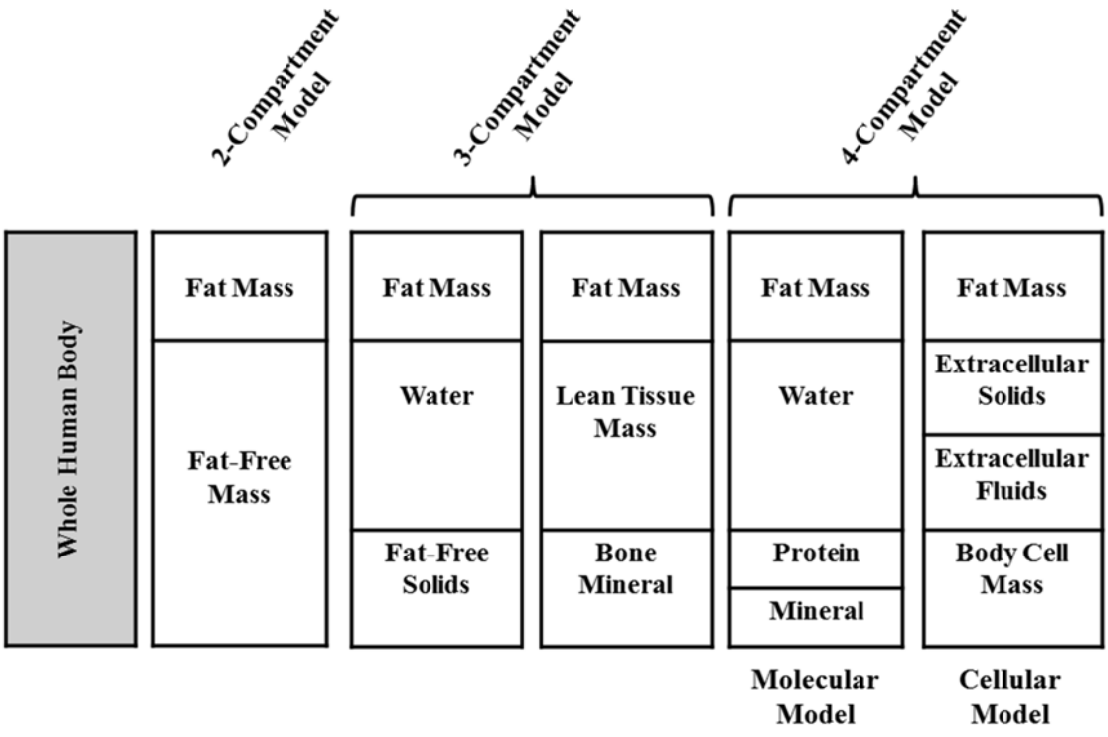
Historically, cadaver analysis is considered to be the gold standard for body composition assessment[1]. Although it is a direct measurement and provides fundamental understanding of body composition, it is the most invasive method involving human dissection[13]. A major drawback to this analysis is the requirement of deceased subjects[13], limiting its practicality in clinical practice. However, cadaver analyses contributed to reference data for developing body composition models[13,14], separating body weight into two or more compartments[13].

1.1.1 The Two-Compartment Body Composition Model

The two-compartment (2-C) model is the basic body composition model, consisting of fat mass and fat-free mass[15], shown in **Figure 1.1**. While fat mass includes all ether-extractable lipids in the body[16], fat-free mass is somewhat heterogeneous, comprising of all

non-fat components such as total body water, protein and bone minerals[15]. The 2-C model measures fat-free mass, then fat mass is derived by subtracting fat-free mass from total body weight. This model assumes known and constant proportions of fat-free mass as water, protein and mineral[17,18]. However, due to inter- and intra- individual variations in these components, especially in pathological states[15,19] e.g. chronic kidney disease (CKD), this model may be inaccurate[17]. Examples of body composition measurements using 2-C model include skinfold thickness, arm muscle measurements, near-infrared interactance, fixed-frequency and non-phase sensitive bio-impedance analysis, underwater weighing, and air-displacement plethysmography.

Figure 1.1. Body Composition Models, adapted from Ellis (2000)[13]



1.1.2 The Three-Compartment Body Composition Model

The three-compartment (3-C) model is an evolution of the 2-C model (shown in **Figure 1.1**). There are two common types of 3-C model. Fat-free mass may be further divided into either:1) water and remaining fat-free solids (i.e. protein and bone mineral)[13]; or 2) bone mineral and lean tissue mass[20]. The 3-C model measures body water and/or solids, with fat mass estimated by subtracting these from the total body weight. The 3-C model assumes constant densities of fat, body solids and hydration[13]. However, when patients present with substantially depleted protein and bone mineral masses e.g. CKD patients[21,22], the assumed density of the bodily solids may be inaccurate, which may invalidate the estimation of fat mass. DEXA scanning and phase sensitive multi-frequency bio-impedance analysis are examples of body composition measurement techniques using the 3-C model.

1.1.3 The Four-Compartment Body Composition Model

The 3-C model may be further extended to the four-compartment (4-C) model. The 4-C or multi-compartment models estimate body composition by combining independent measurements of different body composition compartments with minimal assumptions. There are two common types of 4-C model: molecular and cellular compartment models[13], shown in **Figure 1.1**. In the molecular model, fat-free mass is divided into total body water, protein and bone mineral masses[13]. The cellular model separates fat-free mass into extracellular solids, extracellular fluids, and body cell mass[13]. Both extracellular solids

and extracellular fluids are constituents of fat-free mass outside cells. While extracellular solids represents bone mineral mass, extracellular fluids includes interstitial fluid and blood plasma[13]. Body cell mass consists of skeletal muscle protein and visceral protein[23], both of which are metabolically active[19,23], hence reflecting nutritional status and signs of wasting[19]. The cellular model is considered of greater value in CKD patients due to its unique ability to measure extracellular fluids and body cell mass[19]. In both molecular and cellular compartment models, the different compartments of the fat-free mass are measured, with fat mass predicted by total body weight minus the sum of all the measured fat-free mass compartments. The 4-C model is regarded as the most accurate measure of body composition, and is often used as a reference technique for development of new body composition methods[17]. However, the techniques required for this model are technically challenging, costly, and not readily available in clinical settings. Examples of measurement modalities for each of the 4-C model compartments include deuterium dilution (measurement of total body water); bromide (measurement of extracellular fluids); total body calcium or bone mineral content (measurement of extracellular solids); total body potassium (measurement of body cell mass); and DEXA scanning (measurement of bone mineral component).

1.1.4 Characteristics of Body Composition in Kidney Transplant Recipients

A greater understanding of body composition in kidney transplant recipients (KTRs) may provide insight into its relationships with post-transplant complications, long-term morbidity, mortality, and QoL outcomes. It may also form the basis for future

interventional strategies aiming to improve clinical and QoL outcomes of kidney transplantation. To date, there are no universally recommended methods for body composition assessment in KTRs. Studies investigating the characteristics of body composition in KTRs employed a variety of techniques to ascertain body composition in this patient population. A summary of studies reporting body composition in KTRs are shown in **Table 1.1**.

Table 1.1. Summary of Studies Reporting Body Composition in Kidney Transplant Recipients

Study	Sample Size	Study Type	Body Composition Measurements	Study Protocol	Key Findings in Relation to Body Composition Post-Transplantation
Haggan et al[24]	44	Prospective	▪ Weight ▪ DEXA	Anthropometric measurements and body composition at kidney transplantation and 3, 6 and 12 months post-transplantation.	▪ Significant weight gain in female from baseline to 12 months post-transplantation; but no significant weight change in male during the course of follow-up. ▪ Total fat and lean body masses increased significantly in female from baseline to 12 months post-transplantation. ▪ In male, total fat mass decreased significantly from baseline to 12 months post-transplantation; but no significant change in total lean body mass. ▪ Bone mass did not change significantly in female, but decreased significantly from baseline to 12 months post-transplantation in male. ▪ Weight gain associated with female gender. ▪ Increased fat mass correlated with high energy intake. ▪ Increased lean body mass associated with reduced steroid dose. ▪ Bone loss associated with male gender and high doses of steroids.
Heaf et al[25]	115	Prospective	▪ Weight ▪ BMI ▪ DEXA	Anthropometric and DEXA measurements at baseline and repeated 3 years later.	▪ High prevalence of overweight and obesity observed in KTRs, with 39% and 14% of KTRs being overweight and obese respectively. ▪ No significant change in weight, BMI, lean body mass and bone weight. ▪ Significant increase in fat mass. ▪ Increase in fat mass associated with low fat mass at baseline, high plasma bicarbonate, and shorter time post-transplantation. ▪ Increase in lean body mass associated with high fat mass at baseline, low lean body mass at baseline, reduced intakes of energy and protein, and increase in plasma bicarbonate.

Table 1.1. Summary of Studies Reporting Body Composition in Kidney Transplant Recipients (continued)

Study	Sample Size	Study Type	Body Composition Measurements	Study Protocol	Key Findings in Relation to Body Composition Post-Transplantation
Moreau et al[26]	44	Prospective	▪ DEXA	DEXA measurements pre-transplantation, and followed-up at 1, 2 and 5 years post-transplantation.	▪ In female, significant increase in body weight was observed from baseline to 5 years post-transplantation, due to significant increase in fat mass at 1 and 2 years post-transplantation. ▪ In male, body composition remained stable and closed to baseline measurements during the course of follow-up period. ▪ KTRs on lower corticosteroid doses developed normalised bone mass over the 5 years follow-up period, whereas KTRs on higher doses of corticosteroid developed a decreased bone mass during the first year post-transplantation, and then improved significantly thereafter back to baseline by 2 years post-transplantation.. ▪ Increase in bone mass from 1 to 5 years post-transplantation was only significant in KTRs treated with tacrolimus.
Dolgos et al[27]	102	Prospective	▪ DEXA	DEXA measurements at transplantation, and 10 weeks post-transplantation.	▪ Fat mass increased significantly from baseline to 10 weeks post-transplantation. ▪ Fat-free mass declined significantly from baseline to 10 weeks post-transplantation, with no significant change in body weight. ▪ Independent predictors of increased fat mass were advancing age, low baseline fat mass, elevated C-reactive protein, increasing dialysis vintage and high cumulative steroid dose. ▪ High cumulative steroid dose was the only independent predictor of declined fat-free mass.

Table 1.1. Summary of Studies Reporting Body Composition in Kidney Transplant Recipients (continued)

Study	Sample Size	Study Type	Body Composition Measurements	Study Protocol	Key Findings in Relation to Body Composition Post-Transplantation
Harada et al[28]	55	Prospective	▪ BMI ▪ Bio-impedance spectroscopy	BMI and bio-impedance before and 1 year after kidney transplantation.	▪ No significant change in BMI. ▪ Total body water, percentage body muscle, and bone mass decreased significantly. ▪ Percentage body fat increased significantly.
Netto et al[29]	145	Prospective	▪ BMI ▪ Skinfold thickness ▪ Arm circumference ▪ Arm muscle circumference ▪ Arm muscle area ▪ Percentage body fat	BMI and anthropometry immediately after transplantation, and followed-up at 6 months post-transplantation.	▪ No significant change in BMI. ▪ Female displayed significantly higher muscle mass at baseline compared to male, measured by arm circumference, arm muscle circumference, and arm muscle area. ▪ Percentage body fat at baseline was above the recommended levels in 80% of KTRs. ▪ At 6 months post-transplantation, higher renal function was observed among normal weight compared to overweight and obese KTRs, despite comparable estimated glomerular filtration rate at baseline.
Van den Ham et al[30]	11	Prospective	▪ DEXA	DEXA measurements at 3-4 weeks, 3- and 6- months post-transplantation.	▪ At 6 months post- transplantation, weight gain is predominately due to an increase in fat mass. ▪ Elevated fat mass was evident within 3 months post-transplantation, this include extremity and truncal fat mass, with truncal region being the most prominent.

Table 1.1. Summary of Studies Reporting Body Composition in Kidney Transplant Recipients (continued)

Study	Sample Size	Study Type	Body Composition Measurements	Study Protocol	Key Findings in Relation to Body Composition Post-Transplantation
Miller et al[31]	45	Retrospective	▪ Weight ▪ Height ▪ Triceps skinfold thickness ▪ Mid-arm muscle circumference	Anthropometry pre- and post- transplantation, with a mean post-transplantation follow-up time of 23 months.	▪ Weight and weight for height increased significantly from pre- to post- transplantation. ▪ Post-transplantation, 38% of KTRs had mid-arm muscle circumference below the 5th percentile, and 58% had mid-arm muscle circumference above the 50% percentile. ▪ Following transplantation, 14% of KTRs had triceps skinfold thickness above the 95th percentile.
Qureshi et al[32]	30	Cross-sectional	▪ Skinfold measurements ▪ Percutaneous muscle biopsy	Post-transplant anthropometry and muscle biopsy measurements and comparisons with age-match healthy subjects.	▪ KTRs displayed higher percentage of body fat, triceps, subscapular, and total sum of skinfolds compared with healthy controls at 13 months post-transplantation. ▪ Significant protein depletion at the cellular level in KTRs compared with healthy subjects at 14 days post-transplantation.
Isiklar et al[33]	15	Prospective	▪ DEXA	DEXA measurements immediately prior to transplantation, and repeated at 3 and 6 months post-transplantation.	▪ Fat mass increased following transplantation, and was more prominent at 3 months post-transplantation. ▪ Percentage body fat of the total body weight increased following transplantation. ▪ Decreased lean body mass post-transplantation was observed in 6 patients.

Table 1.1. Summary of Studies Reporting Body Composition in Kidney Transplant Recipients (continued)

Study	Sample Size	Study Type	Body Composition Measurements	Study Protocol	Key Findings in Relation to Body Composition Post-Transplantation
Steiger et al[34]	16	Prospective	▪ DEXA	DEXA measurements immediately post-transplantation, and followed-up at 2, 5, 11 and 16 months after transplantation. Results were compared with age-, sex- and BMI- matched healthy controls.	▪ Compared with healthy controls immediately post-transplantation, lean mass of the trunk was higher in KTRs, lean mass of the limb was lower in KTRs, and no difference in fat mass was observed. ▪ Compared between baseline and 16 months post-transplantation, total fat mass increased in male KTRs including all sub-regions such as trunk, arms, legs, head and neck. ▪ Compared between baseline and 16 months post-transplantation, total fat mass remained unchanged in female KTRs, but head and neck fat mass was higher than healthy controls. ▪ Body fat distribution remained constant in both sexes during the course of 16 months. ▪ Lean mass of the trunk in KTRs decreased during early stages post-transplantation (between 11 and 42 days post-transplantation) and remained constant thereafter. ▪ Following the early stages post-transplantation (after 42 days post-transplantation), lean mass of the arms, legs, head and neck in KTRs increased over the observed follow-up period.

1.1.5 Techniques of Body Composition Assessment in this Thesis

In the research studies to be discussed in this thesis (chapters 2 to 5), techniques from the 3-C model, DEXA scanning and the multi-frequency bio-impedance analysis, were used to measure body composition. Both methodologies serve as the best alternatives in terms of cost, convenience, equipment availability, skill requirement and quality of data.

1.1.5.1 DEXA Scanning

DEXA is a recognised reference method for evaluation of body composition[35]. The system works by transmitting low-dose X-rays at two different energy levels through the individual, and measure the differential attenuation of the X-ray beam at these two energy levels to derive whole body and regional bone mineral content, fat mass and lean tissue mass[36]. In the regions with bone, soft tissue and bone were measured, and the composition of soft tissue was evaluated in relation to the adjoining tissue estimates[36]. In the regions without bone, the transmission at the two energy levels estimates fat mass and lean tissue mass[36].

1.1.5.2 Bio-impedance Analysis

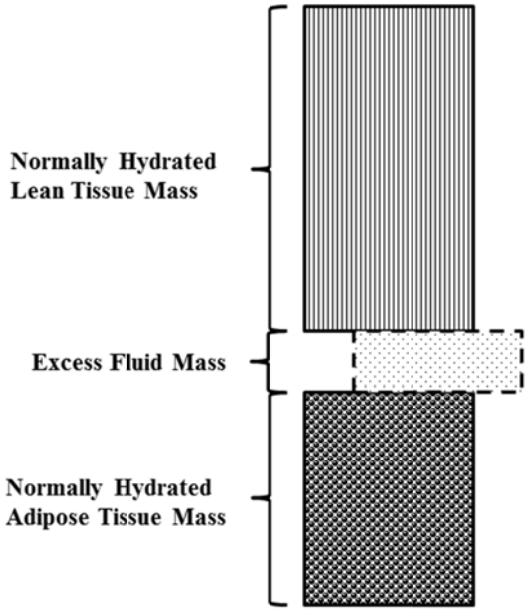
The bio-impedance analysis employed in the present thesis is a relatively new device known as the Body Composition Monitor (BCM, Fresenius Medical Care, Bad Homburg,

Germany). It is a multi-frequency whole-body bio-impedance spectroscopy device that measures adipose tissue mass, lean tissue mass and volume status at 50 frequencies (5 – 1,000 kHz) through electrodes placed on the wrist and ankle[37]. Impedance is a measure of the vector sum of resistance and reactance, and is associated with the length, cross-sectional area, and applied frequency of the conductor[38]. It makes use of the principle that body tissues are capable of conducting low-level alternating electrical current with varying ease, proportional to their water and electrolyte content[39]. Lean tissue mass, contains large amounts of water and electrolytes, is therefore highly conductive and presents low resistance electrical paths[39]. Fat mass is anhydrous, a poor conductor of electricity, and hence offers high resistance electrical pathway[39]. Cell membranes pose reactance to electrical current, opposing the flow of electrical current due to the presence of electrical capacitance found in cell membranes[38]. Since fluid compartments (intra- and extra-cellular fluids) are separated by cell membranes[38], high frequency current passes through the total body water, but low-frequency current is incapable to penetrate through cell membranes and therefore flows exclusively through the extracellular water[39].

The BCM utilises an algorithm based on a new 3-C model[40], shown in **Figure 1.2**. It was modified to reflect the presence of excess fluid accumulated due to pathological reasons[40]. The new 3-C model considered the 3 compartments as normally hydrated adipose tissue mass, normally hydrated lean tissue mass, and excess fluid mass[40]. The hydration fractions within both adipose tissue mass and lean tissue mass were assumed to have zero excess fluid[40]. Therefore, absolute excess fluid mass was determined by calculating the difference between the actual amount of excess fluid in the body detected by the BCM and the

expected amount of excess fluid predicted by the BCM under normal physiological conditions (i.e. euvolemia)[41,42].

Figure 1.2. The New 3-Compartment Body Composition Model, adapted from Chamney et al (2007)[40].



The BCM has not been used extensively in kidney transplant population, and thus far, only one study attempted to compare the utility of this device with DEXA in KTRs[35]. In general, it was found that lean tissue mass and fat mass in KTRs measured by the BCM demonstrated good correlations with DEXA[35]. However, due to the high variability within these associations, its validity for assessing body composition individually remains to be determined[35]. Nevertheless, the body composition monitor may be useful in large-scale studies[35]. Further, the device has been used in dialysis patients extensively[41], and has been validated against reference methods for volume status and body composition assessments

in healthy and haemodialysis populations[43], with methods frequently used in the 4-C model such as bromide dilution, total body potassium, deuterium dilution, DEXA scanning, air displacement plethysmography, and under-water weighing.

1.2 Kidney Transplantation

Kidney transplantation is a surgery to place a healthy kidney into a patient with end-stage renal failure. It may be obtained from a deceased-donor or a living-donor. The latter, in turn, is further classified as living-related or living-unrelated transplants, depending on the existence of a biological relationship between the donor and the recipient.

1.2.1 History and Benefits of Successful Kidney Transplantation

Sixty years ago, on 23rd December 1954, the first successful kidney transplantation was performed at the Peter Bent Brigham Hospital in Boston, USA[44]. It was a living-related donation that occurred between identical twins[44]. Since then, this operation has moved from a medical miracle to part of a routine clinical practice[44]. Kidney transplantation is now considered as the preferred modality of renal replacement therapy for many patients with end-stage renal disease[45]. Compared to remaining on the transplant waiting list, kidney transplantation improves long-term survival[46], enhances QoL[47], demonstrates cost benefit[48], and rectifies uraemia and metabolic abnormalities contributing to an overall sense of well-being[49]. In spite of these advantages, the shortage of organs is corroborated

by a small proportion of patients on the transplant waiting list proceeding to transplantation, with up to 40% dying while waiting on dialysis[50]. It is crucial that the best possible use is made of those transplanted kidneys (grafts); therefore, optimising patient and graft survival after transplantation is a priority in this patient group.

1.3 Barriers to Successful Clinical Outcomes of Kidney Transplantation

Clinical outcomes of kidney transplantation are often defined as post-transplant morbidity and mortality. The most common clinical outcome measures in kidney transplantation are short- and long- term patient- and graft survival. Other examples include hospitalisation rates, hospital re-admission rates, length of hospitalisation, delayed graft function (defined as dialysis requirement within the first week post-transplantation), primary non-function (defined as non-functioning graft within the first 6 weeks following transplantation), and biopsy proven acute rejection[51].

Short-term patient and graft survival have substantially improved over the recent decades, with most centres reporting both survival rates at 1 year of greater than 90%[52]. However, long-term success has been difficult to accomplish as evident by the marginal increase in patient and graft survival rates over the past 15 years[53]. This phenomenon is multifactorial. Firstly, modification of alloimmunity using immunosuppression has reduced short-term early acute rejection rates[53], but the nephrotoxicity of immunosuppressive medication is known to limit long-term graft survival[54]. Secondly, the use of maintenance immunosuppressive therapy is associated with increased risk of infection and

malignancy[55]. Thirdly, cardiovascular disease (CVD) is a well-known long-term complication in KTRs[56]; it is the leading cause of death after transplantation and death with a functioning graft[57].

In order to improve long-term patient- and graft- survival, kidney transplantation research today largely focuses on immunosuppression, immunology of transplantation, and evaluating the risk factors and their contributions to CVD in this population. Several other non-immunological aspects of kidney transplantation, which potentially contribute to adverse long-term outcome of KTRs, have not been fully investigated. This thesis sought to explore different compartments of body composition as a non-immunological factor. A greater understanding of body composition is the prerequisite for developing interventional strategies, aiming to improve long-term patient- and graft survival.

1.3.1 Effects of Body Composition on Clinical Outcomes of Kidney Transplantation

Adiposity, a potential risk factor for CVD[58], has been extensively researched in the field of kidney transplantation. However, majority of the studies investigating the impact of adiposity have used BMI solely to estimate body composition.

1.3.1.1 Effects of Pre-Transplant Body Composition on Clinical Outcomes of Kidney Transplantation

Overweight and obesity are common at the time of transplantation[59]. Currently, 60% of KTRs are overweight at the time of transplantation[59], representing a 116% increase from 1987[59]. With the use of BMI, there is a consensus that pre-transplant obesity predisposes to delayed graft function[52,60-66], which in turn is associated with reduced long-term graft survival[52,60,67]. Also, it unanimously demonstrates a negative effect on post-transplant surgical outcomes[61-66]. However, the effects of pre-transplant obesity on long-term patient- and graft- survival have yielded conflicting conclusions[5,52,61-66,68-72]. Such discrepancies may partly stem from large variations in sample size, definition of "long-term"[73], and follow-up period (2-20 years). The inconsistent results may also reflect the limitation of BMI to distinguish between fat mass, lean muscle mass and volume status.

Specifically, only one study to date examined the impact of pre-transplant muscle mass together with BMI on mortality in KTRs[5]. Streja et al found that pre-transplant obesity, defined as higher pre-transplant BMI values above the reference range of 22 to <25 kg/m^2, did not confer an increased risk of long-term graft loss and mortality[5]. In contrast, sarcopenic obesity, defined as reduced estimated pre-transplant muscle mass represented by lower pre-transplant serum creatinine in patients with BMI ≥ 30 kg/m^2, was associated with increased mortality and graft failure[5]. These findings suggest that pre-transplant obesity is not associated with inferior post-transplant outcomes, but increased muscle mass is associated with patient- and graft- survival benefits[5]. However, it is important to note

that the use of BMI and serum creatinine as surrogates for body composition may be subject to limitations[5]. Indeed, a recent editorial highlighted the need to define accurate and practical measures of body composition that predict clinical outcomes of kidney transplantation[74].

1.3.1.2 Effects of Post-Transplant Body Composition on Clinical Outcomes of Kidney Transplantation

Weight gain after transplantation is very common and occurs in up to 50% of KTRs[75,76], affecting obese and non-obese patients[77]. The average weight gain after transplantation is between 10% and 35% of body weight, with most weight gain within the first 12 months after transplantation[75,78-80]. The characteristics of body composition in KTRs are summarised in **Table 1.1**. In essence, it appears that much of the weight gain post-transplant is attributed to an increase in fat mass, especially in the abdominal area[30].

Excessive weight gain and increased fat mass in KTRs is traditionally attributed to the immunosuppression treatment protocol post-transplantation. This is due to the well-known hyperphagic effect of steroids and their adverse influence on adipocytes, resting energy expenditure, and lipid oxidation, resulting in centripetal obesity (i.e. increased fat deposition in the peritoneum, mediastinum, and subcutaneous sites such as face and neck)[81]. However, recent evidence suggests that the effect of steroids on weight gain is controversial[75,81-86], weight gain post-transplantation may be largely due to lifting of previous dietary restrictions, improved appetite after the correction of uraemia, and the

enhanced sense of well-being that occurs with transplantation[49]. Other contributing factors for post-transplant weight gain have been identified, including age, gender, ethnicity, pre-transplant BMI, dialysis modality, the occurrence and treatment of rejection, and graft function[75,78,81].

Weight gain and post-transplant obesity are commonly known to be associated with reduced long-term patient- and graft- survival[52]. Although it failed to display an independent relationship with risk of CVD[87-90], post-transplant obesity adversely affects cardiovascular risk profile including hypertension, dyslipidaemia, diabetes mellitus, and insulin resistance, all of which are independently associated with increased risk of graft failure[87-90]. These individual conditions also cluster as part of the metabolic syndrome, which is associated with increased risk of graft failure as revealed by the recent sub-analysis of the Assessment of LEscol in Renal Transplantation (ALERT) study[91].

In contrast, a recent study by Kovesdy et al[6] found that higher BMI was associated with lower mortality after adjustment for waist circumference, whereas higher waist circumference was associated with higher mortality after adjustment for BMI. In addition, clinically obese patients with exclusive subcutaneous fat excess were found in a normal metabolic state and demonstrated a limited deposition of fat at visceral sites[92], conferring reduced metabolic risk. Further, Haggan et al[24] found that increased lean tissue mass during the first year post-transplantation was associated with the absence of delayed graft function and acute rejection. Also, recent data from a renal transplant population showed that lower creatinine excretion, a proxy for reduced muscle mass, was associated with

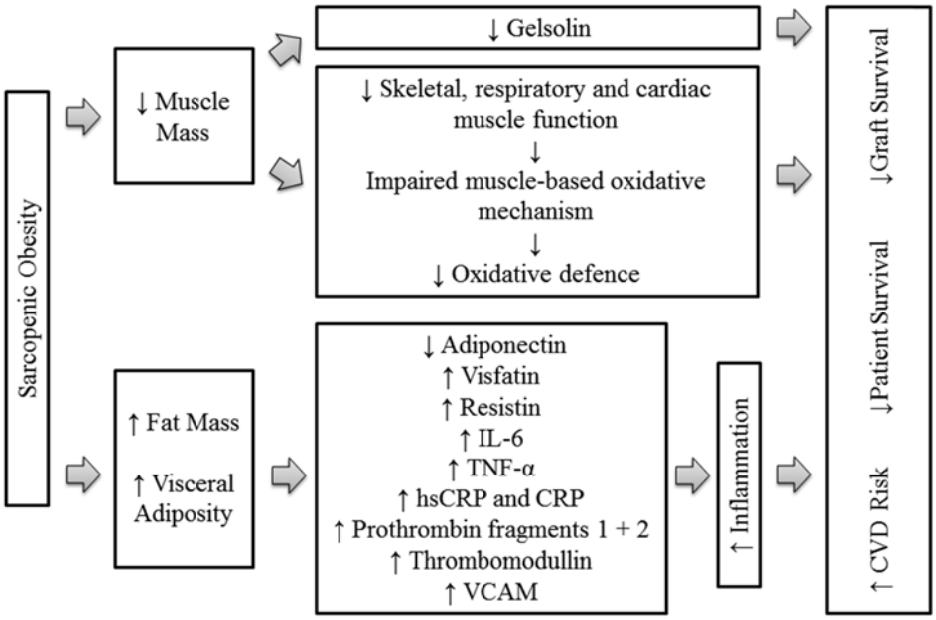
increased mortality and graft failure[7]. These findings suggest that not only does waist circumference appear to be a better prognostic marker for obesity than BMI, visceral adiposity adversely affects kidney transplant outcomes, and increased subcutaneous fat and/or muscle mass may be protective against mortality risk.

1.3.1.3 Potential Mechanisms for Body Composition-Mediated Post-Transplant Clinical Outcomes

A number of mechanisms associating body composition parameters with post-transplant clinical outcomes have been proposed; these are summarised in **Figure 1.3**. In particular, sarcopenic obesity, characterised by reduced muscle mass coupled with increased fat mass, appears to be the driving force behind the adverse events.

One of the survival advantages may derive from the beneficial effects of increased muscle mass, which has been proposed to improve skeletal, respiratory, and cardiac muscle function and consequently improve muscle based-oxidative mechanism, leading to increased antioxidant defense[6]. Correspondingly, these mechanisms may reduce the risk of CVD, cancer, neurodegenerative disorders, and other chronic conditions[93]. Also, gelsolin, produced by the skeletal muscle has been shown to be associated with improved survival in dialysis patients[6]. Although the effect of gelsolin has not been studied in kidney transplantation, the depletion of which may explain higher mortality risk in KTRs.

Figure 1.3. Potential Mechanisms for Body Composition-Mediated Post-Transplant Clinical Outcomes.



Key: IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; hsCRP, high-sensitivity c-reactive protein; CRP, c-reactive protein; VCAM, vascular cell adhesion molecule; CVD, cardiovascular disease.

The negative metabolic effects of elevated adiposity, particularly visceral adiposity, may explain the increased CVD risk and graft failure observed in KTRs. First of all, the ALERT trial showed that raised BMI was associated with inflammation[94]; inflammatory markers including interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hsCRP) were independently and positively associated with major cardiovascular events and all-cause mortality in KTRs[94].

Specifically, visceral adipose tissue is the most metabolically active system that secretes adipokines (cytokines secreted by adipose tissue), and examples include chemerin, IL-6, visfatin, adiponectin, leptin, tumor necrosis factor-alpha (TNF-α), and resistin[95]. The effect of adiponectin remains controversial in KTRs, with Chitalia et al finding no association between adiponectin and CVD risk[96], but Kaisar et al reported that higher adiponectin levels may be protective against the development of CVD, and this relationship may be delineated by the positive association between adiponectin and high-density lipoprotein levels[97]. Nevertheless, hypoadiponectinemia was shown to correlate with inflammation in KTRs, characterised by elevated C-reactive protein (CRP) and hsCRP[97].

Raised levels of visfatin were found to correlate with inflammation and markers of endothelial damage in KTRs, including raised levels of hsCRP, prothrombin fragments 1 and 2, and vascular cell adhesion molecule (VCAM)[98]. Also, raised resistin levels correlated with markers of inflammation and endothelial dysfunction in KTRs, including elevated levels of hsCRP, IL-6, thrombomodulin, and VCAM[99].

The link between inflammation and mortality in KTRs has been previously established by Winkelmayer et al[100]. Inflammation has been proposed to exert its downstream adverse sequelae via the mediation of vascular damage causing vascular inflammation, which in turn leads to pathogenesis of atherosclerosis in KTRs[94].

Another suggested mechanism associated with adiposity is that adipose tissues secrete pro-inflammatory cytokines, including TNF-α and IL-6, which sequentially activate the renin-angiotensin-aldosterone pathway, contributing to the onset and progression of graft damage by sustaining cell growth, inflammation, and fibrosis[52].

In addition, the metabolic load of the graft increases as a result of obesity; the graft consequently adapts by increasing in size and glomerular filtration[52]. The combining effect of fibrosis and glomerulomegaly are the most common histologic lesions in patients with obesity-related glomerulopathy, suggesting that these are important pathways leading to graft failure.

1.4 Barriers to Successful Quality of Life Outcomes of Kidney Transplantation

Whilst improving clinical outcomes of kidney transplantation through maximising patient- and graft- survival are of utmost importance in kidney transplantation, achievement of maximal QoL is also one of the major goals of transplantation[101]. The World Health Organisation defined QoL as "*individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns*"[102]. It includes different health-related aspects from patients' perspective including physical, psychological, social functioning, and overall well-being[101]. Of importance, previous studies have shown that physical health-related QoL predicts long-term mortality and graft failure independently of socio-demographic and clinical risk factors in KTRs[103-105].

Although it is well recognised that QoL improves following successful kidney transplantation compared to remaining on dialysis[106,107], life after transplantation may be subjected to several QoL challenges[104,108,109]. Previous studies have shown that female gender, increasing time post-transplantation, lower education level, reduced functional status, anaemia, comorbidity, immunosuppressive therapy, sleep disorders, fatigue and depression, impair post-transplant QoL[110-112]. Despite this, there is a paucity of research addressing these factors with QoL as the endpoint. The scarce data mainly pertains to minimising physical side effects of immunosuppression by converting immunosuppressive therapy, and reducing symptoms of depression with psychotherapy.

1.4.1 Immunosuppression and Quality of Life in Kidney Transplant Recipients

Modern maintenance immunosuppression therapy has improved long-term patient- and graft- survival, but such treatment protocol has led to medical and physical side effects, compromising QoL[109]. Corticosteroids and calcineurin inhibitors (both tacrolimus and cyclosporine) continued to be the mainstream of contemporary immunosuppression. However, these medications are associated with physical side effects. Corticosteroids commonly trigger weight gain, hirsutism, acne, cushingoid appearance, osteoporosis, and mood disturbance[113]. Calcineurin inhibitors frequently cause hirsutism, gingival hyperplasia, alopecia, and hand tremors[111,114-117]; although substantial differences have been observed between tacrolimus and cyclosporin[111,114-117]. Previous studies have shown that such immunosuppression-related physical side effects exert significant psychological impact on KTRs[111,118], leading to non-adherence to immunosuppression treatment

regimens which may result in acute rejection, graft loss, hospitalisation and mortality[109]. Conversion from cyclosporin to tacrolimus demonstrated positive results in reducing hirsutism and gingival hyperplasia without affecting graft function[119,120], potentially improving QoL.

1.4.2 Depression and Quality of Life in Kidney Transplant Recipients

Depression, although its severity and prevalence post-transplantation is lower compared to remaining on dialysis, it continues to be an important determinant of QoL following transplantation[121,122]. KTRs are subjected to several mental challenges including frequent medical follow-up, the necessity to adhere to a complex regimen of immunosuppressive therapy that may generate distressing side effects, and the anxiety and fears about transplant rejection leading to potential graft loss[123]. Of importance, depression independently predicts mortality in KTRs[122]. On the positive side, psychotherapy has shown promising results in alleviating symptoms of depression in KTRs[124].

1.4.3 Fatigue and Quality of Life in Kidney Transplant Recipients

Similar to the clinical outcomes of kidney transplantation, many other aspects which may impact upon QoL following transplantation have not been well investigated. In particular, fatigue has received little attention in the field of kidney transplantation. Fatigue is an important QoL outcome, it may be measured as part of QoL, or may be evaluated

independently using an assessment tool specially tailored to address the multi-dimensional aspects of fatigue[125].

Fatigue represents an important patient-reported outcome in many medical conditions[126,127] and involves physical, cognitive, emotion, and functional components[128]. It is often medically unexplained[129], persistent[127], and interferes with an individual's ability to function in important roles[130]. As a corollary, fatigue can have a major negative impact upon QoL[131]. In chronic dialysis patients, fatigue is frequently reported as a pervasive and distressing symptom[132-134]. The fact that kidney transplantation improves QoL and results in an enhanced sense of well-being[135], means that it is often assumed that fatigue no longer features as a major problem after transplantation, but in fact there has been very little research to either confirm or refute this assumption. Only one study has specifically examined fatigue after transplantation[112], noting that the symptom was reported in 59% of KTRs and that it negatively impacted on virtually every aspect of the QoL[112].

1.4.3.1 Effects of Body Composition on Fatigue in Kidney Transplant Recipients

Body composition may be an important determinant of fatigue. However, the effects of body composition on post-transplantation fatigue have not been well-investigated. Thus far, only one study determined the contributors to fatigue following kidney transplantation. Poor sleep quality, mood disturbance, and raised BMI were identified as significant predictors for post-transplantation fatigue[112]. However, the impact of different body composition compartments on fatigue was not examined and warrant further investigation.

1.5 Aims and Outline of the Thesis

With this background, evidently, there are significant gaps in the current literature pertaining to the effects of body composition including fat mass, muscle mass and volume status, on both clinical and QoL outcomes in KTRs. The studies presented in this thesis aim to explore the effects of different body composition compartments on morbidity and fatigue, the potential contributing factors to long-term patient- and graft- survival, as well as QoL.

1.5.1 Chapter 2: The Role of Hepcidin-25 in Kidney Transplantation

Chapter 2 investigates the associations between adiposity with inflammation, hepcidin and haemoglobin levels in KTRs.

Anaemia (or low haemoglobin level) remains a common finding post-transplantation[136,137]. Reduced haemoglobin levels are associated with short-term complications, inferior patient and graft survivals[138-140]. Hepcidin is a peptide hormone synthesized by hepatocytes in response to iron repletion and acute phase inflammation[141-143]. Adipose tissue also releases hepcidin in response to systemic inflammation [144]and the local inflammatory milieu within the adipose tissue[145]. Upon hepatic and adipocytic synthesis, hepcidin reduces intestinal iron absorption and sequestration[142], limiting haemoglobin production. Hepcidin may therefore explain the link between inflammation and reduced haemoglobin levels[146,147], a

relationship that remains unestablished in the field of kidney transplantation. Similarly, the relationship between inflammation and hepcidin has not been explored in kidney transplantation[148]. Also, the underlying determinants of inflammation in this setting remain controversial. In particular, both low and high BMI were suggested as potential causes of inflammation in previous studies[94,149,150].

The primary aims of this chapter were to determine the factors influencing hepcidin levels, and to establish the relationship between hepcidin and haemoglobin levels in clinically stable KTRs. The secondary aims were to describe the factors associated with inflammation in this setting, and to assess the correlation between inflammation and adiposity. This will address whether adiposity-related inflammation in KTRs is associated with elevated hepcidin, possibly contributing to reduced haemoglobin by dysregulation of iron homeostasis.

1.5.2 Chapter 3: Hypervolemia and Blood Pressure in Prevalent Kidney Transplant Recipients

Chapter 3 focuses on the effects of hypervolemia (or volume expansion) on blood pressure and levels of N-terminal fragment of pro-hormone B-Type natriuretic peptide (NT-proBNP).

Hypervolemia is associated with hypertension in dialysis patients[151,152], but this relationship has not been studied in KTRs, despite the latter complication being a major risk factor for CVD[153], the leading cause of death in KTRs.

NT-proBNP, is a biologically inactive peptide, cleaved from pro-hormone B-type natriuretic peptide (pro-BNP) that is secreted from ventricles in response to increased stretch of the ventricular wall[154]. NT-proBNP is an independent predictor of mortality in patients with end-stage renal disease[155]. Recent studies have confirmed that it is marker of volume overload rather than cardiac dysfunction *per se* in maintenance dialysis patients[156-159]. However, little research has examined this relationship after transplantation, with limited data showing an inverse relationship between NT-proBNP levels and allograft function[160,161].

The primary objectives of this chapter were to determine the prevalence and predictors for hypervolemia in clinically stable KTRs, and to assess its association with post-transplant hypertension. Secondly, this study sought to explore the utility of NT-proBNP as a marker of hypervolemia and renal dysfunction in this cohort.

1.5.3 Chapter 4: Predictors and Consequence of Fatigue in Prevalent Kidney Transplant Recipients

Chapter 4 explores the role of muscle and fat masses on post-transplantation fatigue.

Fatigue is a common symptom following kidney transplantation[112]. However, it has been under-investigated as discussed in **Section 1.4.3**. Raised BMI was identified as a significant predictor for post-transplantation fatigue[112], but the impact of different body composition compartments remains unknown. Greater insight into fatigue severity, its impact on QoL, and its possible underlying causes are all prerequisites for developing interventions to combat this symptom. In addition, it is important to ascertain the extent to which clinicians are aware of the problem.

The purposes of this chapter were to determine the nature, severity, prevalence, and clinical awareness of fatigue in clinically stable KTRs. Additionally, this study aimed to examine the impact of this symptom upon QoL, determine the associations between fatigue with lean tissue mass and fat mass, and to explore other potential predictors of post-transplantation fatigue.

1.5.4 Chapter 5: Cardiovascular, Muscular and Perceptual Contributions to Physical Fatigue in Prevalent Kidney Transplant Recipients

Chapter 5 specifically examines the mechanistic aetiology of physical fatigue in KTRs by evaluation of muscle mass, muscular and cardiovascular functions, and fatigue perception.

Physical fatigue describes physical sensations of tiredness[162], leading to physical underperformance[163]. One of the major findings from Chapter 4 showed that physical

fatigue represents the dominant component of fatigue in KTRs, outweighing behavioural, emotional, and cognitive aspects.

Conceptually, research on physical fatigue has traditionally been considered as either "cardiovascular", "muscular" or "perceptual" in aetiology. The cardiovascular model refers to insufficient oxygen or nutrient delivery to the muscular system, limiting oxidative phosphorylation and glycolysis, both of which are essential mechanisms for muscle contraction[164]. Accordingly, fatigue with cardiovascular origin results in decreased ability of muscle to generate and to maintain force, hence reducing the capability to sustain muscle contraction, possibly contributing to physical fatigue. The muscular model denotes insufficient muscle mass or reduction in muscle function, leading to failure of muscle force generation[164-166], and/or ability to maintain a certain force or power output[167], plausibly resulting in physical fatigue. The perceptual theory represents the increased perception of effort, characterised by loss of motivation and reluctance to perform a physical task when perception of effort reaches a certain level. In fatigue with perception origin, individuals experience heightened responses to afferent feedback from the working body, resulting in exhaustion[164,168,169], which may be expressed as physical fatigue.

Of interest, it has been recognised that mental fatigue, characterised by inability to focus and maintain cognitive attention, is a crucial determinant of physical limits in healthy individuals[170-172], by heightening the perception of exertion[170,171]. This phenomenon suggests that mental fatigue possibly contributes to physical fatigue by raising perception of exertion.

The cardinal mechanistic aetiology of physical fatigue in KTRs remained unexplored. Therefore, the primary objectives of this chapter were to systematically examine the aetiology of physical fatigue in KTRs, by measuring factors which may be mechanistically linked to symptoms of physical fatigue. These include quantification of muscle mass, assessment of muscular and cardiovascular functions, and by evaluating perceived exertion during a standardised exercise protocol. We also sought to establish the prevalence of physical fatigue and its impact upon QoL in clinically stable KTRs. The key findings are that, physical fatigue, which adversely impacts on QoL, affects 22% of KTRs, and that cardiovascular and muscular factors do not contribute to the aetiology of physical fatigue. These observations point towards increased perception of exertion as the dominant cause of physical fatigue. Such findings arising from the earlier part of this chapter led to further investigation to examine the role of mental fatigue, and other plausible predictors of heightened perception.

1.5.5 Chapter 6: General Discussion

Chapter 6 addresses the major findings from the thesis, discussing the strengths and limitations of the aforementioned studies, the clinical implications and directions for future research.

1.5.6 Chapter 7: Appendices

Chapter 7 includes confirmation of ethical approval, and all the questionnaires used for the research studies presented in this thesis.

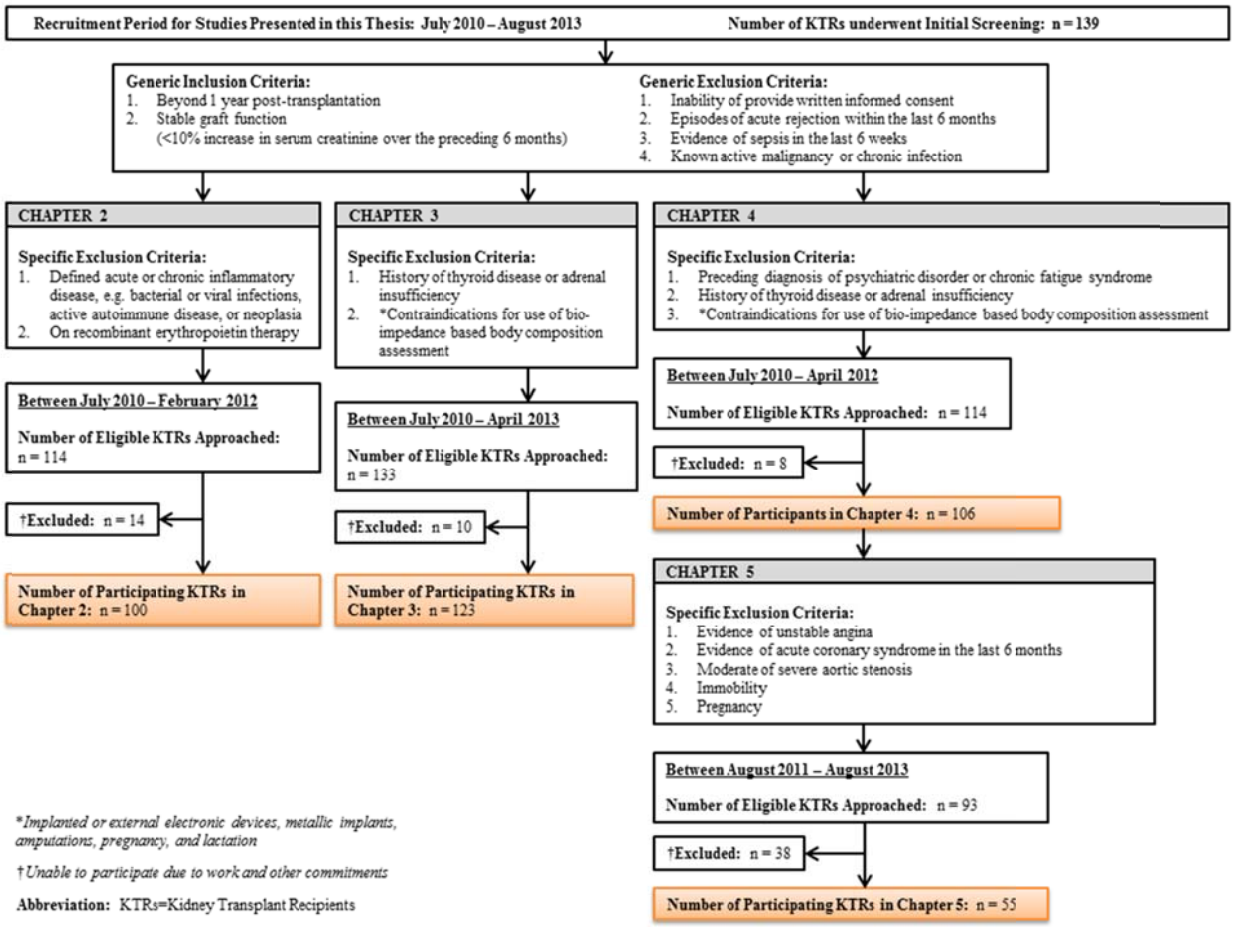
1.6 Study Design and Patient Selection in this Thesis

The studies presented in chapters 2 to 5 are single-centre cross-sectional pilot studies, and recruited KTRs from the out-patient clinic of the Queen Elizabeth Hospital Birmingham, United Kingdom. In total, 139 KTRs underwent initial screening for eligibility between July 2010 and August 2013. The same group of KTRs were used for each of the studies described in this thesis. Generic inclusion and exclusion criteria were employed at first, followed by specific exclusion criteria for each chapter. These are summarised in the flow diagram shown in **Figure 1.4**.

1.7 Ethical Approval of Research Studies presented in this Thesis

The studies presented in chapters 2 to 5 in this thesis were approved by the Staffordshire Research Ethics Committee, with Research Ethics Committee reference number of 10/H1203/16, confirmation of favourable ethical opinion can be found under **Appendices**, **Chapter 7**, **Sections 7.1 to 7.4**.

Figure 1.4. Flow Diagram of Patient Selection for this Thesis



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THE ROLE OF HEPCIDIN-25 IN KIDNEY TRANSPLANTATION

Chapter 2

Effects of Body Composition on Clinical and Quality of Life Outcomes in Kidney Transplant Recipients

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CHAPTER 2: THE ROLE OF HEPCIDIN-25 IN KIDNEY TRANSPLANTATION

2.1 Abstract

Background and Objectives: Hepcidin-25 is a peptide hormone involved in iron absorption and homeostasis. It is found at increased serum levels in conditions involving systemic inflammation, renal dysfunction, and increased adiposity. Hepcidin may be involved in the pathogenesis of anaemia, but its role in kidney transplantation remains undefined. The primary objectives of this study were to determine the factors influencing serum hepcidin levels, and to establish the relationship between hepcidin and haemoglobin levels in clinically stable kidney transplant recipients (KTRs). The secondary objectives were to ascertain the factors associated with inflammation in this setting, and to assess the correlation between adiposity and inflammation.

Materials and Methods: This single-centre cross-sectional study enrolled 100 clinically stable KTRs at least 12 months post-transplantation. Serum hepcidin-25 level, and relevant demographic and laboratory data pertinent to post-transplantation anaemia, were measured and collected.

Results: Independent associations were observed between raised hepcidin levels and allograft dysfunction (reduced estimated glomerular filtration rate), inflammation (elevated high sensitivity C-reactive protein), iron storage (elevated transferrin saturation), and the use of marrow-suppressive medications ($p<0.05$ for all associations). In addition, increased fat tissue index (whole-body multi-frequency bio-impedance spectroscopy

measurement) was associated with elevated hepcidin levels, but this relationship did not persist after adjustment for inflammation. In turn, inflammation was associated with increased fat tissue index (p=0.01) and male gender (p=0.04). Further, a non-linear association between serum hepcidin and haemoglobin levels was established, with progressive decrease in haemoglobin as hepcidin increased to 100 ng/mL, but limited effect thereafter (p=0.009). This association was independent of renal dysfunction and female gender, both of which also displayed independent relationships with reduced haemoglobin level.

Conclusions: This study highlights the possible mechanisms of haemoglobin reduction in KTRs, and the therapeutic opportunities from understanding the role of hepcidin in this context.

2.2 Introduction

Anaemia remains a common finding after kidney transplantation, occurring in approximately 30% of kidney transplant recipients (KTRs) beyond the first year post-transplantation[1,2]. This is not merely an incidental laboratory finding because reductions in haemoglobin levels have been associated with short-term complications, and inferior patient- and graft- survival[3-5]. Furthermore, a causal role for lower haemoglobin levels leading to adverse events was suggested by the patients of the Correction of Anaemia and Progression of Renal Insufficiency in Transplant (CAPRIT) study, which demonstrated a slower progression of kidney disease, a lower incidence of end-stage renal failure, and an improved quality of life in patients targeted to higher haemoglobin concentrations with recombinant erythropoietin[6]. However, reduced renal function and erythropoietin deficiency do not fully account for reduced haemoglobin levels observed in chronic kidney disease (CKD) or transplantation[7], highlighting the importance of understanding the determinants of haemoglobin in this setting.

An important and recently recognised component of haemoglobin homeostasis is hepcidin, the biologically active form of which is a 25 amino acid protein (hepcidin-25), synthesised by liver and adipose tissues, derived from prohepcidin, and degraded to inactive hepcidin-20[8]. Hepcidin has been described as the "master regulator of iron homeostasis" with hepatic synthesis increasing with iron repletion. A primary action of hepcidin-25 is the internalisation and subsequent degradation of ferroportin, the iron transporter found in duodenal enterocytes and macrophages[9]. This results in reduced intestinal iron absorption, and iron sequestration within the reticulo-endothelial system[9]. Thus, hepcidin plays a vital

role in protection from iron overload as there is no recognised mechanism by which iron excretion can be increased. In addition, hepatic hepcidin synthesis is suppressed during times of increased red cell production by the marrow, thereby linking iron availability with requirement[10].

Additionally, it is recognised that hepcidin may be important in certain pathological states. In particular, hepatic hepcidin synthesis is increased in response to an acute phase inflammation[11]. Adipose tissue also releases hepcidin in response to systemic inflammation[12], and the local inflammatory milieu within the adipose tissue[13]. Therefore, it is possible that increased hepcidin production may explain the link between inflammation and reduced haemoglobin levels[14].

CKD, and therefore kidney transplantation, presents an added complexity to these relationships because of the association between renal dysfunction and elevated hepcidin levels[8,15-17]. A previous study in patients with non-transplantation CKD revealed an association between higher circulating hepcidin levels and lower haemoglobin levels, independent of renal function[18]. However, it is currently unknown whether a similar relationship exists in KTRs. Similarly, the described relationship between inflammation and hepcidin has not been established in kidney transplantation[19], although this may provide the "missing link" between inflammation and anaemia in transplanted patients[20]. Also, there are conflicting data pertaining to the underlying determinants of inflammation in this setting. In particular, both low and high body mass index (BMI) were suggested as potential causes of inflammation[21-23].

The primary purpose of this study was to gain a greater understanding of the factors influencing serum hepcidin levels, and the relationship between hepcidin and haemoglobin levels in clinically stable KTRs. Clarifying the role of hepcidin in this context is increasingly relevant, not only to understanding the mechanism of anaemia in transplantation, but also in light of emerging strategies to increase haemoglobin levels by antagonising the production or activity of hepcidin[24,25]. The secondary aims were to determine the factors associated with inflammation in this setting, and to assess the link between adiposity and inflammation.

2.3 Materials and Methods

2.3.1 Study Design and Participants

This prospective, observational cohort study recruited KTRs from the out-patient clinic of the Queen Elizabeth Hospital Birmingham, United Kingdom. Inclusion criteria were KTRs with stable graft function at least 12 months post-transplantation, and with no previous episodes of acute allograft rejection in the 6 months prior to enrolment. KTRs with defined acute or chronic inflammatory disease, such as bacterial or viral infection, active autoimmune disease, or neoplasia, were excluded. KTRs on recombinant erythropoietin therapy were also excluded.

The recruitment target of 100 patients was achieved after 114 patients had been approached to participate. The main reason for decline in participation was work commitment (n=9), participation in other research studies (n=3), and no reason given

(n=2). The study received approval from the research ethics committee, and was conducted in accordance with the principles of the Declaration of Helsinki.

2.3.2 Data Collection

The following measures were evaluated for each participant of the study.

Patient demographics: Age, gender, time post-transplantation, and diabetes status, either documented as a comorbidity prior to transplantation, or the presence of new onset diabetes after transplantation (NODAT).

Other biochemical parameters were determined on the blood samples collected following a 10-hour overnight fast: Serum albumin, transferrin saturation (TSAT), high sensitivity C-reactive protein (hsCRP), hepcidin-25 (see below for methodology), and estimated glomerular filtration rate (eGFR) using the 4-variable modification of diet in renal disease (MDRD) equation with serum creatinine alignment to isotope dilution mass spectrometry-based methodology.

Medication: Use and doses of the antiproliferative agents including mycophenolate mofetil (MMF) or azathioprine versus neither; use of angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) versus neither; and use of statin therapy.

Adipose tissue mass (kg) was assessed using the whole-body multi-frequency bio-impedance spectroscopy, known as the Body Composition Monitor (BCM, Fresenius Medical Care, Hamburg, Germany) as described previously[26]. Adipose tissue mass was normalised to height (m^2) subsequently, and expressed as Fat Tissue Index (FTI, kg/m^2). Additionally, in a subset of 20 patients, dual energy X-ray absorptiometry (DEXA) scanning was undertaken as the "gold standard" method for fat mass estimation, thereby allowing method comparison with bio-impedance measurement.

Documented episodes of biopsy proven prior acute rejection episodes were retrieved from the records of the prospectively collected institutional database.

Only 4 patients admitted current tobacco use, and only 4 patients were not treated with a calcineurin inhibitor, and these variables were not considered for analysis.

2.3.3 Analysis of Hepcidin-25

The active form, hepcidin-25, was measured by surface enhanced lazer desorption / ionization time-of-flight mass spectrometry (SELDI-TOF-MS) using Cu^{2+} loaded IMAC ProteinChip arrays (Ciphergen Biosystems, Fremont, CA) and stable isotope-labelled hepcidin as an internal standard, as described previously[27] and available at http//:www.hepcidin.bham.ac.uk.

2.3.4 Statistical Analysis

SPSS Statistics 21 (Chicago, IL) was used for statistical analysis. Results are expressed as mean ± standard deviation unless otherwise stated. For the major regression analyses, there were three outcome variables (haemoglobin level, serum hepcidin concentration, and serum hsCRP concentration). Each of the variables was measured on a continuous scale and linear regression methods were used for analysis. Haemoglobin levels displayed a normal distribution, and was analysed on its original scale of measurement. Hepcidin and hsCRP concentrations were both positively skewed, and were subjected to logarithmic transformation prior to analysis. Therefore, the regression coefficients for these analyses represent the proportional, rather than absolute, change in hepcidin or hsCRP for the described change in the studied explanatory variable.

The analyses were performed in two stages. Initially, the separate effect of each predictor variables was examined in a series of univariate analyses. Where the relationships were found to be non-linear, quadratic (squared) terms were added to the model in order to improve the capture of the relationship between variables. Variables showing some evidence of association on univariate analysis ($p<0.15$) were subsequently and jointly examined in multivariate analysis. A backwards selection procedure was performed to simplify the final model to include only those variables found to be statistically significant predictors (type I error rate $\leq 5\%$).

Method comparison between the BCM and DEXA was performed by Passing-Bablok regression analysis, a technique specifically developed for method comparison purposes.

2.4 Results

Patient demographics, laboratory and anthropometric data are shown in **Table 2.1**.

Table 2.1. Population Characteristics of the Study Cohort

Age (years)	50.9 ± 13.7
Gender, male (%)	54
Time post-transplantation (years)	8.2 ± 6.7
Diabetes, pre-existing or NODAT (%)	19
Current Tobacco Use (%)	4
Previous Biopsy Proven Acute Rejection (%)	27
Haemoglobin (g/dL)	12.2 ± 1.5
Serum Hepcidin (ng/mL)	43 (29-67)
eGFR (mL/min)	44.1 ± 17.6
hsCRP (mg/L)	2.40 (1.00-4.99)
Transferrin Saturation (%)	25.2 ± 7.2
Serum Albumin (g/L)	44.5 ± 3.1
FTI (kg/m^2)	14.2 ± 6.2
Current calcineurin inhibitor use (%)	96
Current antiproliferative use, MMF or Azathioprine (%)	86
Current use of ACEI or ARB (%)	46
Current use of statin (%)	32

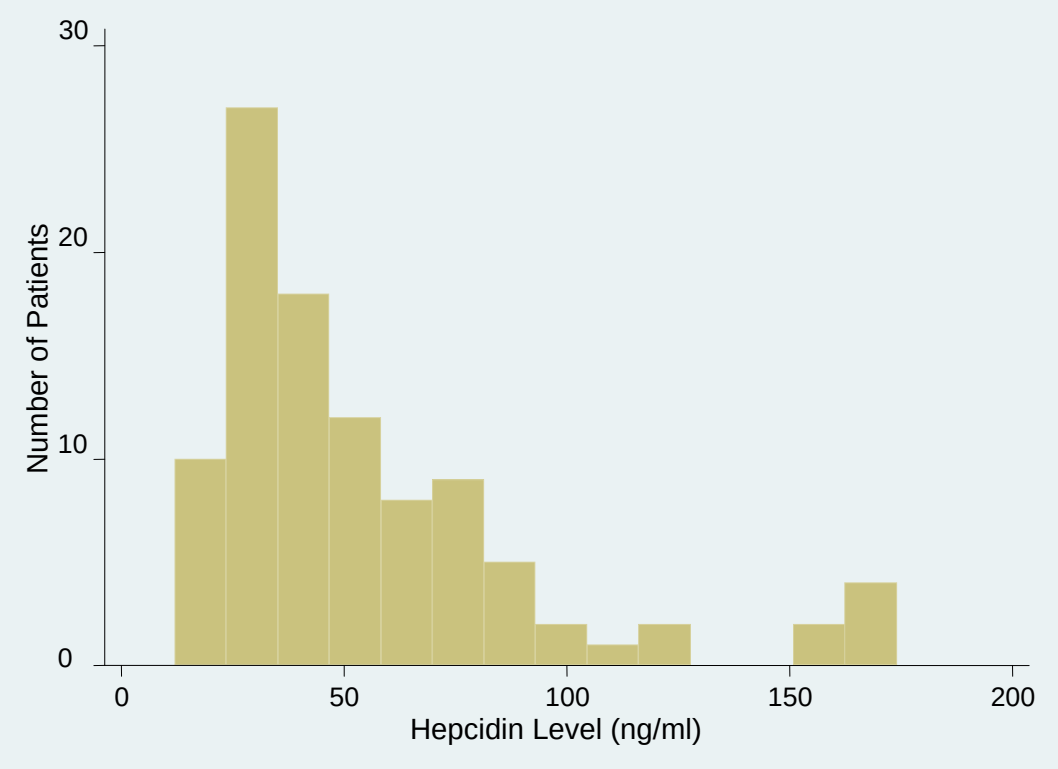
Results expressed as mean ± standard deviation, median (interquartile range), or percentage.

Abbreviations: NODAT=New Onset Diabetes After Transplantation; eGFR=Estimated Glomerular Filtration Rate; hsCRP=high sensitivity C-Reactive Protein; FTI=Fat Tissue Index; MMF=Mycophenolate Mofetil; ACEI=Angiotensin Converting Enzyme Inhibitor; ARB=Angiotensin Receptor Blocker.

2.4.1 Determinants of Serum Hepcidin Level

Hepcidin levels are shown in **Figure 2.1**.

Figure 2.1. Distribution of Serum Hepcidin Levels. Numbers of patients are shown with corresponding ranges of serum hepcidin.



A summary of univariate associations are shown in **Table 2.2**. When examined individually, higher hepcidin levels were associated with increased levels of inflammation (log-transformed hsCRP), the use of ACEI or ARB, the use of antiproliferative agents (MMF or azathioprine), increased iron stores (assessed with serum TSAT), and higher fat mass (assessed by FTI). In addition, hepcidin levels were significantly associated with eGFR. This association was non-linear as shown in **Figure 2.2**. In general, an inverse

association between eGFR and hepcidin was observed, with hepcidin levels rising at eGFR values of less than approximately 40 mL/min, and the relationship tailed off at higher levels of renal function.

Table 2.2. Univariate and Multivariate Associations with Serum Hepcidin Level

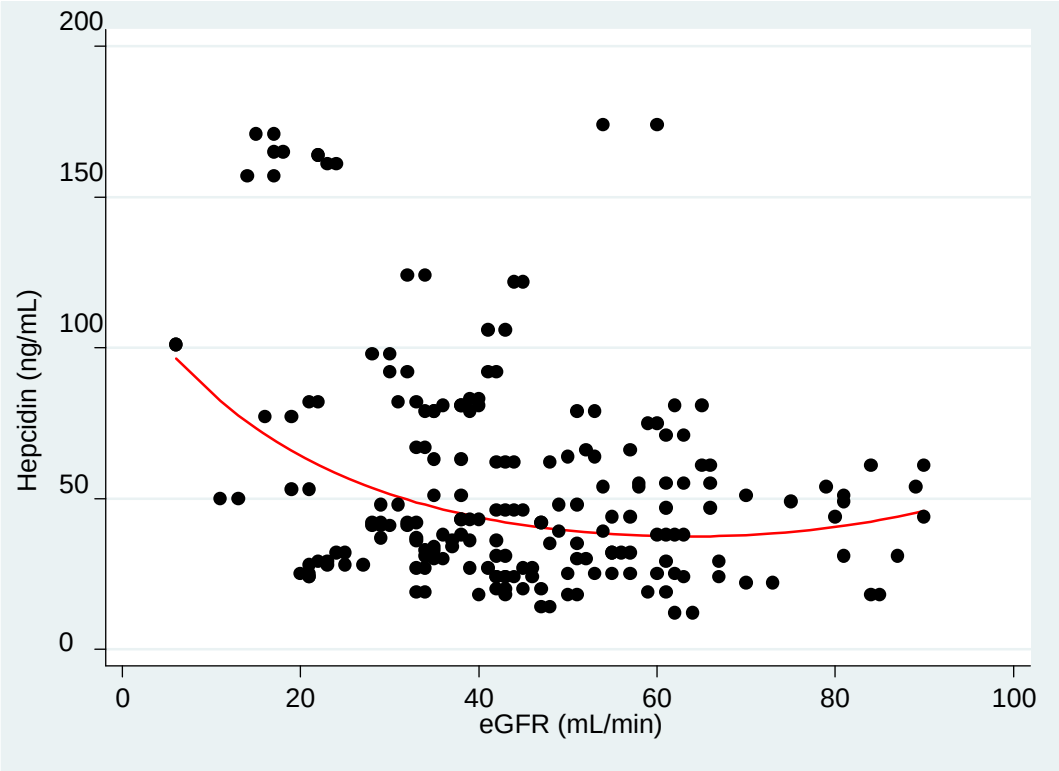
Variable	**Category**	**Univariate Coefficient (95% CI)**	**Univariate *p*-value**	**Multivariate Coefficient (95% CI)**	**Multivariate *p*-value**
*eGFR (mL/min)	Linear term Squared term	0.69 (0.57, 0.84) 1.03 (1.01, 1.05)	0.001 0.001	0.73 (0.61, 0.87) 1.02 (1.01, 1.03)	0.001 0.001
†hsCRP (mg/L)	-	1.15 (1.07, 1.24)	0.001	1.13 (1.06, 1.21)	0.001
*TSAT (%)		1.25 (1.11, 1.40)	0.001	1.27 (1.15, 1.41)	0.002
ACEI / ARB	Yes	1.22 (1.03, 1.44)	0.02	1.18 (1.02, 1.37)	0.03
MMF / Azathioprine	Yes	1.31 (0.55, 3.12)	0.04	1.16 (1.03, 1.31)	0.03
**FTI (kg/m^2)	-	1.07 (1.00, 1.14)	0.05		
Gender	Male	0.96 (0.81, 1.14)	0.63		
*Age (years)	-	1.00 (0.94, 1.07)	0.93		
**Time post-transplantation (years)	-	1.05 (0.98, 1.11)	0.14		
Rejection	Yes	1.05 (0.85, 1.30)	0.62		
Diabetes status	Yes	1.11 (0.89, 1.38)	0.40		
Albumin (g/dL)		0.99 (0.96, 1.02)	0.47		
Statin therapy	Yes	0.96 (0.68, 1.35)	0.33		

(*) Ratio reported for a 10-unit increase in explanatory variable. (**) Ratio reported for a 5-unit increase in explanatory variable. (†) Variable analysed on log scale.
Abbreviations: eGFR=estimated Glomerular Filtration Rate; hsCRP=high sensitivity C-Reactive Protein; TSAT=Transferrin Saturation; ACEI=Angiotensin Converting Enzyme Inhibitor; ARB=Angiotensin Receptor Blocker; MMF=Mycophenolate Mofetil; FTI=Fat Tissue Index.

The final multivariate model shown in **Table 2.2** confirmed the relationships between serum hepcidin and eGFR, log-transformed hsCRP, TSAT, the use of ACEI or ARB, and

the use of antiproliferative agents. These variables explained 45% of the variation in hepcidin levels (R^2: 0.45). After adjustment for these variables, the association between FTI and hepcidin level was no longer significant.

Figure 2.2. Association between Serum Hepcidin Level and Renal Function.



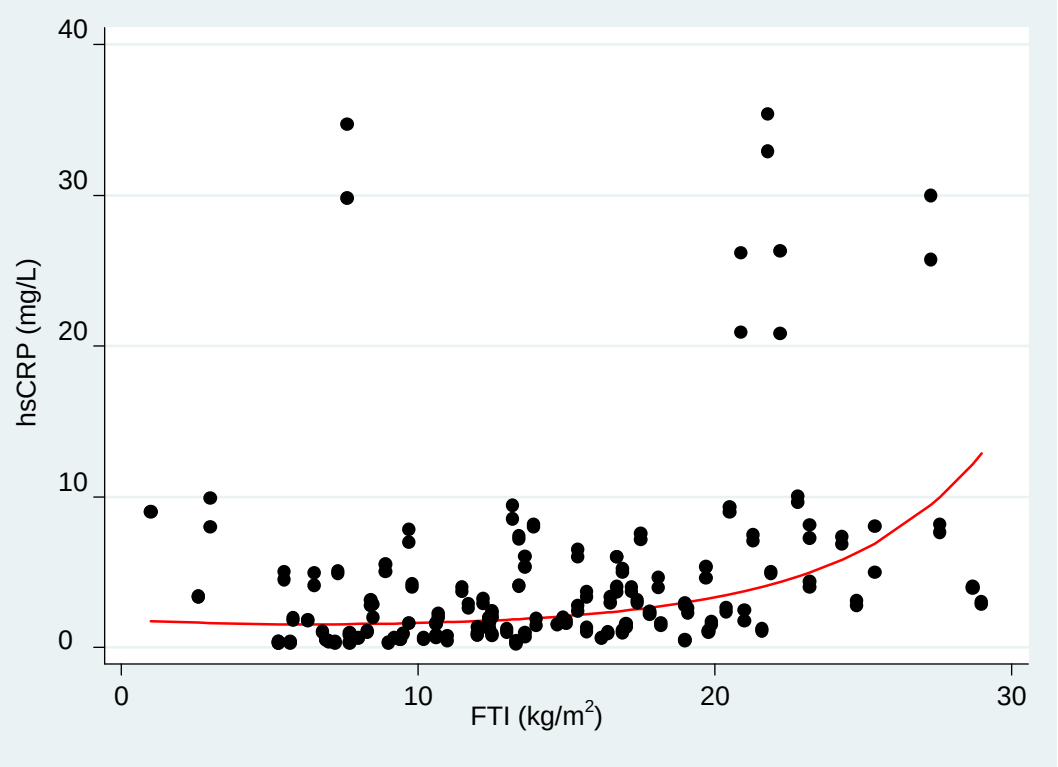
In light of the association between antiproliferative co-medication and hepcidin levels, a subsidiary analysis of medication doses was undertaken. No significant relationship was observed between azathioprine or MMF doses and serum hepcidin level (p=0.09 and p=0.10, respectively, in separate sub-analyses), although the analyses were limited by lower numbers. Due to the varied formulations of ACEI and ARB, no sub-analysis of dose-relationship was undertaken for these agents.

2.4.2 Determinants of Inflammation

Due to the observed relationship between inflammation and hepcidin, and the limited and conflicting data available on to the aetiology of inflammation in KTRs, particularly with regard to low and high BMI[21-23], a secondary analysis was performed, focusing on the factors associated with inflammation, assessed by log-transformed hsCRP level.

When examined individually, FTI was significantly associated with hsCRP. This relationship was non-linear as shown in **Figure 2.3**.

Figure 2.3. Association between Fat Tissue Index (FTI) and Inflammation (hsCRP)



There was a slight increase in hsCRP as FTI increased to 20 kg/m^2, with a stronger positive relationship between the two variables at higher FTI values. In addition, the presence of diabetes (a composite of pre-transplantation diabetes and NODAT) was also associated with increased hsCRP levels, **Table 2.3**.

Table 2.3. Univariate and Multivariate Associations with high-sensitivity C-Reactive Protein (hsCRP)

Variable	**Category**	**Univariate Coefficient (95% CI)**	**Univariate *p*-value**	**Multivariate Coefficient (95% CI)**	**Multivariate *p*-value**
*FTI (kg/m^2)	Linear term Squared term	0.77 (0.38, 1.56) 1.11 (1.06, 1.17)	0.009	0.80 (0.48, 1.33) 1.10 (1.03, 1.17)	0.01
Gender	Male	0.96 (0.81, 1.14)	0.15	1.44 (1.09, 1.91)	0.04
Diabetes status	Yes	1.35 (1.03, 1,77)	0.03		
ACEI / ARB	Yes	1.26 (0.89, 1.78)	0.21		
MMF / Azathioprine	Yes	0.95 (0.58, 1.56)	0.83		
**eGFR (mL/min)	-	0.98 (0.90, 1.07)	0.68		
**Age (years)	-	0.94 (0.84, 1.06)	0.30		
*Time post-transplantation (years)	-	1.05 (0.93, 1.17)	0.45		
Rejection	Yes	0.74 (0.50, 1.10)	0.13		
Statin therapy	Yes	0.90 (0.62, 1.31)	0.27		

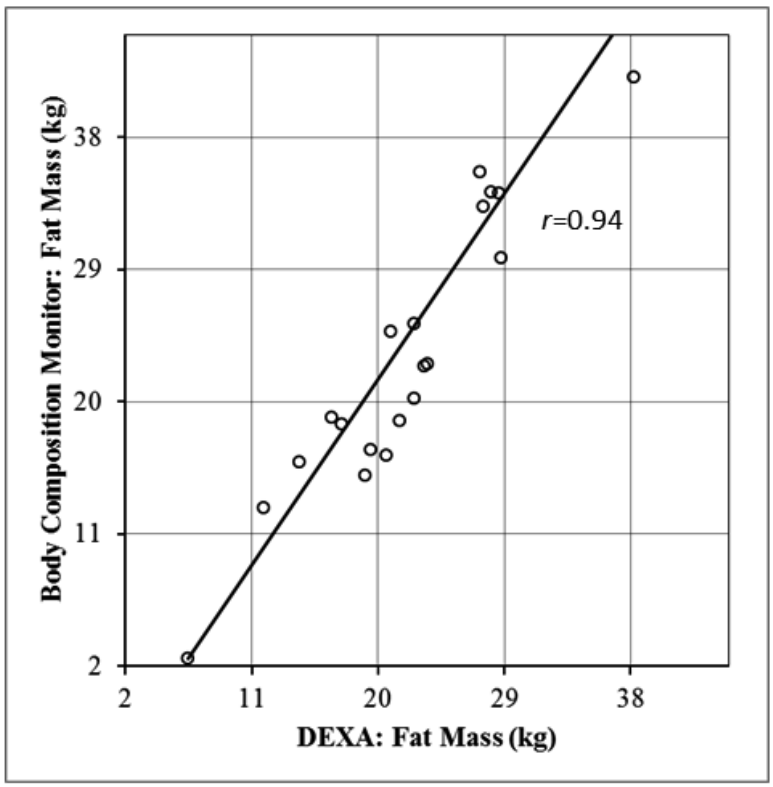
(*) Ratio reported for a 5-unit increase in explanatory variable. (**) Ratio reported for a 10-unit increase in explanatory variable.
Abbreviations: FTI=Fat Tissue Index; ACEI=Angiotensin Converting Enzyme Inhibitor; ARB=Angiotensin Receptor Blocker; MMF=Mycophenolate Mofetil; eGFR=estimated Glomerular Filtration Rate.

In the multivariate model, FTI remained associated with hsCRP, and a similar relationship was demonstrated. In addition, increased hsCRP levels were observed in male KTRs, **Table 2.3**. These two variables explained 27% of the variation in hsCRP levels (R^2: 0.27). Diabetes no longer retained significance in the multivariate model.

2.4.2.1 Comparing Methods of Measurement of Adiposity: Bio-impedance Analysis and Dual Energy X-ray Absorptiometry Scanning

In light of the association between inflammation and FTI assessed using the bio-impedance spectroscopy, a comparison between bio-impedance spectroscopy and DEXA assessment of fat mass was undertaken. Passing-Bablok regression was used to assess the relationship between the 2 methods, and is shown graphically in **Figure 2.4**. Although bio-impedance spectroscopy was associated with slightly lower absolute readings for fat mass when compared with DEXA scanning (as shown by the intercept of the regression line), the 2 methods demonstrated a high degree of correlation (r=0.94). Therefore, for the purposes of the current analyses, bio-impedance spectroscopy using the BCM is an appropriate and robust technique to determine the relationship between fat mass, adjusted to height and expressed as FTI, and the outcome variables assessed.

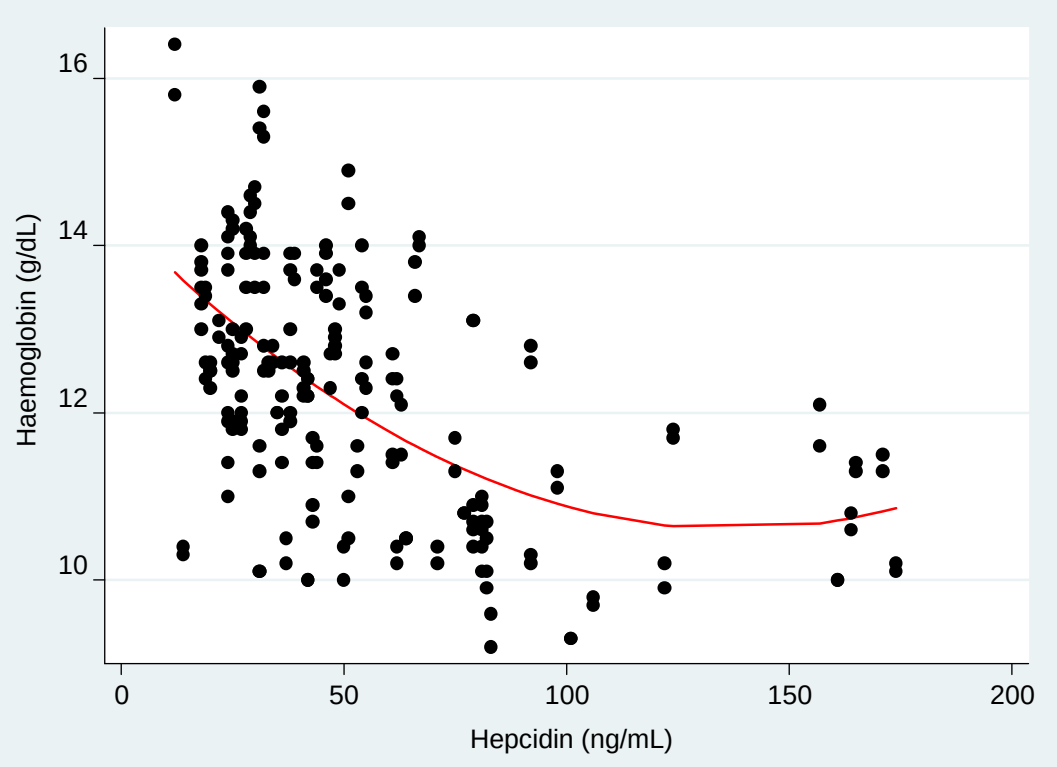
Figure 2.4. Passing-Bablok regression demonstrating the Method of Comparison between Whole-body Multi-frequency Bio-impedance Spectroscopy and Dual Energy X-ray Absorptiometry (DEXA) Scanning for Estimation of Body Fat Mass



2.4.3 Relationship between Hepcidin and Haemoglobin

A significant relationship between haemoglobin and serum hepcidin levels was established. This association was non-linear as shown in **Figure 2.5**. In general, it demonstrated that lower haemoglobin levels at higher serum hepcidin levels. This relationship diminished at higher serum hepcidin concentrations, with limited evidence for an incremental effect with hepcidin levels greater than 100 ng/mL.

Figure 2.5. Association between Serum Hepcidin Level and Haemoglobin Level



On univariate analysis, **Table 2.4**, lower haemoglobin levels were also associated with reduced renal function, inflammation (log transformed hsCRP), female gender, the use of either ACEI or ARB, and the use of antiproliferative immunosuppressants (MMF or azathioprine).

After adjustment for hepcidin level, the effect of inflammation on haemoglobin level was no longer significant. The final multivariate model confirmed the independent association between serum hepcidin and haemoglobin levels. Reduced renal function and female gender were also associated with reduced haemoglobin level. A summary of the results for this analysis is shown in **Table 2.4**, with 35% of the variation in haemoglobin level explained by the variables in the final model (R^2: 0.35). In addition, although not reaching

conventional levels of significance, there was some evidence for associations between lower haemoglobin level with the use of ACEI or ARB (regression coefficient: -0.36; 95% CI: -0.74, 0.02; *p*=0.06), and with the use of antiproliferative agents (regression coefficient: -0.30; 95% CI: -0.63, 0.04; *p*=0.08).

Table 2.4. Univariate and Multivariate Associations with Haemoglobin

Variable	**Category**	**Univariate Beta Coefficient (95% CI)**	**Univariate *p*-value**	**Multivariate Beta Coefficient (95% CI)**	**Multivariate *p*-value**
*Hepcidin (ng/mL)	Linear term Squared term	-1.07 (-1.42, -0.73) 0.08 (0.04, 0.12)	0.009	-1.12 (-1.44, -0.79) 0.09 (0.06, 0.13)	0.009
**eGFR (mL/min)	-	0.31 (0.20, 0.42)	0.002	0.22 (0.12, 0.31)	0.002
Gender	Male	0.57 (0.16, 0.98)	0.01	0.57 (0.24, 0.90)	0.008
†hsCRP (mg/L)	-	-0.21 (-0.38, -0.04)	0.03		
ACEI / ARB	Yes	-0.48 (-0.89, -0.07)	0.03		
MMF / Azathioprine	Yes	-0.42 (-0.70, -0.14)	0.04		
***FTI (kg/m^2)	-	-0.04 (-0.21, 0.13)	0.62		
**Age (years)	-	-0.02 (-0.17, 0.13)	0.79		
***Time post-transplantation (years)	-	-0.11 (-0.27, 0.04)	0.15		
Rejection	Yes	-0.07 (-0.60, 0.46)	0.79		
***TSAT (%)	-	-0.13 (-0.42, 0.16)	0.39		
Diabetes status	Yes	-0.33 (-0.88, 0.12)	0.23		
Albumin (g/dL)	-	-0.02 (-0.09, 0.05)	0.58		
Statin therapy	Yes	0.06 (-0.56, 0.68)	0.44		

(*) Coefficients reported for a 20-unit increase in explanatory variable. (**) Coefficients reported for a 10-unit increase in explanatory variable. (***) Coefficients reported for a 5-unit increase in explanatory variable. (†) Variable analysed on log scale.
Abbreviations: eGFR=estimated Glomerular Filtration rate; hsCRP=high sensitivity C-Reactive Protein; ACEI=Angiotensin Converting Enzyme Inhibitor; ARB=Angiotensin Receptor Blocker; MMF=Mycophenolate Mofetil; FTI=Fat Tissue Index; TSAT=Transferrin saturation.

2.5 Discussion

The major finding of this study is the independent association between elevated serum hepcidin and reduced haemoglobin levels in clinically stable KTRs, with hepcidin levels mostly driven by systemic inflammation and reduced renal function. Whilst the common explanation for low haemoglobin levels in patients with kidney disease is reduced renal function and erythropoietin deficiency, this does not fully explain the phenomenon[7]. Even prior to the recent and still evolving understanding of the role of hepcidin in iron homeostasis and erythropoiesis, it was recognised that other factors must play a role in the determination of haemoglobin levels after kidney transplantation[28,29].

This study represents the first evidence for an independent association between raised serum hepcidin and reduced haemoglobin levels in this setting. A progressive and clinically relevant reduction in haemoglobin was observed with increasing hepcidin levels, and this was independent of renal function and other relevant confounding factors. These observations extend to the field of kidney transplantation, with limited data from non-transplantation CKD showing an association between hepcidin and haemoglobin[18]. This finding suggests that targeting transplant patients with raised hepcidin levels with therapies designed to antagonise hepcidin production or activity may be a useful strategy. Currently, such agents remain in early phases of development, although preliminary clinical data appears encouraging[24,25].

Hepcidin levels were associated with TSAT (the marker of iron storage), reduced renal function, the use of marrow suppressive medication, and inflammation, which is consistent

with the prevailing understanding of the determinants of hepcidin levels[8]. This raises the possibility that, at least in part, such risk factors may exert their effect on haemoglobin via increasing hepcidin levels. No evidence was found for lower haemoglobin levels at the lower end of the spectrum of hepcidin levels, suggesting that iron deficiency was not in general a major mechanism for reduced haemoglobin levels in the current cohort. However, hepcidin may remain a valuable biomarker for identifying true iron deficiency in other patients, as suggested in studies of non-renal cohorts[14].

The observed relationship between reduced renal function and higher serum hepcidin levels in this study confirm and extend similar findings from non-transplantation cohorts[8,15-17,30], and a previous study in KTRs[19]. Although two recent studies in non-transplantation CKD failed to demonstrate this association[18,31], it is likely that differences in the characteristics of the study cohorts are responsible for these conflicting findings, in particular, with regard to levels of haemoglobin and inflammation, the range of renal function studied, and the use of comedication.

An interesting and novel observation was the increase in hepcidin levels associated with the use of ACEI, ARB, MMF and azathioprine. This may relate to bone marrow activity, which, in this setting is reduced due to a recognised effect of these medications, resulting in reduced inhibition of hepcidin secretion, leading to higher circulating levels. However, no measurement of soluble transferrin receptor or reticulocyte count, markers of erythropoietic activity, was undertaken to support this hypothesis. Nevertheless, a recent study of patients on haemodialysis also showed an association between renin-angiotensin system inhibitors and raised hepcidin levels[30], in keeping with the results of this study.

Due to the known association between inflammation with hepcidin, and indirectly haemoglobin, potential factors driving such inflammation in this setting were evaluated in this study. Adiposity, assessed by FTI, was independently and positively associated with inflammation. Furthermore, a univariate association between FTI and hepcidin level was found, although such relationship did not persist when adjusted for inflammation. This notion extends to the field of kidney transplantation, which supports the concept that adipose tissue may itself be a source of hepcidin, produced in response to the effect of inflammatory cytokines released by the fat tissue[13]. The link between adiposity and inflammation is an important observation because previous studies in transplantation examining this association have yielded conflicting results.

A sub-study of the Assessment of LEscol in Renal Transplantation (ALERT) trial showed that inflammation to be associated with raised BMI, a surrogate for fat mass[21]. The current study confirms the results of the ALERT trial, and extends them to a cohort where fat mass was objectively evaluated using bio-impedance derived measurements, which correlated well with DEXA scanning, the "gold standard". In contrast, an association between inflammation and clinically-assessed undernutrition , rather than overnutrition, was found in another study by Molnar and colleagues[22]. However, this study enrolled a mostly unselected cohort, and patients with known underlying chronic inflammatory conditions were not specifically excluded. In the current study, and also from the ALERT trial, participants with known inflammatory conditions were excluded, and this may explain the inconsistent findings between these studies. In addition, the cohort studied by Molnar et al.[22] displayed significant levels of comorbidity, reduced functional status, gastro-intestinal symptomatology, and changes in body weight, pointing to a "sicker" population in general.

A third study found no association between inflammation and BMI[23], but the authors acknowledged that small sample size and lack of a high sensitivity assay for C-Reactive Protein (CRP) may have reduced the power to detect an effect.

Although classically thought of as a marker of liver iron storage, ferritin also represents an acute phase reactant and can indeed be highly correlated with serum hepcidin in transplant recipients[32,33]. Serum ferritin was not evaluated in this study, instead, hsCRP was measured as the marker of inflammation, and TSAT, as the marker of circulating iron stores, the latter adding to the prevailing understanding of the relationship between iron stores and hepcidin levels as described in this study.

It is acknowledged that exclusion of some KTRs from this study, including those treated with erythropoietin, or those with active acute or chronic inflammation, or neoplastic conditions, reduces the generalisability of the results to some extent. However, for these reasons, it may increase the robustness of the findings by reducing certain potential confounders in the analysis, and this strategy is certainly in line with previous study in this field[17]. Nevertheless, the results of the different components of this study are intuitive, biologically plausible, shed light on the possible mechanisms of haemoglobin reduction in KTRs, and point to the opportunities stemming from a greater understanding of the role of inflammation and hepcidin in this context. Further prospective longitudinal follow-up of this cross-sectional study may add further insight into these associations. Finally, these findings require confirmation in larger and independent cohorts.

In summary, this study describes the relationship between hepcidin, inflammation and adiposity in clinically stable KTRs, and demonstrates an independent association between elevated hepcidin and reduced haemoglobin levels in this setting. Hepcidin antagonism may be a strategy for certain patients displaying reduced haemoglobin levels after kidney transplantation.

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HYPERVOLEMIA AND BLOOD PRESSURE IN PREVALENT KIDNEY TRANSPLANT RECIPIENTS

Chapter 3

Effects of Body Composition on Clinical and Quality of Life Outcomes in Kidney Transplant Recipients

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CHAPTER 3: HYPERVOLEMIA AND BLOOD PRESSURE IN PREVALENT KIDNEY TRANSPLANT RECIPIENTS

3.1 Abstract

Background and Objectives: The prevalence and consequences of hypervolemia in kidney transplant recipients (KTRs) have not been investigated. Specifically, its impact on blood pressure and relationship with N-terminal fragment of prohormone B-type natriuretic peptide (NT-proBNP) are unknown. The objectives of this study were to establish the prevalence of hypervolemia among clinically stable KTRs, investigate the predictors of post-transplant hypervolemia, assess its impact on blood pressure, and determine its relationship with NT-proBNP.

Materials and Methods: This single-centre cross-sectional study enrolled 123 clinically stable KTRs. Extracellular volume status was determined by multi-frequency bio-impedance analysis. Mild and severe hypervolemia were defined as percentage volume expansion of greater than 7% and greater than 15% respectively. Systolic blood pressure and diastolic blood pressure were measured, with mean arterial pressure calculated. Serum NT-proBNP was quantified using a non-competitive immunoluminometric assay. Potential demographic, nutritional and clinical predictors of extracellular volume status, blood pressure and NT-proBNP levels were assessed.

Results: Hypervolemia was present in 30% of KTRs, with 5% classified as severe hypervolemia. Significant predictors of volume expansion were increased sodium intake, advancing age, and reduced fat mass ($p<0.01$ for all associations). Hypervolemia was the only independent predictor of elevated mean arterial pressure, systolic and diastolic blood pressure ($p<0.001$ for all associations). Raised NT-proBNP levels were independently associated with both hypervolemia ($p=0.01$) and allograft dysfunction ($p=0.03$).

Conclusions: Hypervolemia is unexpectedly common among clinically stable KTRs. It is closely associated with elevated blood pressure. The relationship with increased sodium intake signals potential therapeutic focus. Further study is warranted to prospectively investigate objective measures of extracellular volume status among KTRs.

3.2 Introduction

Hypervolemia (or volume expansion) represents isotonic expansion of the extracellular fluid compartment caused by abnormal retention of water and sodium, manifesting as fluid accumulation and swelling in the extremities or lung tissues. It is common among patients with end-stage renal disease (ESRD) requiring maintenance dialysis[1-4], and is associated with increased morbidity and mortality[1-3,5]. For many of these patients, kidney transplantation is a preferred option of renal replacement therapy to correct metabolic abnormalities. It is assumed that hypervolemia no longer represents a major problem following transplantation, but no study to date confirms or refutes this.

In addition, hypervolemia is associated with hypertension in patients on haemodialysis[2] and peritoneal dialysis[3], but this relationship has not been studied in kidney transplant recipients (KTRs) despite this complication arising in 75-90% of these patients[6].

B-type Natriuretic Peptide (BNP) is a cardiac hormone that is synthesized as an amino acid precursor protein and undergoes intracellular modification to a Prohormone BNP (pro-BNP)[7]. It is secreted predominately from the ventricles in response to increased stretch of the ventricular wall[7]. Upon release into the circulation, pro-BNP is cleaved into the biologically active 32-amino acid C-terminal fragment BNP, and the biologically inactive 76-amino acid N-terminal fragment (NT-proBNP)[7]. NT-proBNP possesses a longer half-life time than the biologically active counterpart, hence delivering a superior reflection of pathophysiological situation leading to raised BNP levels[8]. Due to renal metabolism of

NT-proBNP, concentrations also rise with the progression of chronic kidney disease (CKD)[9]. NT-proBNP is an independent predictor of mortality in patients with ESRD[10]. Recent studies have confirmed that it is a marker of extracellular volume overload rather than cardiac dysfunction *per se* in maintenance dialysis patients[11-14]. However, little research has examined this relationship following transplantation, with the 2 studies conducted to date highlighting the inverse relationship between NT-proBNP and allograft function[15,16].

The primary objectives of this study were to determine the prevalence and predictors for hypervolemia in a stable kidney transplant cohort, and to assess its association with post-transplant hypertension. Secondly, we sought to explore the utility of serum NT-proBNP as a correlate of hypervolemia and renal dysfunction in this cohort.

3.3 Materials and Methods

3.3.1 Participants and Study Design

KTRs beyond 1 year post-transplantation, with stable graft function (<10% increase in serum creatinine over preceding 6 months), were recruited to this cross-sectional study between July 2010 and April 2013. Exclusion criteria included episodes of acute rejection within the last 6 months, evidence of sepsis in the last 6 weeks, known active malignancy or chronic infection, history of thyroid disease or adrenal insufficiency, and contra-

indications for use of bio-impedance based body composition assessment (implanted or external electronic devices, metallic implants, amputations, pregnancy, and lactation). Of 133 patients approached, 10 did not participate (mainly due to work commitment). The study was approved by the local research ethics committee, and was conducted in accordance with the principles of the Declaration of Helsinki.

3.3.2 Data Collection

3.3.2.1 Demographics and Clinical Parameters

Age, gender, ethnicity, and time post-transplantation were collected from patients' medical records. Smoking status (never smoked, current and ex- smoker) was collected by questionnaire. The following clinical parameters were retrieved from patients' medical records: 1) presence of diabetes, either pre-transplantation (pre-DM) or new onset diabetes after transplantation (NODAT), 2) previous acute rejection episodes, 3) immunosuppressive medication usage, either prednisolone, calcineurin inhibitor or adjunctive antiproliferative agent, 4) use of anti-hypertensive medications, either angiotensin-converting-enzyme inhibitor (ACEI), angiotensin-receptor blocker (ARB), beta-adrenergic blocker (BAB), dihydropyridine calcium-channel blocker (CCB), or alpha-adrenergic blocker (AAB), and 5) use of diuretic.

Systolic and diastolic blood pressure were measured semi-recumbent with a fully-automatic upper-arm digital blood-pressure monitor (Spot Vital Signs ® LXi, Welch Allyn). Six readings over an 8-10 minute period were taken, with the first reading ignored, and the mean of the remaining 5 used for analysis. This protocol for blood pressure monitoring has been shown to produce measurements comparable to that derived from the 24-hour ambulatory blood pressure monitor, the "gold standard" for the diagnosis of hypertension[17]. Mean arterial pressure was subsequently calculated using the formula **[(2 × Diastolic Blood Pressure) + Systolic Blood Pressure] / 3**[18].

3.3.2.2 Laboratory Parameters

Blood samples were collected for measurement of high-sensitivity C-reactive protein (hsCRP), albumin, haemoglobin, and estimated glomerular filtration rate (eGFR) derived using 4-variable modification of diet in renal disease equation[19]. Morning urine was collected for assessment of albumin : creatinine ratio (ACR). Analyses were undertaken in accredited hospital haematology and biochemistry laboratories.

Serum NT-proBNP was measured using a non-competitive immunoluminometric assay as described by Khan and colleagues[20]. This highly specific assay shows no cross-activity with atrial natriuretic peptide, BNP, or C-type natriuretic peptide[20]. The inter- and intra-assay coefficients of variation were 2.3 and 4.8% respectively[20].

3.3.2.3 Sodium and Fluid Intakes

Sodium and fluid intakes were estimated by a 3-day food diary. A multiple-day food diary provides a good estimate of individual's sodium intake[21], comparable to that derived from the mean 24-hour urinary sodium excretion[21,22], and produces a reliable and valid record of fluid intake in free-living humans[23]. Participants were given detailed written instructions on completing an accurate dietary record for a 3-day period, which included one weekend day, within one week prior to attending the research visit. These instructions were accompanied by verbal explanation from the researcher, which included training in portion size estimation and documentation for both dining in and eating out. The dietary records were reviewed by the researcher for accuracy and completeness at the research visit. Data was entered into Dietplan6 P3 (Forestfield Software Ltd) nutrition analysis program by the same researcher, avoiding inter-observer variation. Total daily intakes of fluid, energy, all macro- and micro- nutrients, were calculated by this program. No patients were prescribed sodium-containing oral medication at the time of the study.

3.3.2.4 Measurement of Body Composition and Volume Status; Definition of Volume Status

Body composition and extracellular volume status were assessed by whole body bio-impedance spectroscopy, the Body Composition Monitor (BCM) (Fresenius Medical Care, Germany). This device has been used in dialysis patients extensively[5], and has been validated against reference methods for volume status and body composition[24]. The BCM

utilises an algorithm based on a 3-compartment body model to evaluate extracellular and intracellular fluid volumes[25]. Absolute extracellular volume expansion was determined by calculating the difference between the actual amount of extracellular fluid in the body detected by the BCM and the expected amount of extracellular fluid predicted by the BCM under normal physiological (i.e. normovolemia) conditions[5,26]. Percentage volume expansion (%VE) is therefore defined as: **[(Absolute extracellular volume expansion × 100) / Expected extracellular fluid volume]**.

In a normal reference population, the 90th and the 10th percentiles of %VE is ± 7%[5,27]. Increased mortality in haemodialysis patients is observed when %VE > 15%[28,29]. Hence, established definitions, and those used in the current study, are based on %VE, < -7.0% representing "hypovolemia", within ± 7.0% indicating "normovolemia", between 7.1% and 15.0% denoting "mild hypervolemia", and > 15.0% demonstrating "severe hypervolemia".

Measurements were carried out in a standard manner while the patient was lying supine in a flat and non-conductive bed. The inbuilt physiological body composition model measures whole-body bioimpedance spectroscopy at 50 frequencies (5 to 1000 kHz) via electrodes placed on the wrist (proximal to the transverse) and the ankle (arch on the superior side of the foot) on the same side of the body. Results for %VE, together with Lean Tissue Index (LTI, kg/m^2) and Fat Tissue Index (FTI, kg/m^2), were displayed after each measurement.

3.3.3 Statistical Analysis

Statistical analyses were performed using SPSS Statistics 21 (Chicago IL). Results were presented as mean ± standard deviation (SD) for normally distributed data or median (interquartile range, IQR) for non-normally distributed data.

Unadjusted univariate relationships were evaluated with Pearson's correlation coefficients, and one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple-group comparisons.

Linear regression analysis was used to determine the associations between predictor variables and the continuously-distributed outcome variables, with logarithmic transformation of non-normally distributed data prior to analysis. The analyses were performed in two stages. Initially, the effect of each variable was examined in a series of univariate regression analyses. Subsequently, the joint effect of variables demonstrating some evidence of association on univariate analysis ($p<0.20$) was examined in a multivariable regression analysis, using a backwards selection procedure to derive the final model. A type 1 error rate ≤5% ($p\leq0.05$) was considered significant in the final model.

3.4 Results

3.4.1 Population Characteristics

The characteristics of the studied population are shown in **Table 3.1**. The mean %VE ± SD for the cohort was 2.6 ± 7.7%, ranging from -17.0% to +25.0%. Based on denoted criteria (described in **Materials and Methods**), the prevalence of hypovolemia in KTRs was 11% (13 patients), normovolemia was 59% (73 patients), mild hypervolemia was 25% (31 patients displaying %VE between 7.1 and 15.0%), and 5% suffered from severe hypervolemia (6 patients displaying %VE >15.0%).

Table 3.1. Population Characteristics

	Characteristics
Sample size	n = 123
Gender (%)	Male = 56 Female = 44
[*]Ethnicity (%)	Caucasian = 77 Asian = 16 Afro-Caribbean = 5 Others = 2
[†]Mean age (years)	50 ± 15
[‡]Median time post-transplantation (years)	5 (2-11)
[§]Smoking status (%)	Non-smoker = 63 Current smoker = 8 Ex-smoker = 29
[†]Mean extracellular volume status: %VE (%)	2.6 ± 7.7
[‡]Median level of NT-proBNP (pmol/L)	291.0 (65.0-700.4)
Blood pressure	
[†]Mean systolic blood pressure (mmHg)	141 ± 19
[†]Mean diastolic blood pressure (mmHg)	82 ± 13
[†]Mean arterial pressure (mmHg)	101 ± 13
Immunosuppressive medication usage	
Calcineurin inhibitor (%)	79
Adjunctive antiproliferative agent (%)	87
Prednisolone (%)	77
Dosage of immunosuppressive medications	
[‡]Median dose of Tacrolimus (mg/day)	4.0 (2.5-6.0)
[‡]Median dose of Cyclosporin (mg/day)	150 (150-200)
[†]Mean dose of Mycophenolate Mofetil (mg/day)	987 ± 392
[†]Mean dose of Azathioprine (mg/day)	77 ± 36
[‡]Median dose of Prednisolone (mg/day)	5 (5-5)
Anti-hypertensive medication usage	
ACEI / ARB (%)	43
BAB (%)	21
CCB (%)	48
AAB (%)	39
Diuretic medication usage	
Furosemide, exclusively (%)	15
[‡]Median dosage of Furosemide (mg)	40 (30-40)
Presence of diabetes (%)	Non-diabetic = 75 NODAT = 15 Pre-DM = 10
Previous episodes of acute rejection (%)	Yes = 23 No = 77
[‡]Median hsCRP (mg/L)	2.4 (1.0-4.9)
[†]Mean haemoglobin (g/dL)	12.6 ± 1.6
[†]Mean albumin (g/L)	44.5 ± 3.2
[†]Mean eGFR (mL/min)	44.2 ± 17.3
[‡]Median ACR (mg/mmol)	4.4 (1.6-14.7)
[‡]Median sodium intake (mg)	2725 (2131-3248)
[‡]Median fluid intake (mL)	2567 (2100-3672)
Body Composition	
Body mass index, BMI (kg/m^2)	27.4 ± 5.8
Lean Tissue Index, LTI (kg/m^2)	13.9 ± 3.0
Fat Tissue Index, FTI (kg/m^2)	13.3 ± 6.3

[†]Normally distributed data, results expressed as mean ± standard deviation (SD). [‡]Non-normally distributed data, results expressed as median (interquartile range, IQR).

[*]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 77% "Caucasian" versus 23% "Non-Caucasian". [§]For the purpose of statistical analysis, smoking status was arranged into 2 categories, "non-smoker" versus "current smoker and ex-smoker", 63% and 37% of patients respectively.

Abbreviations: NT-proBNP=N-Terminal pro B-type Natriuretic Peptide; ACEI=Angiotensin-Converting-Enzyme Inhibitor; ARB=Angiotensin-Receptor Blocker; Beta-Adrenergic Blocker; CCB=Calcium Channel Blocker; AAB=Alpha-Adrenergic Blocker; hsCRP=high-sensitivity C-Reactive Protein; eGFR=estimated Glomerular Filtration Rate; ACR=Albumin : Creatinine Ratio; NODAT=New Onset Diabetes After Transplantation; Pre-DM=Presence of Diabetes Mellitus pre-transplantation.

3.4.2 Factors Predicting Extracellular Volume Status

On univariate analysis, increasing values for %VE were associated with the following: higher sodium intake (relationship is shown in **Figure 3.1**), higher fluid intake, older age, pre-DM, male gender, the use of either an ACEI or ARB (grouped as a single category), and the number of antihypertensive medications. The effect sizes for the univariate analyses are shown in **Table 3.2**. In the multivariate analysis, only increased sodium intake (beta coefficient, β = 1.7; 95% confidence interval, CI = 1.2, 2.4; p<0.001) and advancing age (β = 1.8; 95% CI = 1.0, 2.6; p<0.001) retained statistical significance. In addition, an association emerged in the multivariate analysis between increased %VE and reduced FTI (β = -1.4; 95% CI = -2.2, -0.5; p=0.002). A 51% of the variation in extracellular volume status (%VE) was explained by these variables (**R^2**: 51%; **Table 3.2**).

Figure 3.1. Association between Sodium Intake and Extracellular Volume Status (Percentage Volume Expansion, %VE)

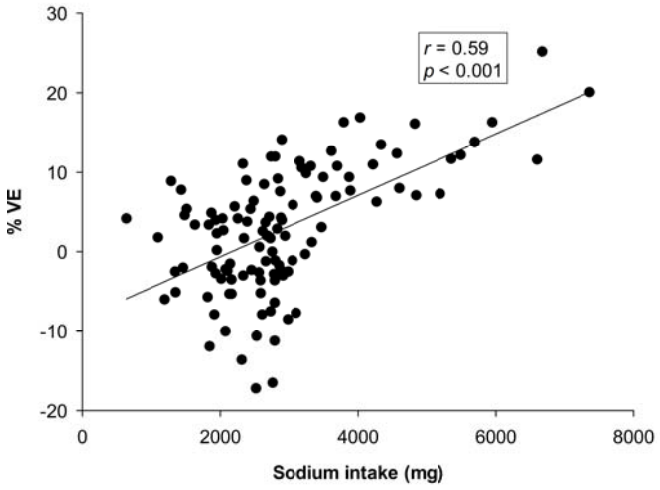


Table 3.2. Predictors of Extracellular Volume Status (Percentage Volume Expansion, %VE)

	Univariate Analysis		Multivariate Analysis[§]	
	Regression Coefficient (95% CI[α])	***p*-value**	**Regression Coefficient (95% CI[α])**	***p*-value**
[(***)] Sodium intake (mg)	1.8 (1.3, 2.3)	<0.001	1.7 (1.2, 2.4)	<0.001
[(***)] Fluid intake (mL)	1.4 (0.6, 2.0)	<0.001		
[(**)] Age (years)	1.9 (0.9, 2.8)	<0.001	1.8 (1.0, 2.6)	<0.001
Presence of diabetes Non-diabetic NODAT Pre-DM	 0 2.4 (-1.2, 5.9) 10.3 (6.0, 14.7)	<0.001		
Gender Female Male	 0 4.3 (1.6, 7.0)	0.002		
Use of ACEI / ARB No Yes	 0 3.6 (0.9, 6.3)	0.01		
Number of antihypertensive medications	1.6 (0.1, 3.2)	0.04		
Albumin (g/L)	-0.4 (-0.8, 0.1)	0.11		
Use of diuretic (furosemide) No Yes	 0 3.5 (-0.8, 7.8)	0.11		
[(*)] FTI (kg/m^2)	-1.0 (-2.0, 0.5)	0.12	-1.4 (-2.2, -0.5)	0.002
[(*)] eGFR (mL/min)	-0.3 (-0.6, 0.1)	0.19		
[(ℓ)] ACR (mg/mmol)	0.5 (-0.4, 1.4)	0.27		
[‡]**Ethnicity** Caucasian Non-Caucasian	 0 -1.8 (-5.1, 1.5)	0.29		
Use of prednisolone No Yes	 0 -1.8 (-5.1, 1.6)	0.29		
[(*)] LTI (kg/m^2)	-0.2 (-0.7, 0.2)	0.31		
[†]**Smoking status** Never smoked Ex-smoker / Current smoker	 0 0.1 (-1.4, 4.3)	0.32		
Use of BAB No Yes	 0 0.2 (-1.3, 3.9)	0.34		
Use of CCB No Yes	 0 0.1 (-1.5, 4.1)	0.34		
Haemoglobin (g/dL)	-0.4 (-1.3, 0.5)	0.44		
Use of AAB No Yes	 0 0.1 (-2.0, 3.9)	0.52		
[(*)] Time post transplantation (years)	0.2 (-0.9, 1.3)	0.76		
Use of calcineurin inhibitor No Yes	 0 -0.3, (-3.7, 3.1)	0.85		
[(ℓ)] hsCRP (mg/L)	0.1 (-1.3, 1.4)	0.94		
Previous episodes of acute rejection No Yes	 0 -0.1 (-3.4, 3.2)	0.95		
Use of adjunctive antiproliferative agents No Yes	 0 -0.0 (-4.6, 4.5)	0.99		
R^2 value from final model			51%	

[§]Results in the final multivariate regression model were presented. [α]CI = Confidence Interval. [†]For the purpose of statistical analysis, smoking status was arranged into 2 categories, "non-smoker" versus "the combination of current smoker and ex-smoker", 63% and 37% of patients respectively. [‡]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 77% "Caucasian" versus 23% "Non-Caucasian". [(*)] Coefficients reported for a 5-unit increase in explanatory variable. [(**)] Coefficients reported for a 10-unit increase in explanatory variable. [(***)] Coefficients reported for a 50-unit increase in explanatory variable. [(ℓ)] Variable analysed on the log scale (base 10).

Abbreviations: NODAT=New Onset Diabetes After Transplantation; Pre-DM=Presence of Diabetes Mellitus pre-transplantation; ACEI=Angiotensin-Converting-Enzyme Inhibitor; ARB=Angiotensin-Receptor Blocker; FTI=Fat Tissue Index; eGFR=estimated Glomerular Filtration Rate; ACR=Albumin : Creatinine Ratio; LTI= Lean Tissue Index; BAB=Beta-Adrenergic Blocker; CCB=Calcium Channel Blocker; AAB=Alpha-Adrenergic Blocker; hsCRP=high-sensitivity C-Reactive Protein.

3.4.3 Extracellular Volume Status and Blood Pressure

Increasing volume status (higher %VE) was associated with progressive increases in all measures of blood pressure (systolic blood pressure, r=0.83, p <0.001; diastolic blood pressure, r=0.60, p<0.001; mean arterial pressure, r=0.78, p<0.001; **Figure 3.2**). A significant difference across categories of volume status ("hypovolemia"; "normovolemia"; "mild hypervolemia"; "severe hypervolemia") was seen, with increased blood pressure at higher degrees of extracellular volume status (**Figure 3.3**).

Figure 3.2. Relationship between Extracellular Volume Status (Percentage Volume Expansion, %VE) and Blood Pressure

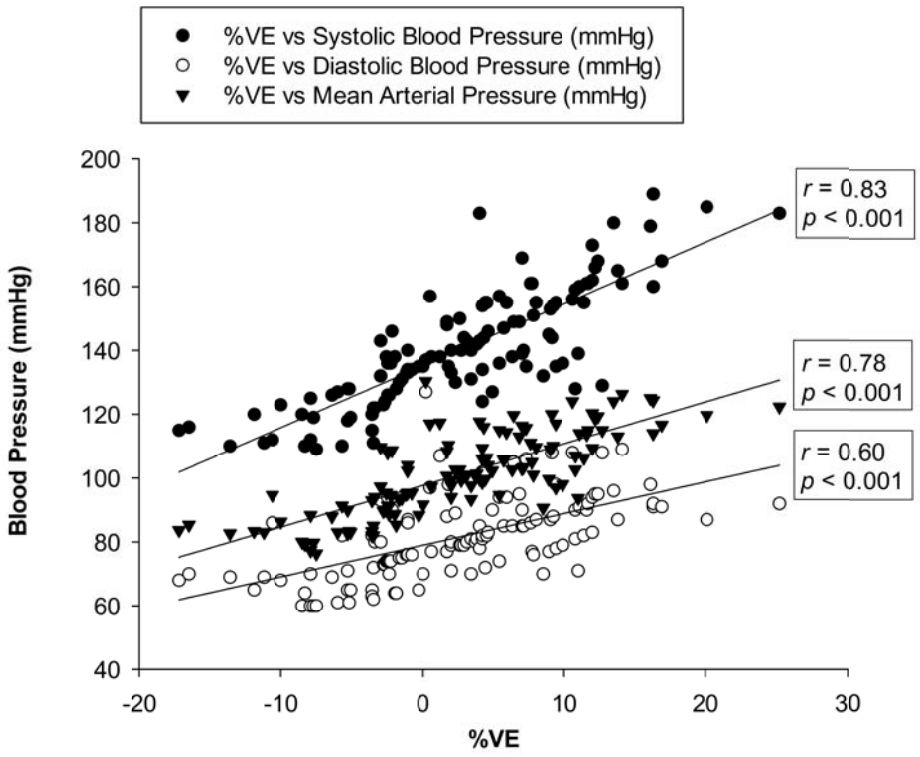
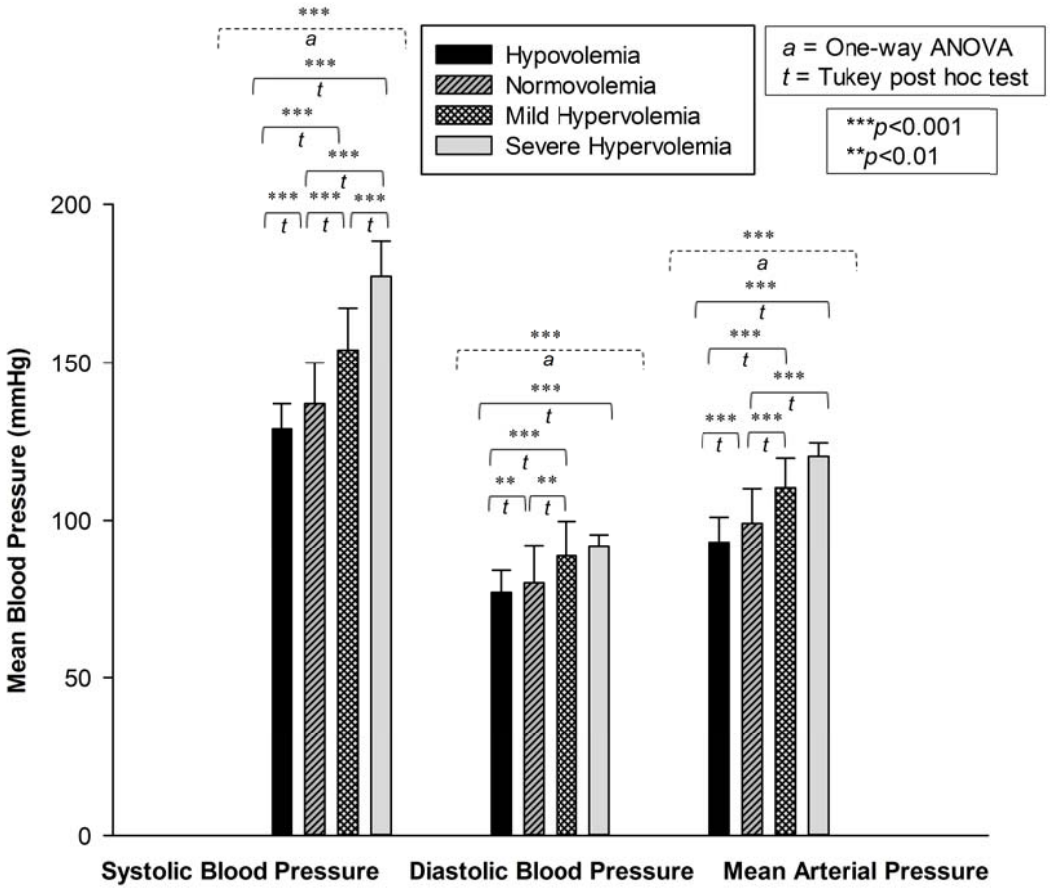


Figure 3.3. Comparisons of Blood Pressure among Kidney Transplant Recipients with different Extracellular Volume Status



The univariate and adjusted analyses describing the predictors of mean arterial pressure, systolic and diastolic blood pressure are shown in **Table 3.3**, **Table 3.4**, and **Table 3.5**, respectively. The following predictor variables displayed univariate, unadjusted associations with higher values for all measures of blood pressure (mean arterial pressure, systolic and diastolic blood pressure): increasing %VE, increased sodium intake (associations shown in **Figure 3.4**), older age, diabetes (pre-DM; or NODAT), the use of either an ACEI or ARB, hypoalbuminaemia, male gender, and number of antihypertensive

medications. In addition, higher fluid intake was associated with higher mean arterial pressure and systolic blood pressure readings, but not diastolic blood pressure. However, in the adjusted model, the only independent predictor of blood pressure was a higher %VE, with this effect seen for mean arterial pressure (β = 6.6; 95% CI = 5.6, 7.6; p<0.001), systolic blood pressure (β = 9.8; 95% CI = 8.5, 11.0; p<0.001), and diastolic blood pressure (β = 4.9; 95% CI = 3.7, 6.2; p<0.001). Of note, a substantial proportion of blood pressure variation could be explained by this single predictor variable (62%, 69% and 35% for mean arterial, systolic and diastolic blood pressure as shown in **Table 3.3**, **Table 3.4**, and **Table 3.5**, respectively).

Figure 3.4. Association between Sodium Intake and Blood Pressure

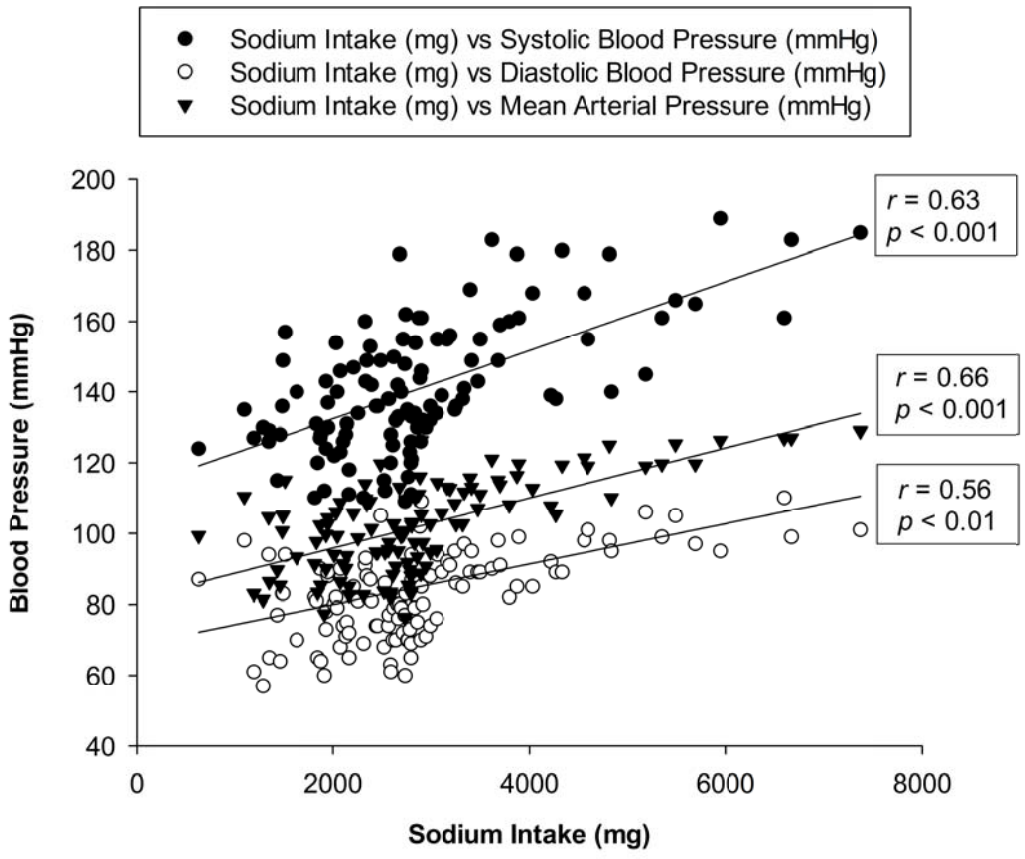


Table 3.3. Predictors of Mean Arterial Pressure

	Univariate Analysis		Multivariate Analysis[§]	
	Regression Coefficient (95% CI[≈])	***p*-value**	**Regression Coefficient (95% CI[≈])**	***p*-value**
[(*)] %VE	6.6 (5.6, 7.5)	<0.001	6.6 (5.6, 7.6)	<0.001
[(***)] Sodium intake (mg)	0.3 (0.2, 0.4)	<0.001		
[(**)] Age (years)	2.5 (0.9, 4.1)	<0.01		
Presence of diabetes Non-diabetic NODAT Pre-DM	 0 5.6 (2.1, 9.0) 11.2 (2.8, 19.5)	<0.01		
Use of ACEI / ARB No Yes	 0 6.7 (2.1, 11.3)	<0.01		
Albumin (g/L)	-0.9 (-1.7, -0.2)	0.01		
Gender Female Male	 0 5.8 (1.1, 10.4)	0.02		
[(***)] Fluid intake (mL)	0.2 (0.0, 0.3)	0.03		
Number of antihypertensive medications	2.7 (0.0, 5.4)	0.05		
[‡]**Ethnicity** Caucasian Non-Caucasian	 0 5.0 (-0.5, 11.0)	0.08		
[(*)] Time post transplantation (years)	1.2 (-0.6, 2.9)	0.18		
[(*)] FTI (kg/m^2)	1.3 (-3.1, 0.6)	0.19		
Use of calcineurin inhibitor No Yes	 0 3.6 (-2.2, 9.3)	0.22		
Use of diuretic (furosemide) No Yes	 0 4.0 (-2.5, 10.5)	0.23		
Use of prednisolone No Yes	 0 -2.5 (-8.1, 3.1)	0.38		
Use of CCB No Yes	 0 2.1 (-2.7, 6.8)	0.39		
Use of BAB No Yes	 0 3.2 (-2.9, 6.2)	0.41		
[(*)] eGFR (mL/min)	-0.2 (-0.9, 0.5)	0.54		
Haemoglobin (g/dL)	-0.5 (-2.0, 1.1)	0.56		
Use of adjunctive antiproliferative agents No Yes	 0 2.2 (-5.3, 9.7)	0.56		
Use of AAB No Yes	 0 1.5 (-3.5, 6.5)	0.56		
[†]**Smoking status** Never smoked Ex-smoker / Current smoker	 0 1.4 (-3.5, 6.3)	0.57		
LTI (kg/m^2)	-0.2 (-1.0, 0.6)	0.63		
[(ℓ)] hsCRP (mg/L)	1.1 (-3.8, 5.9)	0.66		
[(ℓ)] ACR (mg/mmol)	0.6 (-3.0, 4.3)	0.72		
Previous episodes of acute rejection No Yes	 0 -0.5 (-6.1, 5.1)	0.86		
R^2 value from final model			62%	

[§]Results in the final multivariate regression model were presented. [≈]CI = Confidence Interval.
[†]For the purpose of statistical analysis, smoking status was arranged into 2 categories, "non-smoker" versus "the combination of current smoker and ex-smoker", 63% and 37% of patients respectively. [‡]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 77% "Caucasian" versus 23% "Non-Caucasian".
[(*)] Coefficients reported for a 5-unit increase in explanatory variable. [(**)] Coefficients reported for a 10-unit increase in explanatory variable. [(***)] Coefficients reported for a 50-unit increase in explanatory variable. [(ℓ)] Variable analysed on the log scale (base 10).
Abbreviations: %VE=Percentage Volume Expansion; NODAT=New Onset Diabetes After Transplantation; Pre-DM=Presence of Diabetes Mellitus pre-transplantation; ACEI=Angiotensin-Converting-Enzyme Inhibitor; ARB=Angiotensin-Receptor Blocker; FTI=Fat Tissue Index; CCB=Calcium Channel Blocker; BAB=Beta-Adrenergic Blocker; eGFR=estimated Glomerular Filtration Rate; AAB=Alpha-Adrenergic Blocker; LTI= Lean Tissue Index; hsCRP=high-sensitivity C-Reactive Protein; ACR=Albumin : Creatinine Ratio.

Table 3.4. Predictors of Systolic Blood Pressure

	Univariate Analysis		Multivariate Analysis[§]	
	Regression Coefficient (95% CI[∞])	***p*-value**	**Regression Coefficient (95% CI[∞])**	***p*-value**
[(***)] Sodium intake (mg)	0.4 (0.3, 0.6)	<0.001		
[(***)] Fluid intake (mL)	0.2 (0.1, 0.4)	<0.001		
[(**)] Age (years)	4.2 (2.0, 6.3)	<0.001		
[(*)] %VE	9.7 (8.4, 11.0)	<0.001	9.8 (8.5, 11.0)	<0.001
Presence of diabetes				
Non-diabetic	0	<0.001		
NODAT	9.2 (4.3, 14.0)			
Pre-DM	23.9 (12.7, 35.0)			
Use of ACEI / ARB				
No	0	<0.01		
Yes	9.3 (2.8, 15.8)			
Gender				
Female	0	0.02		
Male	8.1 (1.5, 14.6)			
Albumin (g/L)	-1.1 (-2.2, -0.1)	0.03		
Number of antihypertensive medications	3.3 (-0.5, 7.0)	0.09		
[‡]**Ethnicity**				
Caucasian	0	0.20		
Non-Caucasian	5.2 (2.7, 13.1)			
[(*)] FTI (kg/m^2)	-1.6 (-4.2, 1.1)	0.24		
[(ℓ)] ACR (mg/mmol)	2.9 (-2.1, 8.0)	0.25		
Use of diuretic (furosemide)				
No	0	0.28		
Yes	-4.4 (-12.3, 3.5)			
Use of prednisolone				
No	0	0.28		
Yes	-4.4 (-12.3, 3.5)			
LTI (kg/m^2)	-0.6 (-1.8, 0.5)	0.28		
[(*)] Time post transplantation (years)	1.2 (-1.2, 3.7)	0.31		
Haemoglobin (g/dL)	-0.9 (-3.1, 1.3)	0.42		
Use of calcineurin inhibitor				
No	0	0.53		
Yes	2.6 (-5.6, 10.7)			
Use of BAB				
No	0	0.55		
Yes	-2.1 (-4.4, 7.2)			
Previous episodes of acute rejection				
No	0	0.56		
Yes	-2.4 (-10.3, 5.6)			
Use of CCB				
No	0	0.62		
Yes	1.7 (-5.0, 8.4)			
[†]**Smoking status**				
Never smoked	0	0.69		
Ex-smoker / Current smoker	1.4 (-5.5, 8.3)			
[(*)] eGFR (mL/min)	-0.2 (-1.2, 0.8)	0.71		
Use of AAB				
No	0	0.74		
Yes	1.2 (-5.9, 8.3)			
[(ℓ)] hsCRP (mg/L)	0.9 (-6.0, 7.8)	0.80		
Use of adjunctive antiproliferative agents				
No	0	0.95		
Yes	0.4 (-10.2, 11.0)			
R^2 value from final model			69%	

[§]Results in the final multivariate regression model were presented. [∞]CI = Confidence Interval.
[†]For the purpose of statistical analysis, smoking status was arranged into 2 categories, "non-smoker" versus "the combination of current smoker and ex-smoker", 63% and 37% of patients respectively. [‡]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 77% "Caucasian" versus 23% "Non-Caucasian".
[(*)] Coefficients reported for a 5-unit increase in explanatory variable. [(**)] Coefficients reported for a 10-unit increase in explanatory variable. [(***)] Coefficients reported for a 50-unit increase in explanatory variable. [(ℓ)] Variable analysed on the log scale (base 10).
Abbreviations: %VE=Percentage Volume Expansion; NODAT=New Onset Diabetes After Transplantation; Pre-DM=Presence of Diabetes Mellitus pre-transplantation; ACEI=Angiotensin-Converting-Enzyme Inhibitor; ARB=Angiotensin-Receptor Blocker; FTI=Fat Tissue Index; ACR=Albumin : Creatinine Ratio; LTI= Lean Tissue Index; BAB=Beta-Adrenergic Blocker; CCB=Calcium Channel Blocker; eGFR=estimated Glomerular Filtration Rate; AAB=Alpha-Adrenergic Blocker; hsCRP=high-sensitivity C-Reactive Protein.

Table 3.5. Predictors of Diastolic Blood Pressure

	Univariate Analysis		Multivariate Analysis[§]	
	Regression Coefficient (95% CI[α])	***p*-value**	**Regression Coefficient (95% CI[α])**	***p*-value**
[(*)] %VE	5.0 (3.7, 6.2)	<0.001	4.9 (3.7, 6.2)	<0.001
[(***)] Sodium intake (mg)	0.2 (0.1, 0.3)	<0.01		
Use of ACEI / ARB No Yes	 0 5.3 (0.7, 9.9)	 0.02		
Albumin (g/L)	-0.8 (-1.5, -0.1)	0.03		
[(**)] Age (years)	1.7 (0.1, 3.3)	0.04		
Presence of diabetes Non-diabetic NODAT Pre-DM	 0 3.7 (0.2, 7.2) 4.9 (-3.6, 13.4)	 0.04		
Gender Female Male	 0 4.7 (0.0, 9.3)	 0.05		
[‡]**Ethnicity** Caucasian Non-Caucasian	 0 4.9 (-0.6, 10.4)	 0.08		
Number of antihypertensive medications	2.4 (-0.3, 5.0)	0.08		
[(***)] Fluid intake (mL)	0.1 (-0.1, 0.2)	0.16		
Use of BAB No Yes	 0 2.8 (-3.1, 4.8)	 0.16		
Use of calcineurin inhibitor No Yes	 0 4.0 (-1.7, 9.6)	 0.16		
[(*)] Time post transplantation (years)	1.1 (-0.6, 5.6)	0.21		
[†]**Smoking status** Never smoked Ex-smoker / Current smoker	 0 2.9 (-1.9, 7.7)	 0.23		
[(*)] FTI (kg/m^2)	-1.1 (-3.0, 0.7)	0.24		
Use of CCB No Yes	 0 2.3 (-2.4, 7.0)	 0.34		
Use of adjunctive antiproliferative agents No Yes	 0 3.2 (-4.1, 10.6)	 0.39		
[(*)] eGFR (mL/min)	-0.2 (-0.9, 0.5)	0.50		
Use of AAB No Yes	 0 1.6 (-3.3, 6.5)	 0.53		
Use of diuretic (furosemide) No Yes	 0 1.8 (-4.6, 8.2)	 0.58		
Use of prednisolone No Yes	 0 -1.5 (-7.1, 4.0)	 0.59		
[(l)] hsCRP (mg/L)	1.2 (-3.6, 6.0)	0.62		
[(l)] ACR (mg/mmol)	-0.6 (-4.1, 3.0)	0.75		
Haemoglobin (g/dL)	-0.2 (-1.7, 1.3)	0.77		
Previous episodes of acute rejection No Yes	 0 0.4 (-5.1, 6.0)	 0.87		
LTI (kg/m^2)	0.0 (-0.8, 0.8)	0.91		
R^2 value from final model			35%	

[§]Results in the final multivariate regression model were presented. [α]CI = Confidence Interval.
[†]For the purpose of statistical analysis, smoking status was arranged into 2 categories, "non-smoker" versus "the combination of current smoker and ex-smoker", 63% and 37% of patients respectively. [‡]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 77% "Caucasian" versus 23% "Non-Caucasian".
[(*)] Coefficients reported for a 5-unit increase in explanatory variable. [(**)] Coefficients reported for a 10-unit increase in explanatory variable. [(***)] Coefficients reported for a 50-unit increase in explanatory variable. [(l)] Variable analysed on the log scale (base 10).
Abbreviations: %VE=Percentage Volume Expansion; ACEI=Angiotensin-Converting-Enzyme Inhibitor; ARB=Angiotensin-Receptor Blocker; NODAT=New Onset Diabetes After Transplantation; Pre-DM=Presence of Diabetes Mellitus pre-transplantation; BAB=Beta-Adrenergic Blocker; FTI=Fat Tissue Index; CCB=Calcium Channel Blocker; eGFR=estimated Glomerular Filtration Rate; AAB=Alpha-Adrenergic Blocker; hsCRP=high-sensitivity C-Reactive Protein; ACR=Albumin : Creatinine Ratio; LTI= Lean Tissue Index.

3.4.4 NT-proBNP as a Marker of Volume Status and Allograft Function

Median serum NT-proBNP level in this cohort of KTRs was 291.0 (IQR: 65.0-700.4) pmol/L. NT-proBNP levels demonstrated a positively skewed distribution and underwent logarithmic transformation prior to parametric analysis. On univariate analysis, higher %VE, lower eGFR, and reduced haemoglobin level were associated with higher values for NT-proBNP (**Table 3.6**). In the multivariate analysis, increasing %VE (Ratio, R = 1.16; 95% CI = 1.03, 1.29; p=0.01), decreasing eGFR (R = 0.95; 95% CI = 0.90, 0.99; p=0.03), and lower haemoglobin level (R = 0.74; 95% CI = 0.58, 0.96; p=0.02) retained significant associations with NT-proBNP. In addition, the absence of a CCB prescription (R = 0.63; 95% CI = 0.45, 0.89; p<0.01) and either current or previous smoking history (R = 1.46; 95% CI = 1.04, 2.05; p=0.03) were significant predictors of raised NT-proBNP levels in the multivariate model. The relationships of NT-proBNP with %VE and renal allograft function are demonstrated in **Figure 3.5** and **Figure 3.6** respectively. A 21% of the variation in NT-proBNP was explained by the variables in the final multivariate model.

Table 3.6. Predictors of N-Terminal of prohormone B-type Natriuretic Peptide (NT-proBNP)

	Univariate Analysis		Multivariate Analysis[§]	
	Ratio (95% CI[∞])	***p*-value**	**Ratio (95% CI[∞])**	***p*-value**
[(*)] %VE	1.38 (1.07, 1.78)	0.01	1.16 (1.03, 1.29)	0.01
[(*)] eGFR (mL/min)	0.89 (0.80, 0.99)	0.03	0.95 (0.90, 0.99)	0.03
Hb (g/dL)	0.74 (0.57, 0.96)	0.03	0.74 (0.58, 0.96)	0.02
Use of CCB				
No	1	0.09	1	<0.01
Yes	0.84 (0.53, 1.05)		0.63 (0.45, 0.89)	
[(ℓ)] ACR (mg/mmol)	1.24 (0.96, 1.60)	0.10		
Use of adjunctive antiproliferative agents				
No	1	0.11		
Yes	0.85 (0.40, 1.10)			
[†]**Smoking status**				
Never smoked	1	0.12	1	0.03
Ex-smoker / Current smoker	1.16 (0.93, 1.84)		1.46 (1.04, 2.05)	
LTI (kg/m^2)	0.96 (0.91, 1.02)	0.20		
[(**)] Age (years)	1.20 (0.91, 1.59)	0.20		
[(*)] Time post transplantation (years)	1.20 (0.91, 1.60)	0.20		
[‡]**Ethnicity**				
Caucasian	1	0.21		
Non-Caucasian	0.56 (0.23, 1.40)			
Use of prednisolone				
No	1	0.29		
Yes	0.17 (0.01, 4.75)			
[(ℓ)] hsCRP (mg/L)	0.83 (0.58, 1.19)	0.31		
Gender				
Female	1	0.33		
Male	0.68 (0.32, 1.47)			
Use of AAB				
No	1	0.41		
Yes	1.09 (0.82, 1.64)			
Presence of diabetes				
Non-diabetic	1	0.42		
NODAT	2.02 (0.69, 5.96)			
Pre-DM	1.32 (0.37, 4.70)			
Use of BAB				
No	1	0.45		
Yes	0.98 (0.81, 1.28)			
Use of calcineurin inhibitor				
No	1	0.48		
Yes	1.44 (0.52, 4.01)			
[(***)] Fluid intake (mL)	1.07 (0.86, 1.33)	0.53		
Number of antihypertensive medications	0.95 (0.79, 1.16)	0.63		
Alb (g/L)	1.03 (0.91, 1.17)	0.67		
Use of diuretic (furosemide)				
No	1	0.81		
Yes	1.02 (0.67, 1.67)			
Use of ACEI / ARB				
No	1	0.90		
Yes	1.05 (0.48, 2.28)			
[(*)] FTI (kg/m^2)	1.01 (0.88, 1.15)	0.95		
[(***)] Sodium intake (mg)	1.01 (0.84, 1.20)	0.95		
Previous episodes of acute rejection				
No	1	0.98		
Yes	1.01 (0.36, 2.89)			
R^2 value from final model			21%	

[§]Results in the final multivariate regression model were presented. [∞]CI = Confidence Interval.
[†]For the purpose of statistical analysis, smoking status was arranged into 2 categories, "non-smoker" versus "the combination of current smoker and ex-smoker", 63% and 37% of patients respectively. [‡]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 77% "Caucasian" versus 23% "Non-Caucasian".
[(*)] Coefficients reported for a 5-unit increase in explanatory variable. [(**)] Coefficients reported for a 10-unit increase in explanatory variable. [(***)] Coefficients reported for a 50-unit increase in explanatory variable. [(ℓ)] Variable analysed on the log scale (base 10).
Abbreviations: %VE=Percentage Volume Expansion; eGFR=estimated Glomerular Filtration Rate; CCB=Calcium Channel Blocker; ACR=Albumin : Creatinine Ratio; LTI= Lean Tissue Index; hsCRP=high-sensitivity C-Reactive Protein; AAB=Alpha-Adrenergic Blocker; NODAT=New Onset Diabetes After Transplantation; Pre-DM=Presence of Diabetes Mellitus pre-transplantation; BAB=Beta-Adrenergic Blocker; ACEI=Angiotensin-Converting-Enzyme Inhibitor; ARB=Angiotensin-Receptor Blocker; FTI=Fat Tissue Index.

Figure 3.5. Association between Extracellular Volume Status (Percentage Volume Expansion, %VE) and Level of NT-proBNP

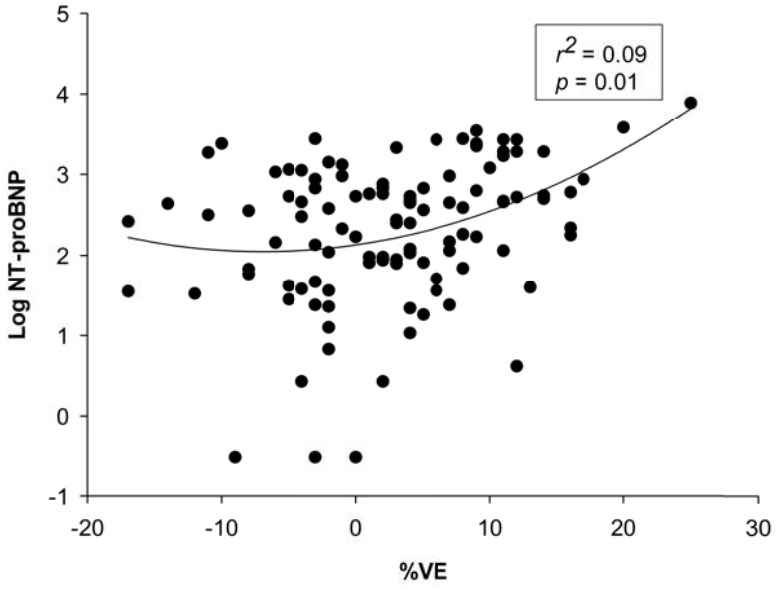
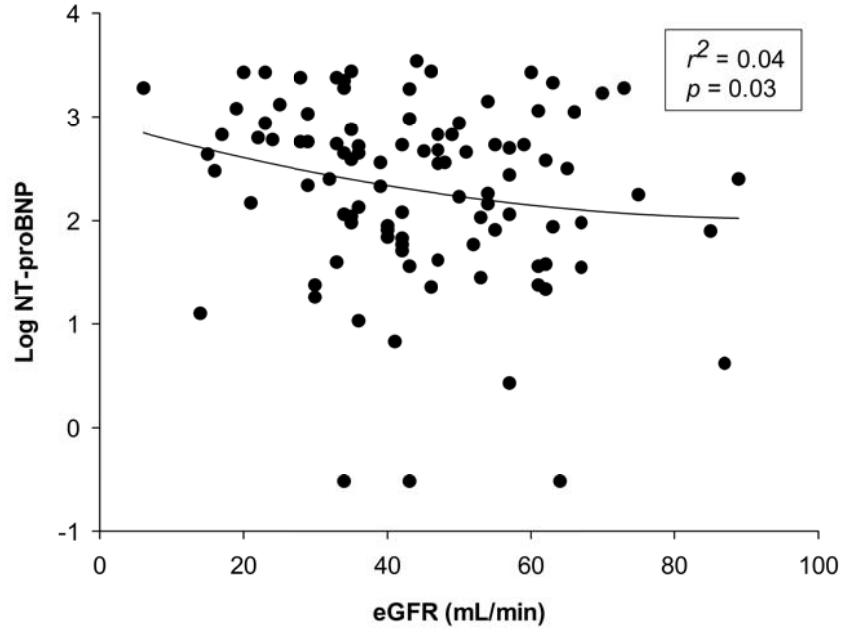


Figure 3.6. Association between Renal Function and Level of NT-proBNP



3.5 Discussion

This is the first study to address in detail the prevalence, predictors, and consequences of hypervolemia in KTRs. Based on the previously established definition of hypervolemia, 30% of KTRs were hypervolemic, of whom 5% suffered from severe hypervolemia. Despite a lower incidence when compared to continuous ambulatory peritoneal dialysis[3] or haemodialysis[30] populations, this degree of hypervolemia was unexpected, and is noteworthy in light of the specific selection of a clinically and biochemically stable kidney transplant cohort for this study. Hypervolemia was associated with increasing sodium intake, highlighting an important target for intervention. Dietary sodium restriction has not been formally examined in KTRs, but has gained attention in other contexts[31]. The daily sodium intake in the current cohort of KTRs was 2725mg (118mmol), lower than previously reported (3588 mg/156mmol per day)[18], but well above the recommendation of Dietary Approach to Stop Hypertension (DASH) guideline (1500-2300 mg/65-100 mmol per day)[32]. Collectively, these findings suggest that reducing sodium intake in line with the DASH diet should be recommended for KTRs presented with hypervolemia.

A recent study demonstrated a relationship between increased sodium intake and higher blood pressure, although the contribution of extracellular volume status was not evaluated therein[18]. Whilst the results of the current study confirmed a univariate association between sodium intake and blood pressure, this relationship did not hold when the effect of extracellular volume status was taken into account. Indeed, hypervolemia was identified as the only independent risk factor for elevated blood pressure, which has a recognised impact

upon long-term patient and graft outcomes[33-35]. Although this relationship between hypervolemia and elevated blood pressure resonates with findings in dialysis patients[2,3,36], this has not been previously demonstrated in KTRs.

Pertinently, the American Society of Hypertension[37] acknowledges the possible role of volume expansion and potential therapeutic role of diuretics in post-transplant hypertension. Other expert review articles also recognise volume expansion as a potential risk factor, although remain guarded over the use of diuretic therapies[38,39]. In the current study, the prevalence of diuretic usage was only 15%, with furosemide being the only diuretic prescription. No association between furosemide usage and volume status was observed, but this may be a reflection of "confounding by indication". Furthermore, the median dosage of furosemide in this study cohort was 40mg, a dosage which may be insufficient to target hypervolemia in KTRs with a mean eGFR of 44 mL/min[40]. Such confounding may also be responsible for the association between renin-angiotensin system blockers (ACEI and ARB), and volume overload, mean arterial pressure, systolic and diastolic blood pressure, although these associations did not persist in the adjusted analysis.

In regard to other determinants of extracellular volume status, an inverse association between fat mass and volume status was observed in the current study. This phenomenon has been demonstrated in a non-transplanted population[25], which now extends to the kidney transplant population. Interestingly renal dysfunction was not identified as one of the predictors of volume status and blood pressure in this study. However, based on the statistical point estimates, eGFR displayed inverse associations with volume overload,

mean arterial pressure, systolic and diastolic blood pressure, and the absence of statistical significance may reflect the study size and range of renal function encountered in this study, and certainly the current results do not exclude the importance of renal function in this setting.

Based on the findings from this study, a multi-modality approach involving the DASH diet and increased diuretic usage may be beneficial in the treatment of volume overload and hypertension in KTRs. Previous studies have shown that synergistic hypotensive effects were achieved when sodium restriction and diuretics were used in combination[41,42]. In particular, the DASH diet, comprising high fruits, vegetables, whole-grains, and low-fat dairy products; and low fat, refined carbohydrates and sodium, has been shown to substantially lower blood pressure in large, randomised, controlled trials[32,43,44]. It has also been proven to potentiate the benefits of antihypertensive medication treatment[43]. Diuretic therapy should be titrated in accordance with volume status and blood pressure. Crucially, meticulous monitoring of both volume status and blood pressure should be in place to ensure optimal management of hypertension in KTRs. In particular, increasing fluid intake is often promoted particularly in the early period post-transplantation, yet also displayed univariate association with volume overload, mean arterial pressure and systolic blood pressure, thereby highlighting the importance of judicious assessment of extracellular volume in these patients. Indeed, the findings from this study suggest that more widespread and accurate evaluation of extracellular volume status may facilitate the clinical management of KTRs, and sets the scene for interventional measures which have shown benefit in a recent haemodialysis-based trial[45]. It is hoped that the findings of this study will highlight the importance of extracellular volume status assessment in the

management of hypertension, a tool yet to be incorporated into international guidelines from Kidney Disease: Improving Global Outcomes (KDIGO)[46], European Renal Best Practice (ERBP) Work Group[47] and United Kingdom Renal Association (UKRA)[48].

The independent association between an objective measure of hypervolemia and raised NT-proBNP level is a novel and noteworthy finding of this study, confirming and extending findings from the non-transplanted populations, predominantly patients undergoing dialysis[11-14]. Additionally, reduced allograft function was independently associated with raised NT-proBNP levels, in keeping with findings from previous studies among KTRs[15,16], due to a reduced renal clearance of NT-proBNP. Although previous studies have suggested NT-proBNP as a marker of cardiac dysfunction in dialysis patients[49,50], interpretation of these studies is limited by a lack of concomitant and objective measurement of volume status, and by the variation in NT-proBNP levels depending on the timing of blood sampling relative to dialysis treatment. In fact, the most detailed study in dialysis, which employed standardised sampling times, simultaneous echocardiography, and bio-impedance based extracellular fluid volume measurements, showed that NT-proBNP was dependent on volume overload *per se*, rather than the echocardiographic parameters of cardiac dysfunction[11,12]. The single study in KTRs addressing the relationship between echocardiography and NT-proBNP level likewise found no relationship between the two parameters[15]. Whilst cardiac function was not assessed in the current study, the findings from this study certainly support the concept that NT-proBNP levels reflect volume status. However, an important caveat is the high variability in the relationship between NT-proBNP levels and both %VE and eGFR. This suggests that although NT-proBNP may be a marker of volume expansion and renal

dysfunction, it cannot yet be considered as an accurate surrogate for either. The utility of serial NT-proBNP measurements cannot be discerned by the current study.

Other factors independently associated with elevated NT-proBNP levels included smoking (current or ex- smoker, or both), reduced level of haemoglobin, and the absence of CCB prescription as an antihypertensive agent. Although the mechanisms behind these findings are not fully understood and were not the focus of the present study, these results are in keeping with previous observations in non-transplant cohorts[51-57], and reflecting the "face validity" of the current findings.

This study has limitations that should be acknowledged. It represents a single-centre experience, and validations of the findings are needed in other cohorts. Also, transplant renal artery stenosis is a potential cause for post-transplant hypertension and volume expansion. However, it was not systematically sought in this study due to an estimated prevalence of only 5-10%[58], and the lack of detection is unlikely to have confounded the results. The cross-sectional nature of this study is unable to establish the causal relationship between predictor and outcome variables. Long-term longitudinal follow-up and experimental interventions are now required to robustly evaluate the impact of extracellular volume status on relevant end-points in kidney transplantation.

In summary, this is the first study to investigate the prevalence, predictors, consequences, and biochemical markers of hypervolemia in KTRs. It points at potential targets for intervention, thereby expanding future avenues for basic and clinical research.

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PREDICTORS AND CONSEQUENCES OF FATIGUE IN PREVALENT KIDNEY TRANSPLANT RECIPIENTS

Chapter 4

Effects of Body Composition on Clinical and Quality of Life Outcomes in Kidney Transplant Recipients

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CHAPTER 4: PREDICTORS AND CONSEQUENCES OF FATIGUE IN PREVALENT KIDNEY TRANSPLANT RECIPIENTS

4.1 Abstract

Background and Objectives: Fatigue has been under-investigated in clinically stable kidney transplant recipients (KTRs). The objectives of this study were to investigate the nature, severity, prevalence and clinical awareness of fatigue in medically stable KTRs, examine the impact of fatigue on quality of life (QoL), and explore the underlying causes of post-transplantation fatigue.

Materials and Methods: This single-centre cross-sectional study enrolled 106 stable KTRs. Multi-Dimensional Fatigue Inventory-20 (MFI-20) was used to measure 5 fatigue dimensions: General Fatigue, Physical Fatigue, Reduced Activity, Reduced Motivation, and Mental Fatigue. Clinical awareness of fatigue was determined by reviewing medical records. QoL was assessed by Medical Outcomes Study Short Form-36 (SF-36) Questionnaire. Demographic, clinical, psychosocial and behavioural parameters were evaluated as fatigue predictors.

Results: Fatigue was found in 59% of KTRs. Only 13% had this symptom documented in medical records. Fatigue in KTRs was in the same range as chronically unwell patients,

with Physical Fatigue, Reduced Activity and Reduced Motivation approached levels observed in chronic fatigue syndrome. All fatigue dimensions significantly and inversely correlated with QoL ($p<0.001$ for all associations). Demographic predictors were male, older age and non-Caucasian ethnicity ($p\leq0.05$ for all associations). Clinical predictors included elevated high-sensitivity C-Reactive Protein (hsCRP, inflammation), decreased estimated Glomerular Filtration Rate (eGRF, graft dysfunction), and reduced Lean Tissue Index (LTI) ($p\leq0.05$ for all associations). Psychosocial and behavioural predictors were inferior sleep quality, anxiety and depression ($p<0.01$ for all associations).

Conclusions: Fatigue is common and pervasive in clinically stable KTRs. It is strongly associated with reduced QoL. This study identified modifiable fatigue predictors, and sets the scene for future interventional studies.

4.2 Introduction

Fatigue is an important patient-reported outcome in many medical conditions[1,2] and involves physical (e.g. feeling exhausted and tired), cognitive (e.g. impaired concentration), emotional (e.g. lack of motivation) and functional components[3]. It is often medically unexplained[4] and persistent[2], and interferes with an individual's ability to function in important roles (e.g. work, family, social life, self-care)[5]. As a corollary, fatigue can have a major negative impact upon quality of life (QoL)[6].

In chronic dialysis patients, fatigue is frequently reported as a pervasive and distressing symptom[7-9]. For many of these patients, kidney transplantation is the preferred modality of renal replacement therapy[10]. Kidney transplantation increases long term survival[10], improves QoL[11], demonstrates cost benefits[12], and results in enhanced sense of well-being. Consequently, it might be assumed that fatigue no longer feature as a major problem following kidney transplantation, but in fact there has been very little research to either confirm or refute this assumption. Only one study has specifically examined fatigue following transplantation[13], noting the symptom was reported in 59% of kidney transplant recipients (KTRs) and that it negatively impacted on virtually every aspect of the QoL[13]. Poor sleep quality, mood disturbance and raised body mass index (BMI) were identified as significant predictors for post-transplantation fatigue[13]. However, other potentially modifiable contributors to fatigue such as body composition, inflammation, renal function, and other biochemical markers were not examined and warrant further investigation.

Greater insight into fatigue severity, its impact on QoL, and its possible underlying causes are all pre-requisites for developing interventions to combat this symptom. In addition, it is also important to know the extent to which clinicians are aware of the problem. Therefore, the objectives of this study were to determine the nature, severity, prevalence and clinical awareness of post-transplantation fatigue in a clinically stable prevalent kidney transplant cohort. Additionally, this study aimed to examine the impact of this symptom upon QoL, and to explore the predictors of post-transplantation fatigue.

4.3 Materials and Methods

4.3.1 Participants and Study Design

Stable KTRs beyond 1 year post-transplantation, with stable graft function (<10% increase in serum creatinine over preceding 6 months) were recruited to this cross-sectional study from the renal transplant outpatient clinic at Queen Elizabeth Hospital Birmingham UK, between July 2010 and April 2012. Exclusion criteria included episodes of acute rejection within the last 6 months, evidence of sepsis in the last 6 weeks, known active malignancy or chronic infection, preceding diagnosis of psychiatric disorder or chronic fatigue syndrome, and history of thyroid disease or adrenal insufficiency.

Of 114 eligible patients approached, n=6 refused to participate and n=2 did not attend the research visit. Reasons for declining entry were work commitment (n=4) and participation in other studies (n=2). The study was approved by the local research ethics committee, and was conducted in accordance with the principles of the Declaration of Helsinki.

Patients attended the research visit following a 10-hour overnight fast. The order of tests was standardised. A fasting blood sampling was taken, followed by a light breakfast before bio-impedance body composition assessment, and self-completion of questionnaires under supervision of the researcher (see below).

4.3.2 Fatigue Measurement

Severity and nature of fatigue were determined using the MFI-20, which is a 20-item self-report questionnaire that measures fatigue in 5 primary dimensions: General Fatigue; Physical Fatigue; Reduced Activity; Reduced Motivation; and Mental Fatigue. The physical aspects of fatigue are captured by General Fatigue and Physical Fatigue; and the behavioural, emotional and cognitive aspects of fatigue are represented by Reduced Activity, Reduced Motivation, and Mental Fatigue[14]. MFI-20 is among the most commonly utilized measures of fatigue in patient studies. It shows good reliability in patients with end-stage renal disease[15], and demonstrates validity in several medical conditions[16]. Each fatigue dimension is assessed by 4 items, each using a 5-point Likert scale. Scores for each dimension range from 4 to 20, with higher scores indicating greater

fatigue. General Fatigue describes the individual's reported functioning (e.g. "I feel tired"); Physical Fatigue refers to physical sensations of tiredness (e.g. "physically I feel I am in a bad condition"); Reduced Activity describes the reduction in activity (e.g. "I get little done"); Reduced Motivation describes the individual's lack of motivation or initiative (e.g. "I don't feel like doing anything"); and Mental Fatigue signifies cognitive symptoms such as concentration difficulties (e.g. "my thoughts easily wander").

A consensus definition for clinically meaningful fatigue is lacking. In this study, KTRs were considered fatigued if scores for any dimension was ≥ upper 95th percentile for the general population as reported by Lin[16] (General Fatigue ≥ 15; Physical Fatigue ≥ 14; Reduced Activity ≥ 12; Reduced Motivation ≥ 12; Mental Fatigue ≥ 13). The present data were also compared with two other clinical groups, similarly derived from Lin[16], namely patients with chronic fatigue syndrome (CFS) and patients with other chronic (> 6 months) diseases.

Reporting of fatigue by clinicians was assessed by retrieving medical records for the 4 clinic visits prior to participation in this study. Medical records were reviewed in search of any description of fatigue or synonymous term (e.g. exhaustion; lethargy; sleepiness; weariness; tiredness; weakness; sluggish; and lack of energy).

4.3.3 Quality of Life Assessment

QoL was assessed using the Medical Outcomes Study Short Form 36 (SF-36) questionnaire. The SF-36 is among the most commonly used instruments to assess QoL and is regarded as valid and reliable in different population groups, and with both medical and psychiatric conditions[17,18] including patients undergoing renal replacement therapy[19]. SF-36 consists of 36 questions grouped into 8 life domains: physical functioning; social functioning; role limitation due to physical problems; role limitation due to emotional problems; mental health; energy and vitality; bodily pain; and general health perception. For each tested domain, item scores were coded, summed, and transformed into a scale from 0 (worst QoL) to 100 (best QoL) using the standard SF-36 scoring algorithm[18]. As well as the total score for QoL, these sub-scales are subsumed under 2 subscores, i.e. physical health summary score and mental health summary score. Physical health is represented by the physical functioning, role limitation due to physical problems, bodily pain, general health perception, and energy and vitality subscales of SF-36. Mental health is represented by the mental health, role limitation due to emotional problems, social functioning, energy and vitality, and general health perception subscales of SF-36[20].

4.3.4 Factors Associated with Fatigue

4.3.4.1 Demographics and Clinical Parameters

Age, gender, marital status, ethnicity, and time post-transplantation were collected from patients' medical records. Smoking status (never smoked, current smoker, ex-smoker) and alcohol intake (units per week) were collected by questionnaire. Co-morbidity was assessed by Index of Co-Existing Disease (ICED), using the algorithm described by the Hemodialysis (HEMO) Study[21], with data extracted from patients' medical records. Presence of diabetes, either pre-transplantation (pre-DM) or new onset diabetes after transplantation (NODAT), prior acute rejection episodes, and immunosuppressive medication usage were retrieved from patients' medical records.

4.3.4.2 Laboratory Parameters

Fasting blood sample was taken for analysis of high sensitive C-reactive protein (hsCRP), haemoglobin and estimated glomerular filtration rate (eGFR) derived using the 4-variable modification of diet in renal disease equation[22].

4.3.4.3 Body Composition

Body composition was assessed by multi-frequency bio-impedance spectroscopy, Body Composition Monitor (BCM), made by Fresenius Medical Care, Bad Homburg, Germany. Measurements were carried out in a standard manner while the patient was lying supine in a flat and non-conductive bed. The inbuilt physiological body composition model measures whole-body bio-impedance spectroscopy at 50 frequencies (5 to 1000 kHz) via electrodes placed on the wrist (proximal to the transverse) and ankle (arch on the superior side of the foot). Body composition data including Lean Tissue Index (LTI, kg/m^2) and Fat Tissue Index (FTI, kg/m^2) were displayed after each measurement. This device has been validated against reference methods including dual-energy X-ray absorptiometry (DEXA), air displacement plethysmography and 4-compartment modelling[23]

4.3.4.4 Self-Reported Outcome Measures

4.3.4.4.1 Anxiety and Depression

Anxiety and Depression were assessed using the Hospital Anxiety and Depression Scale (HADS)[24]. HADS was developed to identify anxiety and depression among patients in non-psychiatric hospital settings. It has been validated against clinical diagnoses of anxiety and depression[25] including patients with end-stage renal disease[26]. HADS is a self-

administered 14-item scale, with 7 items measuring anxiety and 7 items measuring depression. Items were scored on a 4-point scale ranging from 0 to 3. The sum-scores for anxiety and depression range from 0 to 21, with higher scores indicating higher levels of anxiety or depression.

4.3.4.4.2 Sleep Quality

Sleep quality was assessed by the Pittsburgh Sleep Quality Index (PSQI)[27]. The PSQI is valid, reliable and widely used[27,28]. In particular, it demonstrated reliability and validity in KTRs[29]. PSQI consists of a 24-item questionnaire measuring sleep disturbances during the previous month in 7 dimensions: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and day-time dysfunction. Each dimension generates a component score, ranging from subscale scores 0 to 3. The addition of the 7 component scores yields a global score of subjective sleep quality ranging from 0 to 21, with higher scores indicating worsening subjective sleep quality.

4.3.5 Statistical Analysis

Statistical analysis was performed using SPSS Statistics 21 (Chicago, IL). Results are presented as mean ± standard deviation (SD) or median (interquartile range, IQR).

Independent sample *t*-tests were used to compare continuous variables, and Pearson correlation coefficients to assess relationship.

Linear regression analysis was used to determine predictor variables associated with different domains of fatigue. The analyses were performed in two stages. Initially, the effect of each variable was examined in a series of univariate analyses. Subsequently, the joint effect of variables was examined in a multivariate analysis, using a backwards selection procedure to derive the final model. A type 1 error rate ≤5% ($p \leq 0.05$) was considered significant. Results for General Fatigue, Physical Fatigue and Reduced Activity revealed normal distributions and were analysed on the original scale of measurement. Results for Reduced Motivation and Mental Fatigue demonstrated positively skewed distributions and underwent logarithmic transformation prior to analysis. In the multivariate regression analyses, only the explanatory variables with univariate *p*-values of <0.20 were included. The figures reported in **Tables 4.5**, **4.6**, **4.7**, **4.8** and **4.9**, were regression coefficients or odds ratios, and their corresponding confidence intervals. The regression coefficients and odds ratios describe the change in fatigue for the described increase (or category) of the predictor variable.

4.4 Results

4.4.1 Patient Characteristics

The characteristics of the studied population are shown in **Table 4.1**.

4.4.2 Relationship between Different Domains of Fatigue

The correlations between different domains of fatigue are shown in **Table 4.2**.

Table 4.2: Correlation between Different Domains of Fatigue

Pearson correlation (r); p-value for each correlation

	General Fatigue	**Physical Fatigue**	**Reduced Activity**	**Reduced Motivation**	**Mental Fatigue**
General Fatigue		r=0.74; p<0.001	r=0.68; p<0.001	r=0.65; p<0.001	r=0.46; p<0.001
Physical Fatigue	r=0.74; p<0.001		r=0.76; p<0.001	r=0.69; p<0.001	r=0.34; p<0.001
Reduced Activity	r=0.68; p<0.001	r=0.76; p<0.001		r=0.62; p<0.001	r=0.32; p=0.001
Reduced Motivation	r=0.65; p<0.001	r=0.69; p<0.001	r=0.62; p<0.001		r=0.46; p<0.001
Mental Fatigue	r=0.46; p<0.001	r=0.34; p<0.001	r=0.32; p=0.001	r=0.46; p<0.001	

Table 4.1: Population Characteristics

	Characteristics
Sample size	n = 106
†**Mean age**	51 ± 14 years
Gender	56% Male; 44% Female
Marital status	Single 21%; Married 71%; Divorced/Widowed 8%
Immunosuppressive medication usage	
Calcineurin inhibitor	89% (55% Tacrolimus, 34% Cyclosporin)
Adjunctive antiproliferatives	87% (58% Mycophenolate Mofetil, 29% Azathioprine)
Prednisolone	74%
Dosage of immunosuppressive medications	
‡Median dose of Tacrolimus	4.0 (2.5-6.0) mg/day
‡Median dose of Cyclosporin	150 (150-200) mg/day
†Mean dose of Mycophenolate Mofetil	987 ± 392 mg/day
†Mean dose of Azathioprine	77 ± 36 mg/day
†Mean dose of Prednisolone	5.2 ± 1.0 mg/day
***Ethnicity**	
Caucasian	76%
Afro-Caribbean	7%
Asian	15%
Other	2%
‡**Median time post transplantation**	6.5 (3.0-14.0) years
‡**Median alcohol intake per week**	0.0 (0.0-3.0) units
Smoking status	
Never smoked	63%
Current smoker	7%
Ex-smoker	30%
†**Mean ICED score (co-morbidity)**	2.1 ± 0.4
Score = 1	2%
Score = 2	85%
Score = 3	13%
†**Mean haemoglobin**	12.6 ± 1.6 g/dl
‡**Median hsCRP**	2.5 (1.0-4.9) mg/l
†**Mean eGFR**	43.9 ± 18.5 ml/min
Body composition	
†Mean LTI	13.9 ± 3.0 kg/m^2
†Mean FTI	14.2 ± 6.2 kg/m^2
HADS	
‡Median anxiety score	6.0 (2.5-9.5)
‡Median depression score	3.0 (1.0-7.0)
PSQI	
†Mean global score	7.2 ± 4.1

†Normally distributed data, results expressed as mean ± SD. ‡Non-normally distributed data, results expressed as median (IQR).
*For the purpose of the statistical analysis, the ethnicity of 2% of patients classified as "Other" was grouped as "Caucasian". **For the purpose of the statistical analysis, ICED scores were arranged into 2 categories (≤2 versus >2, 87% and 13% of patients respectively).
Abbreviations: ICED=Index of Coexisting Disease; hsCRP=high-sensitivity C-Reactive Protein; eGFR=estimated Glomerular Filtration Rate; LTI=Lean Tissue Index; FTI=Fat Tissue Index; HADS=Hospital Anxiety and Depression Scale; PSQI=Pittsburgh Sleep Quality Index.

4.4.3 Nature, Severity and Prevalence of Fatigue

The nature and severity of fatigue are shown in **Table 4.3**, alongside normative data obtained from Lin's study[16]. Comparison of the MFI-20 subscales indicated that significant differences were found between the following dimensions: General Fatigue and Reduced Activity (p=0.002); General Fatigue and Reduced Motivation (p<0.001); General Fatigue and Mental Fatigue (p<0.001); Physical Fatigue and Reduced Activity (p=0.002); Physical Fatigue and Reduced Motivation (p<0.001); Physical Fatigue and Mental Fatigue (p<0.001); Reduced Activity and Reduced Motivation (p<0.001); Reduced Activity and Mental Fatigue (p<0.001). The differences between the following dimensions were not statistically significant: General Fatigue and Physical Fatigue (p=0.881); Reduced Motivation and Mental Fatigue (p=0.801). In summary, physical aspects of fatigue (General Fatigue and Physical Fatigue) in KTRs were scored significantly higher than behavioural, emotional and cognitive aspects of fatigue (Reduced Activity, Reduced Motivation and Mental Fatigue). Overall, the mean MFI-20 scores in KTRs exceeded the mean scores found in the general population and were comparable with the mean scores reported by chronically unwell patients. In fact, the mean scores for Physical Fatigue, Reduced Activity and Reduced Motivation approached the mean values reported by CFS patients.

Based on the dichotomous classification of fatigue (≥ upper 95th percentile for the general population, see **Materials and Methods**), a total of 63 patients (59%) reported fatigue on at least one MFI-20 subscale. Of these 63 patients, 24% experienced General Fatigue,

38% displayed Physical Fatigue, 35% demonstrated Reduced Activity, 29% indicated Reduced Motivation, and 25% revealed Mental Fatigue. Importantly, only 8 patients (13%) had complaints of fatigue documented in medical records.

Table 4.3: Nature and Severity of Fatigue

Mean Fatigue Score ± SD by Dimensions

	Transplant Patients	**Healthy Population†**	**Chronically Unwell Patients†**	**CFS-like Patients†**
General Fatigue	11.78 ± 4.05	8.42 ± 3.59	12.84 ± 3.84	16.38 ± 2.73
Physical Fatigue	11.73 ± 4.74	7.77 ± 3.36	10.39 ± 3.76	13.63 ± 3.79
Reduced Activity	10.69 ± 4.70	6.76 ± 2.67	9.06 ± 3.75	11.32 ± 4.37
Reduced Motivation	9.36 ± 3.61	6.82 ± 2.91	9.29 ± 3.35	11.95 ± 3.53
Mental Fatigue	9.67 ± 4.54	7.23 ± 3.07	10.98 ± 4.00	13.77 ± 3.77

†Original unpublished normative data provided by Lin et al[16].
Abbreviation: CFS=Chronic Fatigue Syndrome

4.4.4 Fatigue and Quality of Life

As shown in **Table 4.4,** all dimensions of fatigue (General Fatigue, Physical Fatigue, Reduced Activity, Reduced Motivation and Mental Fatigue) were significantly and inversely correlated with all aspects of QoL including SF-36 physical health, SF-36 mental health and SF-36 total score. To exclude the confounding effect of the SF-36 "energy and vitality" subscale, which is a general measure of fatigue within the SF-36[30], results were reanalysed after removal of this subscale, results were comparable after this exclusion (shown in parentheses in **Table 4.4**).

Table 4.4: Association between Fatigue and Quality of Life

	SF-36 Physical Health	**SF-36 Mental Health**	**SF-36 Total Score**
General Fatigue	†r=-0.68; p<0.001 *(r=-0.62; p<0.001)	†r=-0.70; p<0.001 *(r=-0.63; p<0.001)	†r=-0.68; p<0.001 *(r=-0.64; p<0.001)
Physical Fatigue	†r=-0.78; p<0.001 *(r=-0.74; p<0.001)	†r=-0.71; p<0.001 *(r=-0.65; p<0.001)	†r=-0.74; p<0.001 *(r=-0.72; p<0.001)
Reduced Activity	†r=-0.72; p<0.001 *(r=-0.69; p<0.001)	†r=-0.67; p<0.001 *(r=-0.62; p<0.001)	†r=-0.71; p<0.001 *(r=-0.68; p<0.001)
Reduced Motivation	†r=-0.66; p<0.001 *(r=-0.64; p<0.001)	†r=-0.69; p<0.001 *(r=-0.66; p<0.001)	†r=-0.69; p<0.001 *(r=-0.68; p<0.001)
Mental Fatigue	†r=-0.33; p<0.001 *(r=-0.29; p<0.01)	†r=-0.49; p<0.001 *(r=-0.48; p<0.001)	†r=-0.42; p<0.001 *(r=-0.41; p<0.001)

†Correlation and p-value derived from comparisons between all domains of fatigue and all SF-36 subscales in the analysis.
*Correlation and p-value in parentheses derived from comparisons between all domains of fatigue and SF-36 excluding "energy and vitality" subscale in the analysis.

4.4.5 Factors Predicting Dimensions of Fatigue

Linear regression analyses, to identify predictors of each fatigue dimension, were performed in 3 stages. First, univariate analyses tested the predictive value of each parameter individually. Second, multivariate analyses tested the independent prediction of all parameters. Third, the multivariate analysis was repeated excluding the patient-reported outcome data (HADS and PSQI), thereby focusing on clinical, anthropometric and laboratory parameters.

4.4.5.1 General Fatigue

The univariate analyses are shown in **Table 4.5**. In multivariate analysis, only depression (β=2.8; 95% CI=1.9, 3.7; p<0.001) and inferior sleep quality (β=1.1; 95% CI=0.2, 1.9; p=0.01) were independently associated with General Fatigue (**Figure 4.1** and **Figure 4.2**). Repeating the multivariate analysis excluding HADS and PSQI revealed that increasing time post-transplantation (β=0.6; 95% CI=0.0, 1.1; p=0.04), inflammation (β=1.8; 95% CI=0.3, 3.3; p=0.02), and renal dysfunction (β=-0.4; 95% CI=-0.8, 0.0; p=0.04) were independently associated with increasing General Fatigue.

Table 4.5: Results of Univariate and Multivariate Analyses for General Fatigue

	Univariable Analysis		Multivariable Analysis[†,©]		Multivariable Analysis[§,©]	
	Regression Coefficient (95% CI*)	***P*-value**	**Regression Coefficient (95% CI*)**	***P*-value**	**Regression Coefficient (95% CI*)**	***P*-value**
[a]**Depression**	3.3 (2.5, 4.2)	<0.001	2.8 (1.9. 3.7)	<0.001		
[a]**Sleep quality**	2.3 (1.4, 3.1)	<0.001	1.1 (0.2, 1.9)	0.01		
[a]**Anxiety**	2.0 (1.3, 2.8)	<0.001				
[a]**Time post-transplantation (years)**	0.8 (0.3, 1.4)	0.004			0.6 (0.0, 1.1)	0.04
[c]**hsCRP (mg/L)**	2.2 (0.6, 3.8)	0.008			1.8 (0.3, 3.3)	0.02
[a]**FTI (kg/m^2)**	0.8 (0.2, 1.5)	0.009				
[b]**eGFR (ml/min)**	-0.5 (-0.9, -0.1)	0.02			-0.4 (-0.8, 0.0)	0.04
[b]**Age (years)**	0.6 (0.1, 1.1)	0.03				
LTI (kg/m^2)	-0.3 (-0.5, 0.0)	0.05			-0.2 (-0.5, 0.0)	0.07
Alcohol intake (unit)	-0.2 (-0.5, 0.0)	0.06				
Use of calcineurin inhibitor		0.06				
None	0					
Cyclosporin	0.1 (-0.6, 2.7)					
Tacrolimus	-0.2 (-2.3, 0.1)					
ICED						
≤2	0	0.08				
>2	2.1 (-0.3, 4.5)					
Use of prednisolone						
No	0	0.19				
Yes	-0.1 (-3.0, 0.6)					
Presence of diabetes						
None	0	0.23				
Pre-DM	2.0 (-0.6, 4.5)					
NODAT	-0.8 (-3.0, 1.5)					
Haemoglobin (g/dL)	-0.2 (-0.7, 0.3)	0.37				
Use of adjunctive antiproliferatives						
None	0	0.45				
Mycophenolate Mofetil	-0.2 (-3.1, 0.0)					
Azathioprine	0.1 (-0.8, 1.8)					
Previous episodes of acute rejection						
No	0	0.56				
Yes	0.8 (-1.8, 3.3)					
Marital status						
Single	0	0.65				
[∝]Married	0.2 (-0.4, 3.4)					
[◊]Divorced/Widowed	-0.2 (-5.3, 0.6)					
Ethnicity						
Caucasian	0	0.71				
Asian	0.8 (-1.4, 3.0)					
Afro-Caribbean	-0.6 (-3.8, 2.6)					
Smoking status						
Never smoked	0	0.75				
Current smoker	0.0 (-1.5, 1.8)					
Ex-smoker	0.1 (-2.7, 4.8)					
Gender						
Female	0	0.85				
Male	-0.2 (-1.8, 1.4)					
R^2 value from final model			41%		19%	

[†]All predictor variables were included in multivariable analysis.

[§]Patient-reported outcomes (HADS and PSQI) were excluded in multivariable analysis.

[©]Results in the final multivariable regression model were presented.

*CI = Confidence Interval

[∝]"Living with partner" was classified under the "Married" category.

[◊]"Separated" was classified under the "Divorced/Widowed" category.

[a]Coefficients reported for 5-unit increase in explanatory variable.

[b]Coefficients reported for 10-unit increase in explanatory variable.

[c]Variable analysed on log scale (base 10)

Figure 4.1: Association between General Fatigue and Depression

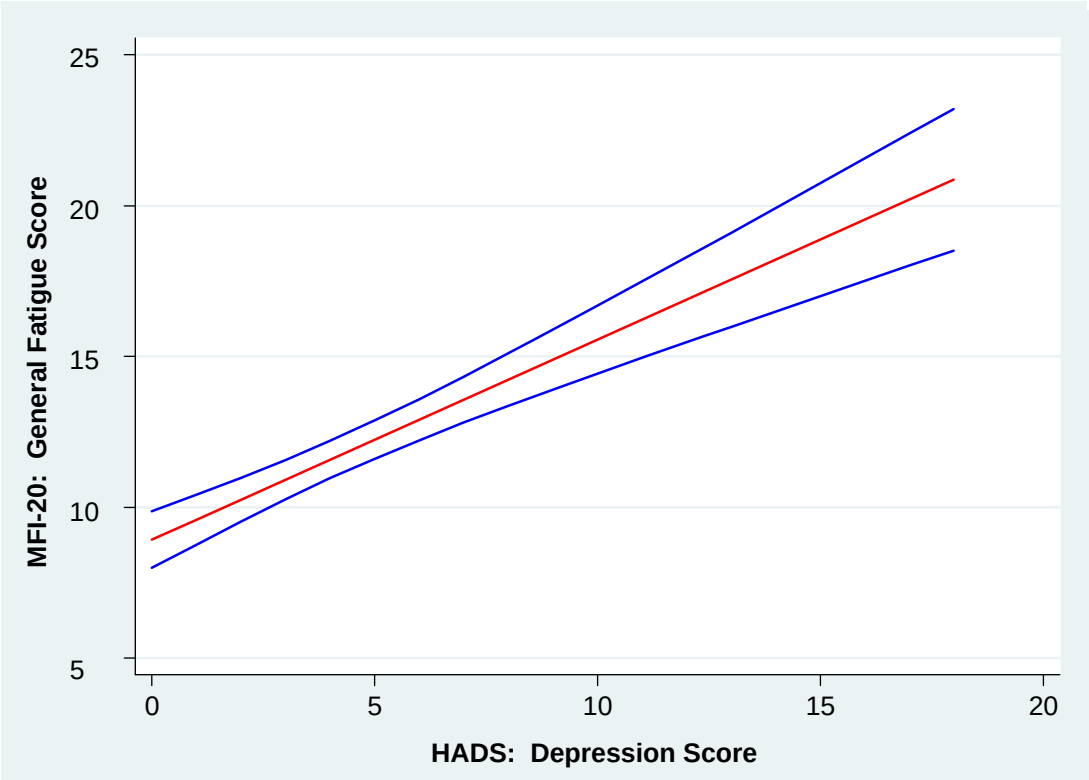
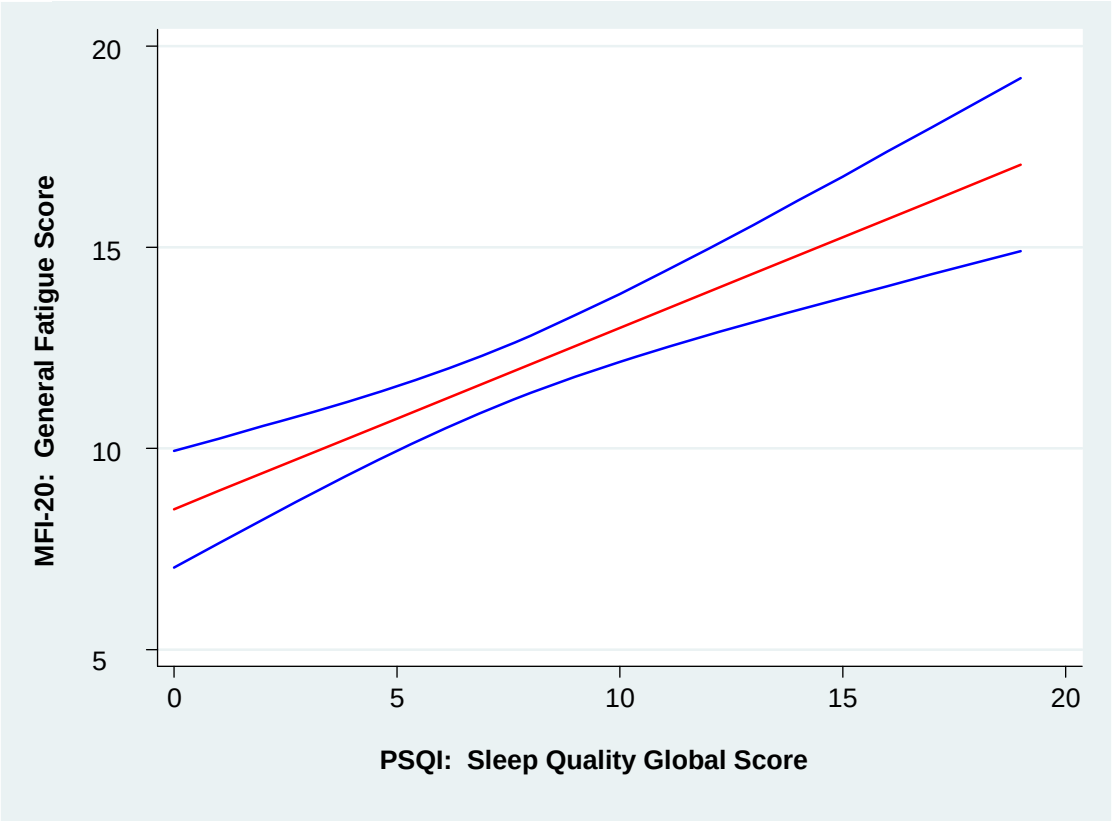


Figure 4.2: Association between General Fatigue and Sleep Quality



4.4.5.2 Physical Fatigue

The univariate analyses are shown in **Table 4.6**. In the multivariate model, depression (β=3.2; 95% CI=2.3, 4.1; p<0.001), renal dysfunction (β=-0.7; 95% CI=-1.4, -0.5; p<0.001; **Figure 4.3**), inflammation (β=1.4; 95% CI=0.0, 2.7; p=0.05), reduced LTI (β=-0.5; 95% CI=-0.8, -0.3; p<0.001; **Figure 4.4**) and male (β=2.4; 95% CI=0.9, 4.0; p=0.003) were independently associated with Physical Fatigue. Repeating the multivariate analysis excluding HADS and PSQI showed that renal dysfunction (β=-0.8; 95% CI=-1.2, -0.4; p<0.001), inflammation (β=2.6; 95% CI=1.0, 4.1; p=0.002), increasing time post-

transplantation (β=0.7; 95% CI=0.2, 1.3; p=0.01), reduced LTI (β=-0.6; 95% CI=-0.9, -0.3; p<0.001), and male (β=3.1; 95% CI=1.2, 5.0; p=0.001) were independently associated with Physical Fatigue.

Figure 4.3: Association between Physical Fatigue and Estimated Glomerular Filtration Rate (eGFR)

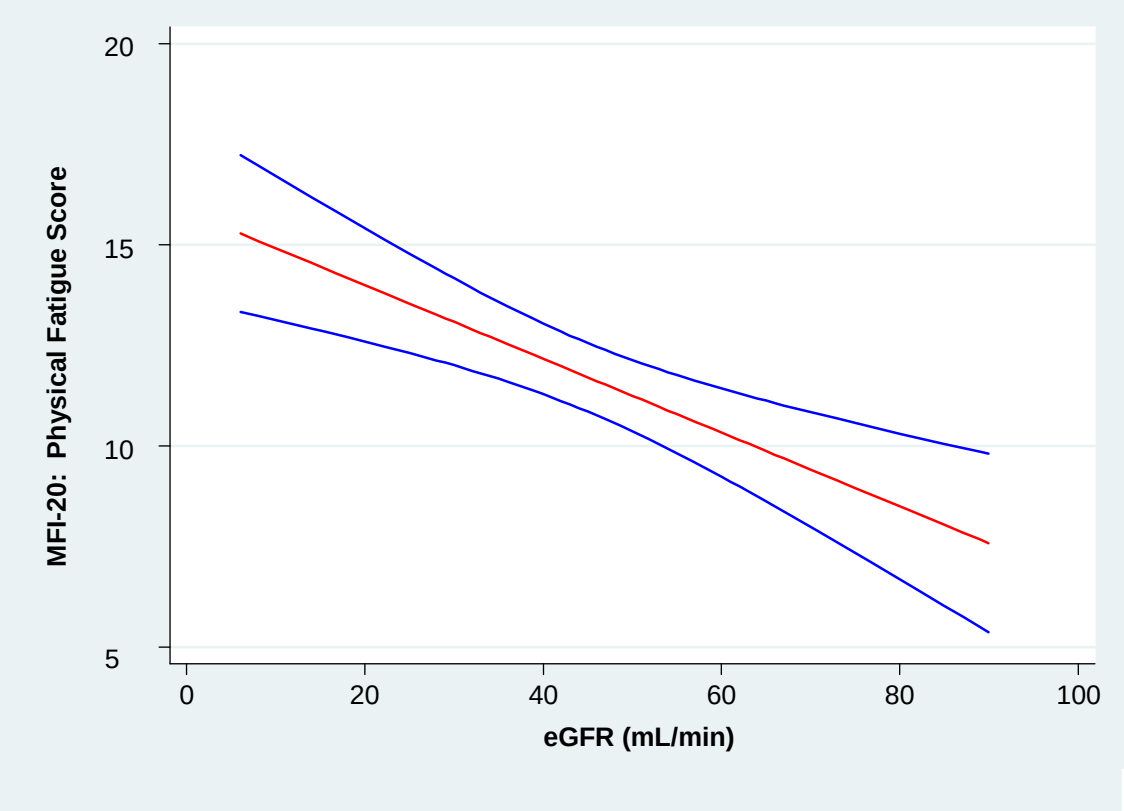


Table 4.6: Results of Univariate and Multivariate Analyses for Physical Fatigue

	Univariable Analysis		Multivariable Analysis[†,©]		Multivariable Analysis[§,©]	
	Regression Coefficient (95% CI*)	***P*-value**	**Regression Coefficient (95% CI*)**	***P*-value**	**Regression Coefficient (95% CI*)**	***P*-value**
[a]**Depression**	4.1 (3.2, 5.1)	<0.001	3.2 (2.3, 4.1)	<0.001		
[b]**eGFR (ml/min)**	-0.9 (-1.4, -0.5)	<0.001	-0.7 (-1.4, -0.5)	<0.001	-0.8 (-1.2, -0.4)	<0.001
[c]**hsCRP (mg/L)**	3.6 (1.8, 5.4)	<0.001	1.4 (0.0, 2.7)	0.05	2.6 (1.0, 4.1)	0.002
[a]**Time post-transplantation (years)**	1.2 (0.6, 1.9)	<0.001			0.7 (0.2, 1.3)	0.01
[a]**Sleep quality**	2.3 (1.2, 3.3)	<0.001				
[a]**Anxiety**	2.1 (1.1, 3.0)	<0.001				
[a]**FTI (kg/m^2)**	1.4 (0.7, 2.1)	<0.001				
[b]**Age (years)**	0.8 (0.2, 1.5)	0.01				
Use of calcineurin inhibitor None Cyclosporin Tacrolimus	 0 0.9 (0.8, 4.5) -0.8 (-3.0, -0.4)	 0.01				
ICED ≤2 >2	 0 3.2 (0.5, 5.9)	 0.02				
Alcohol intake (unit)	-0.2 (-0.6, 0.0)	0.04				
LTI (kg/m^2)	-0.3 (-0.6, -0.0)	0.05	-0.5 (-0.8, -0.3)	<0.001	-0.6 (-0.9, -0.3)	<0.001
Use of prednisolone No Yes	 0 -0.2 (-3.8, 0.3)	 0.09				
Haemoglobin (g/dL)	-0.5 (-1.0, 0.1)	0.11				
Gender Female Male	 0 1.5 (-0.4, 3.3)	 0.12	 0 2.4 (0.9, 4.0)	 0.003	 0 3.1 (1.2, 5.0)	 0.001
Presence of diabetes None Pre-DM NODAT	 0 1.7 (-1.3, 4.8) -0.6 (-3.3, 2.0)	 0.44				
Use of adjunctive antiproliferatives None Mycophenolate Mofetil Azathioprine	 0 -0.2 (-3.6, 0.1) 0.1 (-1.1, 1.9)	 0.58				
Previous episodes of acute rejection No Yes	 0 -0.6 (-3.6, 2.4)	 0.69				
Marital status Single [∝]Married [◊]Divorced/Widowed	 0 0.1 (-1.0, 3.5) -0.1 (-5.3, 1.8)	 0.69				
Smoking status Never smoked Current smoker Ex-smoker	 0 0.1 (-3.1, 6.0) 0.1 (-0.7, 1.3)	 0.85				
Ethnicity Caucasian Asian Afro-Caribbean	 0 -0.5 (-3.1, 2.1) -0.6 (-4.3, 3.2)	 0.91				
R^2 value from final model			58%		41%	

[†]All predictor variables were included in multivariable analysis.

[§]Patient-reported outcomes (HADS and PSQI) were excluded in multivariable analysis.

[©]Results in the final multivariable regression model were presented.

*CI = Confidence Interval

[∝]"Living with partner" was classified under the "Married" category.

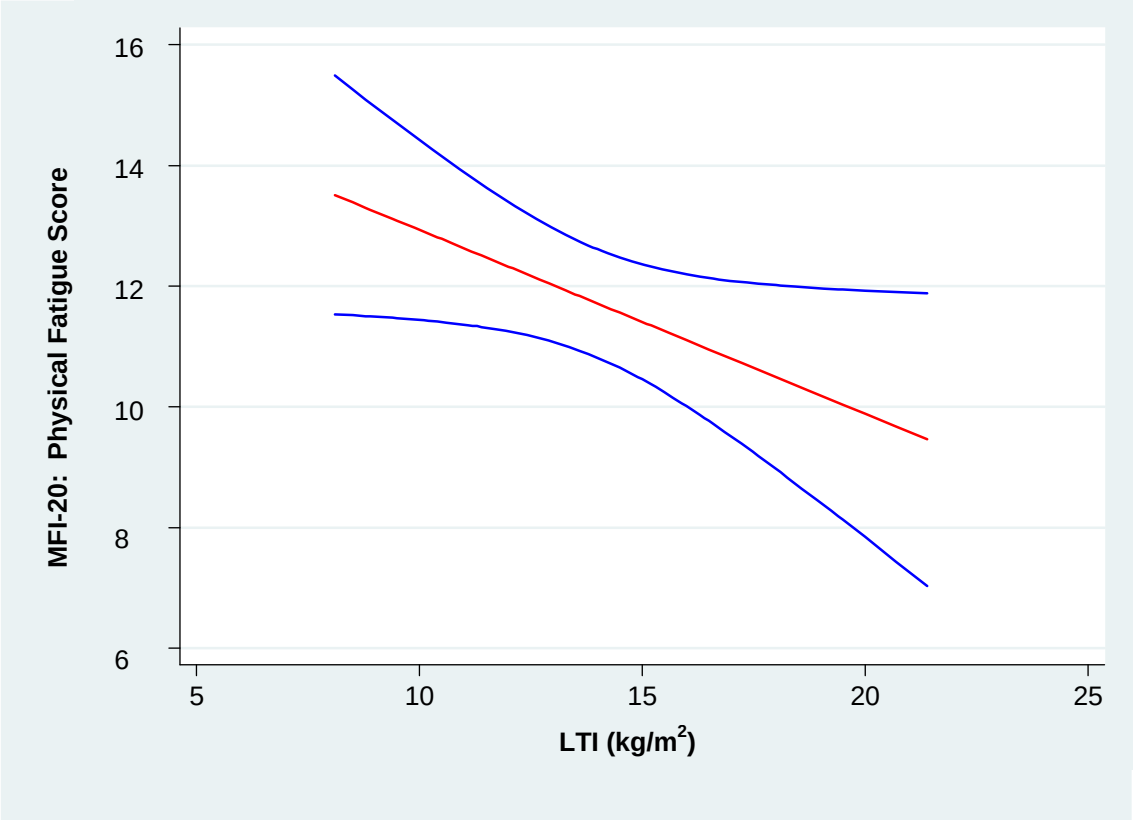
[◊]"Separated" was classified under the "Divorced/Widowed" category.

[a]Coefficients reported for 5-unit increase in explanatory variable.

[b]Coefficients reported for 10-unit increase in explanatory variable.

[c]Variable analysed on log scale (base 10)

Figure 4.4: Association between Physical Fatigue and Lean Tissue Index (LTI)



4.4.5.3 Reduced Activity

The univariate analyses are shown in **Table 4.7.** In the multivariate model, depression (β=3.4; 95% CI=1.9, 3.7; p<0.001), inflammation (β=2.7; 95% CI=1.2, 4.1; p<0.001; **Figure 4.5**), and increasing age (β=0.7; 95% CI=0.2, 1.1; p=0.003) were independent predictors for Reduced Activity. Following exclusion of HADS and PSQI, inflammation (β=3.8; 95% CI=2.2, 5.4; p<0.001), increasing comorbidity (β=3.4; 95% CI=1.0, 5.7; p=0.006), increasing time post-transplantation (β=0.6; 95% CI=0.0, 1.2; p=0.04), and increasing age (β=0.6; 95% CI=0.2, 1.1; p=0.04) were independently associated with Reduced Activity.

Table 4.7: Results of Univariate and Multivariate Analyses for Reduced Activity

	Univariable Analysis		Multivariable Analysis[†,©]		Multivariable Analysis[§,©]	
	Regression Coefficient (95% CI*)	***P*-value**	**Regression Coefficient (95% CI*)**	***P*-value**	**Regression Coefficient (95% CI*)**	***P*-value**
[a]**Depression**	4.1 (3.1, 5.0)	<0.001	3.4 (1.9, 3.7)	<0.001		
[c]**hsCRP (mg/L)**	4.2 (2.5, 5.9)	<0.001	2.7 (1.2, 4.1)	<0.001	3.8 (2.2, 5.4)	<0.001
[a]**Sleep quality**	2.3 (1.2, 3.3)	<0.001				
[a]**Anxiety**	1.8 (0.9, 2.7)	<0.001				
ICED						
≤2	0	0.001			0	0.006
>2	4.7 (2.1, 7.4)				3.4 (1.0, 5.7)	
[a]**FTI (kg/m^2)**	1.2 (0.5, 1.9)	0.001				
[a]**Time post-transplantation (years)**	1.0 (0.4, 1.7)	0.002			0.6 (0.0, 1.2)	0.04
Use of calcineurin inhibitor						
None	0	0.005				
Cyclosporin	0.2 (-0.2, 3.6)					
Tacrolimus	-0.3 (-4.3, -0.3)					
[b]**Age (years)**	0.8 (0.2, 1.4)	0.01	0.7 (0.2, 1.1)	0.003	0.6 (0.2, 1.1)	0.04
[b]**eGFR (ml/min)**	-0.5 (-0.9, 0.0)	0.06				
Gender						
Female	0	0.08				
Male	1.6 (-0.2, 3.4)					
Alcohol intake (unit)	-0.2 (-0.5, 0.0)	0.12				
LTI (kg/m^2)	-0.2 (-0.5, 0.1)	0.14				
Presence of diabetes						
None	0	0.21				
Pre-DM	0.0 (-1.5, 2.4)					
NODAT	-1.6 (-4.2, 1.0)					
Haemoglobin (g/dL)	-0.2 (-0.8, 0.4)	0.50				
Smoking status						
Never smoked	0	0.54				
Current smoker	0.0 (-1.5, 2.4)					
Ex-smoker	0.1 (-3.0, 5.4)					
Use of prednisolone						
No	0	0.62				
Yes	-0.1 (-2.6, 1.5)					
Marital status						
Single	0	0.70				
[∝]Married	0.1 (-1.0, 3.5)					
[◊]Divorced/Widowed	-0.1 (-5.2, 1.8)					
Previous episodes of acute rejection						
No	0	0.71				
Yes	-0.6 (-3.6, 2.4)					
Ethnicity						
Caucasian	0	0.85				
Asian	0.6 (-2.0, 3.2)					
Afro-Caribbean	0.7 (-3.0, 4.5)					
Use of adjunctive antiproliferatives						
None	0	0.85				
Mycophenolate Mofetil	-1.6 (-3.3, 0.4)					
Azathioprine	0.0 (-1.3, 1.6)					
R^2 value from final model			52%		34%	

[†]All predictor variables were included in multivariable analysis.

[§]Patient-reported outcomes (HADS and PSQI) were excluded in multivariable analysis.

[©]Results in the final multivariable regression model were presented.

*CI = Confidence Interval

[∝]"Living with partner" was classified under the "Married" category.

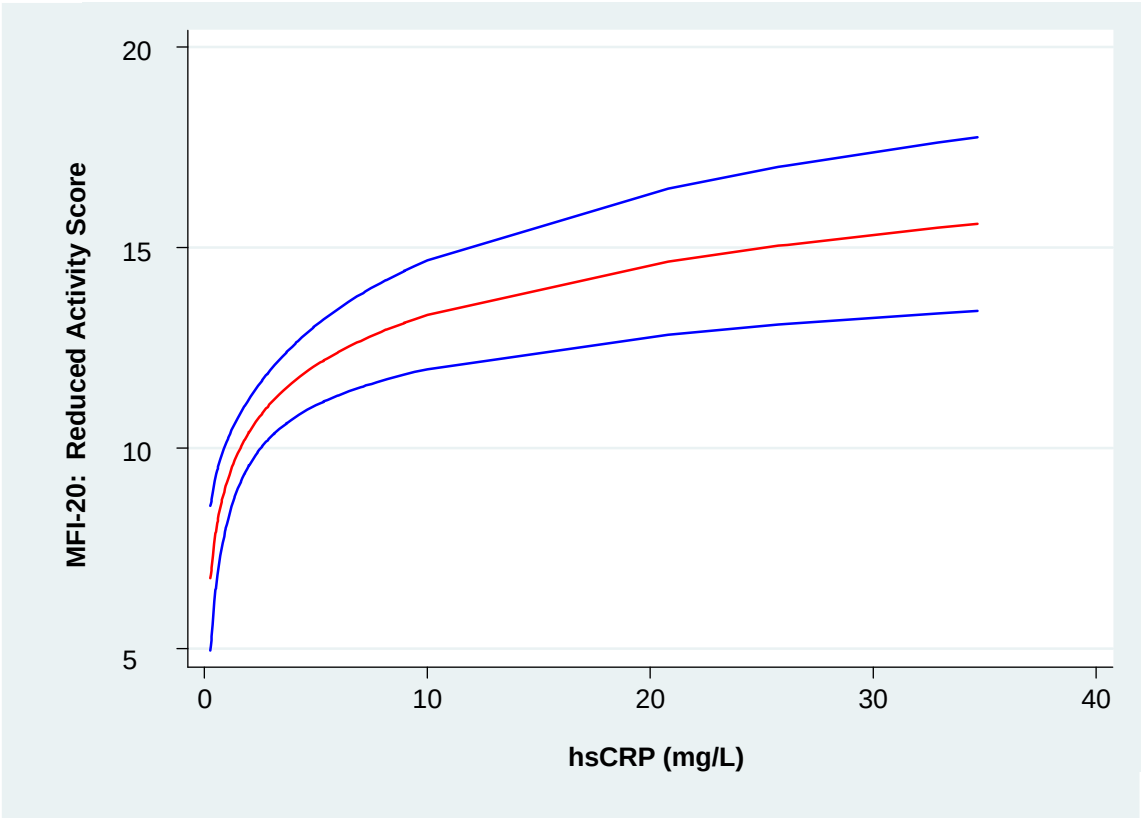
[◊]"Separated" was classified under the "Divorced/Widowed" category.

[a]Coefficients reported for 5-unit increase in explanatory variable.

[b]Coefficients reported for 10-unit increase in explanatory variable.

[c]Variable analysed on log scale (base 10)

Figure 4.5: Association between Reduced Activity and high-sensitivity C-Reactive Protein (hsCRP)



4.4.5.4 Reduced Motivation

The univariate models are shown in **Table 4.8.** In the multivariate analysis, depression (Odds Ratio = 1.40; CI=1.30, 1.52; p<0.001), renal dysfunction (Odds Ratio = 0.96; CI=0.93, 1.00; p=0.03), and reduced LTI (Odds Ratio = 0.98; CI=0.96, 1.00; p=0.05) were associated with Reduced Motivation independently. Following exclusion of HADS and PSQI, increasing time post-transplantation (Odds Ratio = 1.07; CI=1.01, 1.13; p=0.02), renal dysfunction (Odds Ratio = 0.95; CI=0.92, 0.99; p=0.02), and inflammation (Odds Ratio = 1.22; CI=1.05, 1.43; p=0.01) were independent predictors for Reduced Motivation.

Table 4.8: Results of Univariate and Multivariate Analyses for Reduced Motivation

	Univariable Analysis		Multivariable Analysis[†,©]		Multivariable Analysis[§,©]	
	Odds Ratio (95% CI*)	***P*-value**	**Odds Ratio (95% CI*)**	***P*-value**	**Odds Ratio (95% CI*)**	***P*-value**
[a]**Depression**	1.44 (1.32, 1.56)	<0.001	1.40 (1.30, 1.52)	<0.001		
[a]**Sleep quality**	1.25 (1.14, 1.36)	<0.001				
[a]**Anxiety**	1.23 (1.14, 1.33)	<0.001				
[a]**Time post-transplantation (years)**	1.09 (1.03, 1.16)	0.003			1.07 (1.01, 1.13)	0.02
[b]**eGFR (ml/min)**	0.94 (0.90, 0.98)	0.005	0.96 (0.93, 1.00)	0.03	0.95 (0.92, 0.99)	0.02
[c]**hsCRP (mg/L)**	1.26 (1.07, 1.48)	0.006			1.22 (1.05, 1.43)	0.01
[a]**FTI (kg/m^2)**	1.09 (1.02, 1.16)	0.008				
Use of calcineurin inhibitor						
None	1	0.02				
Cyclosporin	1.27 (1.02, 1.30)					
Tacrolimus	0.79 (0.70, 0.99)					
ICED						
≤2	1	0.03				
>2	1.30 (1.02, 1.66)					
[b]**Age (years)**	1.06 (1.00, 1.12)	0.05				
Alcohol intake (unit)	0.83 (0.81, 1.00)	0.06				
LTI (kg/m^2)	0.98 (0.95, 1.00)	0.10	0.98 (0.96, 1.00)	0.05		
Use of prednisolone						
No	1	0.10				
Yes	0.85 (0.83, 1.01)					
Gender						
Female	1	0.12				
Male	1.00 (0.85, 1.17)					
Use of adjunctive antiproliferatives						
None	1	0.19				
Mycophenolate Mofetil	0.87 (0.84, 1.02)					
Azathioprine	1.14 (0.98, 1.29)					
Haemoglobin (g/dL)	0.98 (0.93, 1.03)	0.34				
Marital status						
Single	1	0.39				
[∝]Married	0.99 (0.91, 1.09)					
[◊]Divorced/Widowed	0.84 (0.79, 1.02)					
Presence of diabetes						
None	1					
Pre-DM	0.99 (0.76, 1.29)	0.69				
NODAT	0.90 (0.72, 1.14)					
Ethnicity						
Caucasian	1	0.79				
Asian	1.03 (0.94, 1.09)					
Afro-Caribbean	0.90 (0.82, 1.12)					
Smoking status						
Never smoked	1	0.86				
Current smoker	0.98 (0.76, 1.28)					
Ex-smoker	0.90 (0.82, 1.12)					
Previous episodes of acute rejection						
No	1	0.91				
Yes	0.98 (0.76, 1.28)					
R^2 value from final model			47%		19%	

[†]All predictor variables were included in multivariable analysis.

[§]Patient-reported outcomes (HADS and PSQI) were excluded in multivariable analysis.

[©]Results in the final multivariable regression model were presented.

*CI = Confidence Interval

[∝]"Living with partner" was classified under the "Married" category.

[◊]"Separated" was classified under the "Divorced/Widowed" category.

[a]Odds ratios reported for 5-unit increase in explanatory variable.

[b]Odds ratios reported for 10-unit increase in explanatory variable.

[c]Variable analysed on log scale (base 10)

4.4.5.5 Mental Fatigue

Finally, the univariate analyses predicting Mental Fatigue are shown in **Table 4.9.** In the multivariate model, only anxiety was independently associated with fatigue (Odds Ratio = 1.36; CI=1.24, 1.49; $p<0.001$; **Figure 4.6**). A borderline effect of ethnicity was found (Odds Ratio = 1.42; CI=1.01, 1.99; $p=0.05$). When the multivariate analysis was repeated excluding the HADS and PSQI results, no predictor variables retained statistical significance.

Figure 4.6: Association between Mental Fatigue and Anxiety

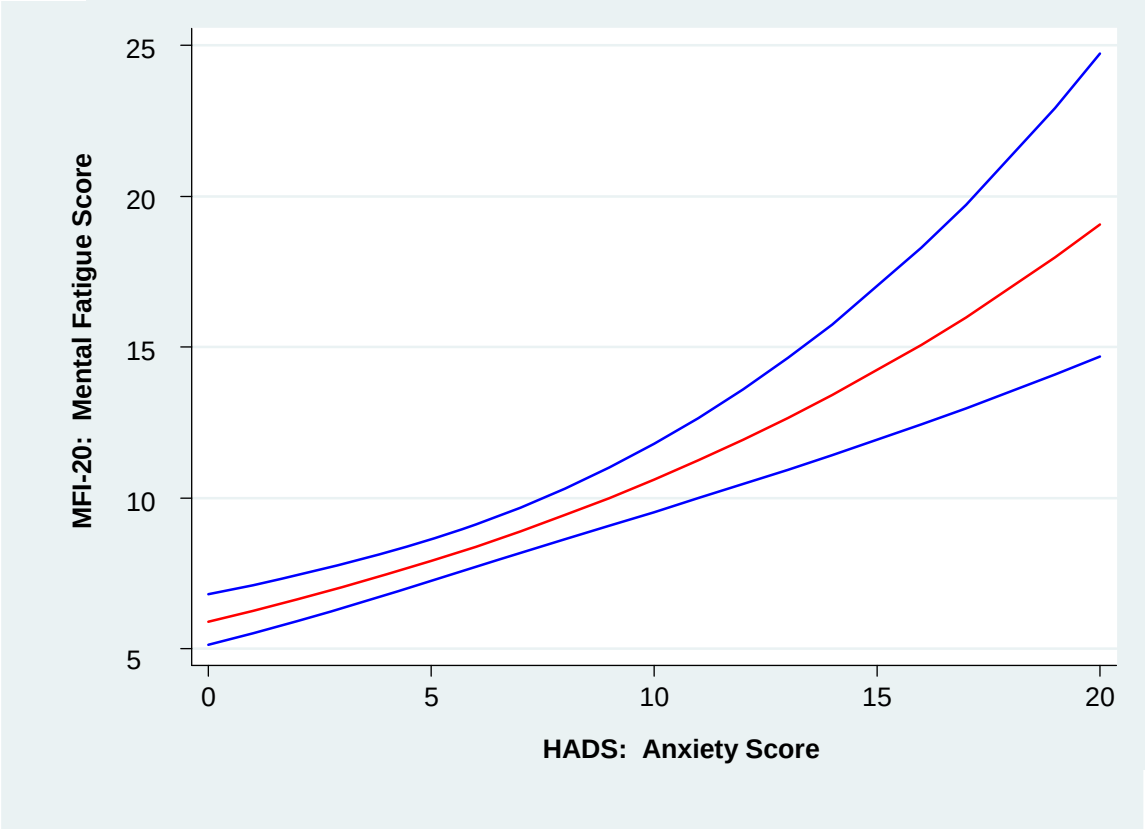


Table 4.9: Results of Univariate and Multivariate Analyses for Mental Fatigue

	Univariable Analysis		Multivariable Analysis[†,©]		Multivariable Analysis[§,©]	
	Odds Ratio (95% CI*)	***P*-value**	**Odds Ratio (95% CI*)**	***P*-value**	**Odds Ratio (95% CI*)**	***P*-value**
[a]**Anxiety**	1.34 (1.22, 1.47)	<0.001	1.36 (1.24, 1.49)	<0.001		
[a]**Depression**	1.36 (1.20, 1.53)	<0.001				
[a]**Sleep quality**	1.17 (1.04, 1.32)	0.01				
Ethnicity						
Caucasian	1	0.19	1	0.05		
Asian	1.27 (0.97, 1.68)		1.20 (0.95, 1.52)			
Afro-Caribbean	1.16 (0.78, 1.73)		1.42 (1.01, 1.99)			
[c]**hsCRP (mg/L)**	1.12 (0.91, 1.38)	0.28				
Smoking status						
Never smoked	1	0.29				
Current smoker	0.90 (0.87, 1.04)					
Ex-smoker	0.97 (0.79, 1.22)					
[b]**eGFR (ml/min)**	0.97 (0.92, 1.03)	0.33				
Alcohol intake (unit)	0.91 (0.88, 1.01)	0.36				
Use of calcineurin inhibitor						
None	1	0.51				
Cyclosporin	1.18 (0.94, 1.24)					
Tacrolimus	0.93 (0.91, 1.04)					
Marital status						
Single	1	0.55				
[∝]Married	0.90 (0.85, 1.05)					
[◊]Divorced/Widowed	1.06 (0.89, 1.22)					
Use of prednisolone						
No	1	0.59				
Yes	0.95 (0.88, 1.07)					
Use of adjunctive antiproliferatives						
None	1	0.60				
Mycophenolate Mofetil	0.88 (0.87, 1.03)					
Azathioprine	1.05 (0.95, 1.09)					
[a]**FTI (kg/m^2)**	1.02 (0.94, 1.11)	0.64				
Haemoglobin (g/dL)	1.01 (0.95, 1.08)	0.64				
ICED						
≤2	1	0.67				
>2	0.94 (0.69, 1.27)					
Previous episodes of acute rejection						
No	1	0.67				
Yes	0.93 (0.67, 1.29)					
Gender						
Female	1	0.74				
Male	0.97 (0.79, 1.18)					
[b]**Age (years)**	0.99 (0.93, 1.06)	0.84				
LTI (kg/m^2)	1.00 (0.97, 1.04)	0.88				
[a]**Time post-transplantation (years)**	1.00 (0.93, 1.08)	0.91				
Presence of diabetes						
None	1	0.93				
Pre-DM	0.96 (0.69, 1.33)					
NODAT	0.96 (0.72, 1.28)					
R^2 value from final model			32%		-	

[†]All predictor variables were included in multivariable analysis.

[§]Patient-reported outcomes (HADS and PSQI) were excluded in multivariable analysis.

[©]Results in the final multivariable regression model were presented.

*CI = Confidence Interval

[∝]"Living with partner" was classified under the "Married" category.

[◊]"Separated" was classified under the "Divorced/Widowed" category.

[a]Coefficients reported for 5-unit increase in explanatory variable.

[b]Coefficients reported for 10-unit increase in explanatory variable.

[c]Variable analysed on log scale (base 10)

4.5 Discussion

This study aimed to investigate the nature, severity, prevalence and clinical awareness of post-transplantation fatigue, to determine the association between fatigue and QoL, and to identify main predictors of post-transplantation fatigue. The results revealed that, in clinically stable KTRs without evidence of intercurrent disease, fatigue is common, severe, and clinically under-appreciated. It has a close association with inferior QoL. These results confirm and significantly extend the findings of the single previous study on post-transplantation fatigue[13], and advances understanding of the possible determinants of fatigue by showing associations with anthropometric and clinical variables not previously evaluated. Depression, anxiety, inferior sleep quality, inflammation, reduced muscle mass, and renal dysfunction were identified as risk factors, forming potential targets for future interventional studies.

The significant correlations between different domains of fatigue suggest that treatment of behavioural, emotional and cognitive aspects of fatigue may improve physical aspects of fatigue, or vice versa. However, a recent study provides experimental evidence that mental fatigue limits exercise tolerance in humans via higher perception of effort rather than cardiorespiratory and musculoenergetic mechanisms[31], implying that the overall focus of fatigue management should be on the behavioural, emotional and cognitive aspects.

Compared with normative data from healthy population[16,32], KTRs suffer from higher levels of fatigue on all dimensions. In comparison with normative data from Lin et al[16], fatigue levels in KTRs were similar to "chronically unwell" patients, defined as having chronic (≥ 6 months) unwellness with or without fatigue, but not meeting criteria for CFS[16]. Indeed, severity in certain domains, such as Physical Fatigue, Reduced Activity and Reduced Motivation, approached that of CFS[16], further highlighting the burden of fatigue in KTRs. Of note, the level of Mental Fatigue was higher in KTRs compared with cancer patients with mild anaemia undergoing chemotherapy[32], and chronic heart failure patients with and without anaemia[33]. Also, KTRs suffer from higher levels of Physical Fatigue, Reduced Activity and Reduced Motivation compared to cancer patients without anaemia[32]. Physical aspects of fatigue outweighed behavioural, emotional and cognitive aspects, resembling findings in liver transplant recipients[34].

Using a dichotomous fatigue definition, 24-38% of participants reported fatigue in at least one of the five dimensions, and 59% in any dimension. This prevalence is comparable to that found by Rodrigue[13] using the one-dimensional "Fatigue Symptom Inventory". Despite the high prevalence, only 13% of patients had fatigue documented in medical records prior to participation in this study, suggesting that this symptom is either under-reported or under-acknowledged. Furthermore, the close correlation between all fatigue domains and QoL resonates with the clinical and social relevance of this symptom.

The assessment of multiple domains of fatigue, and the measurement from the clinically validated HADS extends the findings of Rodrigue[13] where fatigue severity significantly

correlated with a composite mood score incorporating depression, vigour, anger, confusion, anxiety and fatigue itself. The current study highlights the specific, independent importance of depression as a risk factor for all dimensions of fatigue except for Mental Fatigue. This exception is surprising as previous study on multiple sclerosis (MS) related fatigue found that depression was related to Mental Fatigue[35,36]. However, depression and Mental Fatigue can occur independently or simultaneously[37], and this phenomenon has been demonstrated in stroke patients[37] . Many symptoms for depression and Mental Fatigue overlap, but the core symptoms are different. The lack of association in this study may be explained by the distinction between the core symptoms. Depression is an illness or mood disorder with a variety of symptoms, the most defining being an inexplicable, enduring feeling of sadness, and loss of positive effect[38]. The collective symptoms may not manifest as Mental Fatigue, which is a psychobiological state caused by prolonged periods of demanding cognitive activity such as concentration, attention and increased mental load[31]. In Mental Fatigue, mental effort can only be sustained for a short time-frame, and recovery period is disproportionally long[37]. Accompanying symptoms include irritability, sensitivity to stress, concentration difficulties, and emotional instability[37]. Anxiety was a significant predictor for Mental Fatigue, similar to other chronic conditions such as MS[36]. KTRs are subjected to several mental challenges, including fears about transplant rejection and the necessity to adhere to a complex regimen of immunosuppression therapy that may generate distressing side effects[39]. To an extent, the unpredictable clinical course post-transplantation is reminiscent of the relapsing and remitting nature of MS. While acknowledging the limitations of cross-sectional data to make causal inferences, the present results are in line with evidence showing that

psychological interventions addressing disease-related anxiety and depression *per se* may yield added benefit in modifying fatigue.

While inferior sleep quality may intuitively be expected to have a pervasive and broad effect on multiple aspects of fatigue[13], a significant association was only observed for the General Fatigue dimension. This finding suggests that mere sleep difficulties do not explain a large spectrum of the fatigue complaints in KTRs, and interventions aiming to improve sleep quality may have limited effect on fatigue.

An important caveat with the interpretation of the associations between self-reported data, such as depression, sleep difficulties and symptoms of fatigue is that common-method variance may partly drive the observed associations, and may account for 25% of shared variance[40]. In common-method variance, patients high in negative effect (i.e. negative mood) perceive, remember and report more physical and psychological symptoms, and report those symptoms to be more severe than patients with a less negative mood[41]. Additionally, individual items on questionnaires measuring fatigue, depression or sleep problems tend to show conceptual overlap, which further enhances co-variation. While these would not render self-reports unimportant, and neither would refute that sleep and depression may have strong bidirectional links with fatigue, potential interpretational difficulties may result. Therefore, this study's objective and detailed anthropometric and biochemical data represents an important extension of the previous study in the field[13]. When multivariate regression analysis excluded adjustment for mood and sleep, reduced

LTI, renal impairment and inflammation were identified as potentially reversible predictors.

The association between inflammation and fatigue is particularly notable as the studied cohort consisted of clinically stable KTRs, without overt evidence of ongoing acute or chronic inflammatory conditions. Evidence from studies of healthy volunteers, elderly populations and other disease groups have shown that inflammatory cytokines possess potent neurological effects and are mediators of fatigue[13,42-45]. Modifying inflammation may therefore represent an attractive target in future studies.

The independent association between physical fatigue and reduced LTI is intuitively plausible, but not previously reported in KTRs. It replicates results from cancer-related fatigue[46], and fatigue associated with end-stage renal disease on haemodialysis[47,48]. Reduced muscle mass coupled with increased fat mass ("sarcopenic obesity") is a common characteristic of body composition following kidney transplantation[49]. Despite significant univariate associations between FTI and different dimensions of fatigue (General Fatigue, Physical Fatigue, Reduced Activity and Reduced Motivation), this relationship did not hold when adjusted for inflammation, suggesting inflammation as the driver for fatigue, rather than adiposity *per se*. This study advances understanding from Rodrigue et al[13] where raised BMI (a proxy for fat mass) was identified as a predictor of fatigue, but detailed anthropometric and inflammatory evaluation was not undertaken. However, it is possible that the systemic low-grade inflammation present in obesity triggers adipocyte release of pro-inflammatory cytokines[50], this in turn accelerates muscle catabolism[51],

leading to muscle wasting[51]. The current study suggests that lifestyle interventions with a strong focus on increased physical activity and dietary modification aiming to reverse this phenotype should be valuable for patients displaying symptoms of fatigue. Apart from promoting favourable changes in body composition, lifestyle modification is particularly important in light of the inverse associations between all domains of fatigue and SF-36 physical health subscale, which is a representation of self-perceived physical functioning. Recent studies reported that self-perceived physical functioning is significantly and positively correlated with physical activity level[52,53]. Although physical activity level was not measured in the current study, this finding suggests that striving to be physically active enhances functional capacity and improves self-perception of physical functioning, leading to improved fatigue and QoL.

Although fatigue is a common and important symptom for patients on dialysis[7,8], the present results show, for the first time, a relationship between allograft dysfunction and physical fatigue in KTRs. Clinical strategies exist to improve allograft function[54] and fatigue may represent an important patient-reported outcome in future interventional studies.

Other non-modifiable, but important, risk factors for varying domains of fatigue included male, older age, ethnicity, comorbidity, and increasing time post-transplantation.

The lack of association between haemoglobin level and fatigue is unsurprising as the results from the Trial to Reduce cardiovascular Events with Aranesp Therapy (TREAT) study[55] only showed a small improvement in fatigue with haemoglobin normalisation, using recombinant erythropoietin in non-transplant, diabetic, chronic kidney disease.

The use of immunosuppressive medication was not associated with fatigue in KTRs. Of relevance, no link between immunosuppression and fatigue was seen in previous studies of liver transplant recipients[34] and KTRs[13].

This study has limitations that should be acknowledged. It is a single-centre study with a small sample size. The progression and regression of fatigue over time could not be evaluated due to the study design of cross-sectional nature. The results may not be representative of "sicker" patients within the transplanted population. It is recognised that hyperparathyroidism occurs in a substantial proportion of KTRs (17%)[56], with fatigue as a possible manifestation. Regrettably, serum parathyroid hormone concentrations were not measured in this study.

Whilst kidney transplantation is associated with a variety of benefits compared with dialysis, this study shows that fatigue remains a common and relevant problem in otherwise stable KTRs. As the medical complexity of KTRs increases, it is important not to lose sight of important patient-reported outcomes such as fatigue. This study

demonstrates potential targets for intervention, and future research should focus on evaluating the effectiveness and impact of such interventions upon fatigue and QoL.

4.6 References

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CARDIOVASCULAR, MUSCULAR & PERCEPTUAL CONTRIBUTIONS TO PHYSICAL FATIGUE IN PREVALENT KIDNEY TRANSPLANT RECIPIENTS

Chapter 5

Effects of Body Composition on Clinical and Quality of Life Outcomes in Kidney Transplant Recipients

Submitted to Kidney International for Consideration

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CHAPTER 5: CARDIOVASCULAR, MUSCULAR AND PERCEPTUAL CONTRIBUTIONS TO PHYSICAL FATIGUE IN PREVALENT KIDNEY TRANSPLANT RECIPIENTS

5.1 Abstract

Background and Objectives: Physical fatigue is a debilitating and common symptom in clinically stable kidney transplant recipients (KTRs). This study investigates the aetiology of physical fatigue in this setting through examinations of muscle mass, muscular and cardiovascular functions, and perceived exertion. The prevalence of physical fatigue, its association with quality of life (QoL), and the predictors of perceived exertion, were also evaluated.

Materials and Methods: This single-centre cross-sectional study enrolled 55 KTRs. Physical fatigue was measured using Multi-Dimensional Fatigue Inventory-20. QoL was assessed using Medical Outcomes Study Short Form-36. Muscle mass was quantified using dual-energy x-ray absorptiometry. Muscular function was assessed by jumping mechanography. Cardiovascular function (maximal oxygen consumption and oxygen pulse) was estimated during submaximal exercise test, with perceived exertion determined using age-adjusted Borg scale-ratings. Potential demographic, clinical, nutritional, psychosocial and behavioural predictors of perceived exertion were assessed.

Results: Of clinical importance, increased perceived exertion was the only independent predictor of physical fatigue (p=0.001). Physical fatigue occurred in 22% of KTRs, and negatively impacted QoL (p<0.001). Predictors of heightened perception included mental fatigue, anxiety, new-onset diabetes after transplantation, absence of cyclosporine, and very light alcohol intake (p<0.05 for all).

Conclusion: Physical fatigue in KTRs is associated with increased perceived exertion. This study identified predictors of perception, paving the way for future interventions.

5.2 Introduction

Fatigue is the subjective sensation of profound and persistent tiredness, weakness, and lack of energy[1,2]. It is a complex and multi-dimensional phenomenon involving physical, cognitive, and emotional components that interfere with individuals' abilities to function normally[2,3]. Fatigue is a prevalent patient-reported outcome among kidney transplant recipients (KTRs), occurring in up to 59% of these patients[3-5] and substantially impacting upon quality of life (QoL)[4,5]. Yet it is clinically under-recognised and often untreated[4].

Physical fatigue describes physical sensations of tiredness[6], leading to physical underperformance[7]. It represents the dominant component of fatigue in KTRs, outweighing behavioural, emotional, and cognitive aspects[4]. It is found in 38% of KTRs, impacting all aspects of QoL[4].

Conceptually, research on physical fatigue has traditionally been considered as either "cardiovascular", "muscular", or "perceptual" in aetiology. The cardiovascular model refers to insufficient cardiovascular oxygen or nutrient delivery to the muscular system, limiting oxidative phosphorylation and glycolysis, both of which are essential mechanisms for muscle contraction[8]. Accordingly, fatigue with cardiovascular origin results in decreased ability of muscle to generate and to maintain force, hence reducing the capability to sustain muscle contraction, possibly contributing to physical fatigue. The muscular model denotes insufficient muscle mass or reduction in muscle function, leading to failure of muscle force generation[8-10], and/or inability to maintain a certain force or power output[11], plausibly resulting in physical fatigue. The perceptual theory represents the

increased perception of effort, characterised by loss of motivation and reluctance to perform a physical task when perception of effort reaches a certain level. In fatigue with perceptual origin, individuals experience heightened responses to afferent feedback from the working body, resulting in exhaustion[8,12,13], which may be expressed as physical fatigue.

Of interest, it has been recognised that mental fatigue, characterised by inability to focus and maintain cognitive attention, is a crucial determinant of physical limits in healthy individuals[14-16], by heightening the perception of exertion[14,15]. This phenomenon suggests that mental fatigue possibly contributes to physical fatigue by raising perception of exertion.

The cardinal mechanistic aetiology of physical fatigue in KTRs remained unexplored. Therefore, the primary objectives of this study were to systematically examine the aetiology of physical fatigue in KTRs, by measuring factors which may be mechanistically linked to symptoms of physical fatigue. These include quantification of muscle mass, assessment of muscular and cardiovascular functions, and by evaluating perceived exertion during a standardised exercise protocol. We also sought to establish the prevalence of physical fatigue and its impact on QoL in clinically stable KTRs. The key findings are that, physical fatigue, which adversely impacts on QoL, affects 22% of KTRs, and that cardiovascular and muscular factors do not contribute to the aetiology of physical fatigue. These observations point towards increased perception of exertion as the dominant cause of physical fatigue. Such findings arising from the earlier part of this study led to further

investigation to examine the role of mental fatigue, and other plausible predictors of heightened perception.

5.3 Materials and Methods

5.3.1 Participants and Study Design

Prevalent KTRs were recruited from the renal transplant outpatient clinic at Queen Elizabeth Hospital Birmingham UK, between August 2011 and August 2013. Inclusion and exclusion criteria are detailed in **Table 5.1**. Age and gender-matched healthy subjects (control group A) were recruited from Queen Elizabeth Hospital Birmingham and University of Birmingham, UK. However, control group A did not perform jumping mechanography (described below), and consequently, jumping mechanography data from KTRs were compared with normative data from 146 healthy subjects (control group B) collected for a study of muscle and ageing[17].

The study was approved by the local research ethics committee, and was conducted in accordance with the principles of the Declaration of Helsinki.

Table 5.1. Inclusion and Exclusion Criteria

Inclusion Criteria
▪ KTRs beyond 1 year post-transplantation ▪ Stable graft function (<10% increase in serum creatinine over the preceding 6 months)
Exclusion Criteria
▪ Inability to provide written informed consent ▪ Episodes of acute rejection within the last 6 months ▪ Evidence of sepsis in the last 6 weeks ▪ Known active malignancy or chronic infection ▪ History of thyroid disease of adrenal insufficiency ▪ Evidence of unstable angina (occurring at rest, severe and of new onset, or crescendo pattern) ▪ Evidence of acute coronary syndrome in the last 6 months ▪ Moderate or severe aortic stenosis (mean transvalvular gradient of >25mmHg or valve area of <1.5cm^2 on echocardiogram) ▪ Immobility ▪ Pregnancy

5.3.2 Protocol Overview

Patients attended the research visit in the morning following an overnight rest and a light breakfast (260kcal and 12g protein). Upon arrival and prior to initiating the research

protocol, the testing procedures including the blood test, the use of different questionnaires, tools and equipment were explained to the participants.

The order of tests was standardised. First, blood sampling was undertaken. Self-completion of questionnaires (Multi-Dimensional Fatigue Inventory-20, Hospital Anxiety and Depression Scale, Pittsburgh Sleep Quality Index, and Medical Outcomes Study Short Form-36) followed, and then dual-energy x-ray absorptiometry scanning and jumping mechanography were undertaken. Finally, participants rested for one-hour prior to performing an incremental submaximal exercise test, which included a measure of exertion estimated using the Borg Rating of Perceived Exertion (RPE) scale.

5.3.3 Multi-Dimensional Fatigue Inventory and Definitions of Physical and Mental Fatigue

Severity of physical and mental fatigue were determined subjectively using the Multi-Dimensional Fatigue Inventory-20 (MFI-20), a 20-item self-report questionnaire measuring fatigue in 5 dimensions including physical and mental fatigue. Both physical and mental fatigue were assessed by 4 items, each using a 5-point Likert scale. Scores for both physical and mental fatigue ranged from 4-20, with higher scores indicating greater fatigue. It demonstrates reliability in patients with end-stage renal disease[18], and proves validity in several medical conditions[19].

The prevalence of physical fatigue was determined based on the previously established definition of physical fatigue, with fatigue defined as ≥95th percentile for healthy subjects[4,19] (control group A).

5.3.4 Quality of Life Assessment

QoL was assessed using the Medical Outcomes Study Short Form-36 (SF-36). The SF-36 consists of 36 questions grouped into 8 life domains: physical functioning; social functioning; role limitation due to physical problems; role limitation due to emotional problems; mental health; energy and vitality; bodily pain; and general health perception. For each tested domain, item scores were coded, summed, and transformed into a scale from 0 (worst QoL) to 100 (best QoL) using the standard SF-36 scoring algorithm[20]. As well as the total score for QoL, these sub-scales are subsumed under 2 subscores, i.e. physical health summary score and mental health summary score. Physical health is represented by the physical functioning, role limitation due to physical problems, bodily pain, general health perception, and energy and vitality subscales. Mental health is represented by the mental health, role limitation due to emotional problems, social functioning, energy and vitality, and general health perception subscales[21]. The SF-36 is among the most commonly used instruments to assess QoL, and is regarded as valid and reliable in different population groups, including patients undergoing renal replacement therapy[22].

5.3.5 Dual-Energy X-ray Absorptiometry Scanning

Dual-Energy X-ray Absorptiometry (DEXA) Scanning provided measures of whole-body lean tissue mass (LTM), lower limb lean tissue mass (LLTM), and fat mass (FM)[23,24]. Prior to DEXA scanning, body weight was measured for individualised estimation of the most appropriate acquisition mode. Scan acquisition was carried out by trained personnel. Analysis and reporting of the scans were performed by a single trained bone densitometry clinical scientist. Coefficients of variation were <3% for body composition assessment in the local laboratory.

Both LTM and LLTM were normalised to height squared (Ht^2) to account for differences in body size: ***Normalised LTM or LLTM = (LTM or LLTM / Ht^2)***.

5.3.6 Jumping Mechanography

The Leonardo Mechanography Ground Reaction Force Platform, software version 4.2 (Novotec Medical GmbH, Pforzheim, Germany) was used to assess lower limb muscle power, an indication of muscular function. Participants performed three two-legged counter movement jump (CMJ) on the force platform with freely-moving arms. A one-minute rest interval was incorporated between each jump. Participants were instructed to jump as high as possible, producing maximum elevation of centre of mass. The jump with the highest automatic peak detection was selected for data analysis. The coefficient of variation of muscle power measurement using Jumping Mechanography is 3.6%[25] .

Peak power of the vertical movement was computed by the system as the product of force and velocity[25-29], which subsequently provides an important outcome parameter, the peak power normalised to total body mass (BM): ***Peak Power adjusted to BM = Peak Power from CMJ / BM***. In particular, given that jumping mechanography predominately investigates kinetic factors of lower limb muscle function (i.e. mechanical power) in both paediatric and adult populations[30,31], peak power was also adjusted to LLTM: ***Peak Power adjusted to LLTM = Peak Power from CMJ / LLTM***.

5.3.7 Incremental Submaximal Exercise Test

Cardiovascular function, represented by maximal oxygen consumption (VO_2max) and oxygen pulse (O_2 pulse), were measured by performing a submaximal incremental exercise test on an electrically braked cycle ergometer (Lode Corival, Cranlea, UK).

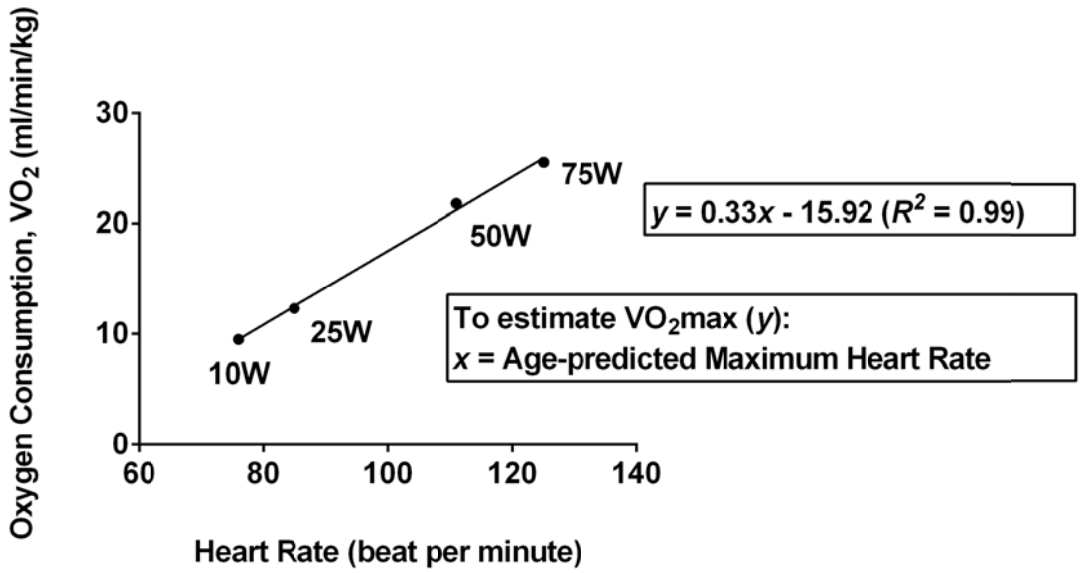
The exercise protocol was preceded and followed by a two-minute warm-up and cool-down period at 10 watts (W). The test started at 25W, and the work rate increased by 25W at three-minute intervals until voluntary exhaustion or the end of three-minutes at 75W. Participants were encouraged verbally to maintain a cadence of ≥65 revolutions per minute.

During the exercise protocol, participants were required to have a nose-clip fitted, and breathe through a mouthpiece attached to a two-way non-rebreathing valve, which is connected to a metabolic cart with a breath-by-breath online gas collection system (MOXUS Modular Metabolic System, AET Technologies, Pittsburgh, USA). The

MOXUS demonstrates validity and reliability compared with the "Douglas bag method", the gold standard[32]. Calibration procedures were performed on the MOXUS metabolic cart prior to exercise testing in accordance with the manufacturer instructions. Ventilation and expired gas were collected continuously and analysed every 30 seconds, calculating oxygen consumption (VO_2). The coefficient of variation for VO_2 measurement is 3.0%[32]. Heart rate (HR) was monitored continuously (Polar Vantage, Kempele, Finland) and recorded every 30 seconds.

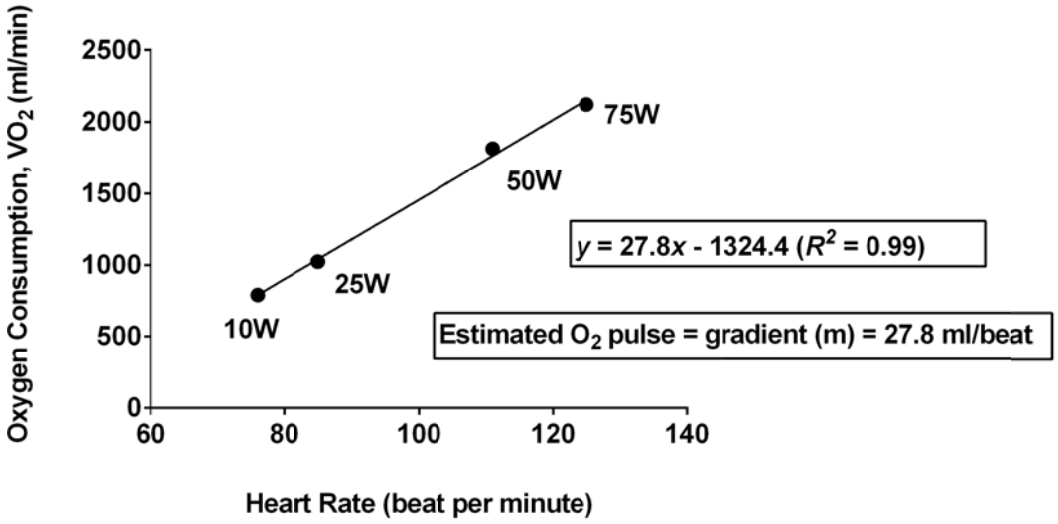
The VO_2 and HR measurements were averaged over the final minute of each of the three-minute workloads (25W, 50W and 75W). Consequently, VO_2max was estimated by linear regression, extrapolating VO_2 to the age-predicted maximum HR: ***Age-predicted Maximum HR = 205.8 – (0.685 × Age)***[33-35]. It is known that estimated VO_2max correlates highly with measured VO_2max when calculated with this approach[36]. An example of the linear regression is shown in **Figure 5.1**, the mean r^2 of the linear regression for the studied cohort is 0.97 (ranged from 0.86 to 1.00).

Figure 5.1. An Example of Linear Regression for Estimation of Maximal Oxygen Consumption (VO_2max)



O_2 pulse, oxygen consumed per heartbeat, was determined by calculating the gradient of the linear regression of VO_2 versus HR[37], **Figure 5.2**, the mean r^2 of the linear regression for the studied cohort is 0.97 (ranged from 0.86 to 1.00). O_2 pulse was expressed as absolute volume and adjusted for BM. In addition, to account for the effects of body size on the magnitude of O_2 pulse response during exercise, the O_2 pulse was adjusted to LTM: ***Adjusted O_2 pulse = O_2 pulse / LTM***.

Figure 5.2. An Example of Linear Regression for Estimation of Oxygen Pulse (O_2 pulse)



5.3.8 Perception of Exertion

Perception of exertion was evaluated using the 15-point Borg Ratings of Perceived Exertion (RPE) Scale[36]. This is a self-reported measure, which evaluates the subjective perception of exertion on a scale from 6 to 20 with 6 representing "no exertion at all", and 20 denoting "maximal exertion". The Borg's RPE Scale was administered using the standardised instructions provided by Borg[38]. Participants were familiarised with the scale prior to exercise testing. Although subjects received strong concurrent verbal encouragement throughout the exercise protocol, memory anchoring was not used. The RPE scale was displayed on a large cardboard. It remained in sight of the participant during the entire exercise test, and was held directly in front of the participants in the last 5

seconds of each minute of the exercise test, where participants reported an instantaneous RPE by pointing at the scale.

For comparisons to be made between different subjects and exercise conditions, the Borg RPE scale should be related to a standardised workload. This may be possible if the cohort is homogenous, but in the present study, there are differences in age, gender, body size, and levels of cardiovascular fitness. There were also variations in the workload achieved among participants. To overcome these issues, RPE index (RPE_{index}) was adopted, in which the ***actual RPE*** at the end of the exercise protocol (or volitional fatigue) was compared to the ***expected RPE*** based on the ***subject's HR at that time*** as a fraction of ***estimated age-predicted maximum HR*** and ***assuming that RPE would be 20 at maximum heart rate***. The RPE_{index} allows for differences in body size, fitness and age, it is therefore a true reflection of the subject's sense of effort ***relative to other people***.

The derivation is summarised below:

RPE_{index} = (Actual RPE / Expected RPE of 20) × (Estimated Age-predicted Maximum HR / Actual HR at exhaustion or end of exercise)

5.3.9 Demographic, Clinical, Psychosocial and Behavioural Data Collection

Age, gender, marital status, ethnicity, and time post-transplantation were collected from patients' medical records. Smoking status (never smoked, current smoker, ex-smoker) and alcohol intake (units per week) were enquired by questionnaire. Co-morbidity was

assessed by Index of Co-Existing Disease (ICED), using the algorithm described by Hemodialysis (HEMO) Study[39], with data extracted from patients' medical records. Presence of diabetes, either pre-transplantation (pre-DM) or new-onset diabetes after transplantation (NODAT), prior acute rejection episodes, and immunosuppressive medication usage were retrieved from patients' medical records.

Blood sampling was undertaken for analysis of high-sensitivity C-reactive protein (hsCRP), haemoglobin, and creatinine-derived estimated glomerular filtration rate (eGFR) using the 4-variable modification of diet in renal disease equation[40].

Symptoms of anxiety and depression were assessed using Hospital Anxiety and Depression Scale (HADS). It has been validated against clinical diagnosis of anxiety and depression including patients with end-stage renal disease[41,42]. HADS is a self-administered 14-item scale, with 7 items measuring anxiety and 7 items measuring depressive symptoms. Items were scored on a 4-point scale ranging from 0 to 3. The sum-scores for anxiety and depression range from 0 to 21, with higher scores indicating increased symptoms of anxiety and depression.

Sleep Quality was assessed by the Pittsburgh Sleep Quality Index (PSQI). It is valid, reliable and widely used[43,44]. In particular, it demonstrated reliability and validity in KTRs[45]. PSQI consists of a 24-item questionnaire measuring sleep disturbances during the previous month, and generates a global score of subjective sleep quality ranging from 0 to 21, with higher scores indicating worsening subjective sleep quality.

5.3.10 Statistical Analysis

Statistical analyses were performed using SPSS Statistics 21 (Chicago, IL). Unless otherwise stated, results were presented as mean ± standard deviation (SD) for normally distributed data, or median (interquartile range, IQR) for non-normally distributed data. Data with positively skewed distribution were given a logarithmic transformation prior to analysis. Pearson correlation coefficient was used to assess relationships. Independent-samples t-test was used to compare differences of continuous variables between groups.

Linear regression analysis was used to determine the association between predictor variables and the continuously-distributed outcome variables. There were two outcomes variables in this study, physical fatigue and RPE_{index}. Both variables were found to be normally distributed, and were analysed on its original scale of measurement. The analyses were performed in two stages. Initially, the effect of each variable was examined in a series of univariate regression analyses. Subsequently, the joint effect of variables demonstrating some evidence of association in univariate analyses ($p<0.20$) was examined in a multivariate regression analysis, using a backward selection procedure to derive the final model. A type 1 error rate ≤5% ($p\leq0.05$) was considered significant in the final model.

5.4 Results

The characteristics of KTRs are shown in **Table 5.2**. Comparison of population characteristics between KTRs and healthy control subjects (control groups A and B) are shown in **Table 5.3**.

Table 5.3. Comparison of Basic Population Characteristics between Kidney Transplant Recipients (KTRs), Control Group A and Control Group B

	KTRs	**Control Group A**	**Control Group B**	***p*-value**
Sample size	n = 55	n = 41	n = 146	---------
Gender (%)	Male = 58	Male = 57	Male = 52	[a]0.61
†Mean age (years)	†46 ± 14	†56 ± 10	†51 (range 18-82)	[b]0.18
†Mean or ‡Median weight (kg)	‡73.0 (64.2-88.5)	†76.6 ± 15.0	‡66.7 (60.1-76.5)	[b]0.59
†Mean height (m)	†1.70 ± 0.10	†1.71 ± 0.09	†1.73 ± 0.09	[b]0.20
†Mean or ‡Median BMI (kg/m^2)	†26.8 ± 5.1	‡24.8 (23.6-27.9)	‡22.4 (19.0-27.1)	[b]0.21

[†]Normally distributed data, results expressed as mean ± standard deviation (SD), unless otherwise stated. ‡Non-normally distributed data, results expressed as median (interquartile range, IQR).
[a]Cochran's Q test was used to test differences on the dichotomous dependent variable between 3 groups.
[b]Kruskal-Wallis One-Way Analysis of Variance (ANOVA) was used to test differences of the continuous variable between groups.

Table 5.2. Population Characteristics for Kidney Transplant Recipients

	Characteristics
Sample size	n = 55
Gender (%)	Male = 58
†Mean age (years)	46 ± 14
Ethnicity (%)	Caucasian = 80 Asian = 13 Afro-Caribbean = 5 Others = 2
Marital status (%)	Single = 22 Married = 67 Divorced/Widowed = 11
Alcohol intake (units per week)	2 (0-3)
Smoking status (%)	Non-smoker = 60 Current smoker = 11 Ex-smoker = 29
‡Median time post-transplantation (years)	2 (1-7)
Previous episodes of acute rejection (%)	Yes = 7 No = 93
Immunosuppressive medication usage	
Calcineurin inhibitor (%)	93
Adjunctive antiproliferative agent (%)	87
Prednisolone (%)	86
Dosage of immunosuppressive medications	
†Mean dose of tacrolimus (mg/day)	5.8 ± 3.2
†Mean dose of cyclosporine (mg/day)	184 ± 47
†Mean dose of mycophenolate mofetil (mg/day)	1147 ± 456
†Mean dose of azathioprine (mg/day)	85 ± 36
‡Median dose of prednisolone (mg/day)	5.3 (5.0-5.0)
Physical fatigue	
†Mean MFI-20 score	10 ± 4
MFI-20 score ≥95th percentile of control group A	22
Mental fatigue	
†Mean MFI-20 score	10 ± 5
MFI-20 score ≥95th percentile of control group A	20
Quality of Life	
†Mean total score	77 ± 18
†Mean physical health summary score	73 ± 20
†Mean mental health summary score	77 ± 18
Body composition (DEXA measurements)	
†Mean LTM (kg)	50.7 ± 11.5 [Male: 58.0 ± 9.6; Female: 41.2 ± 4.9]
†Mean LTM adjusted to Ht^2 (kg/m^2)	17.5 ± 2.5 [Male: 18.7 ± 2.4; Female: 15.8 ± 1.6]
†Mean LLTM (kg)	16.0 ± 3.7 [Male: 18.1 ± 3.1; Female: 13.4 ± 2.4]
†Mean LLTM adjusted to Ht^2 (kg/m^2)	5.5 ± 0.9 [Male: 5.8 ± 0.8; Female: 5.0 ± 0.8]
†Mean FM (kg)	23.2 ± 8.9 [Male: 22.2 ± 9.3; Female: 24.5 ± 8.6]

Table 5.2. Population Characteristics for Kidney Transplant Recipients (continued)

	Characteristics
Jumping mechanography	
†Mean muscle power from CMJ (W)	2641 ± 756 [Male: 3008 ± 727; Female: 2171 ± 493]
†Mean muscle power per BM from CMJ (W/kg)	35 ± 7 [Male: 37 ± 8; Female: 32 ± 6]
†Mean muscle power per LLTM from CMJ (W/kg)	169 ± 31 [Male: 172 ± 33; Female: 166 ± 29]
Incremental submaximal exercise test	
†Mean VO_2max (ml/min/kg)	27.7 ± 10.4 [Male: 30.1 ± 9.7; Female: 21.9 ± 5.0]
†Mean O2 Pulse (ml/beat)	16.8 ± 5.8 [Male: 21.6 ± 7.4; Female: 12.0 ± 4.2]
†Mean O2 Pulse (ml/beat/kg BM)	0.22 ± 0.07 [Male: 0.26 ± 0.08; Female: 0.18 ± 0.06]
†Mean O_2 Pulse (ml/beat/kg LTM)	0.35 ± 0.14 [Male: 0.39 ± 0.13; Female: 0.31 ± 0.14]
Borg scale	
†Mean RPE_{index}	1.0 ± 0.3 [Male: 0.9 ± 0.3; Female: 1.0 ± 0.2]
Presence of diabetes (%)	Non-diabetic = 73 NODAT = 14 Pre-DM = 13
Co-morbidity	
‡Median ICED score	2 (2-2)
HADS	
†Mean anxiety score	8 ± 5
†Mean depression score	4 ± 3
PSQI	
†Mean global score	6 ± 3
‡Median hsCRP (mg/L)	1.67 (0.61-3.96)
†Mean Hb (g/dL)	12.6 ± 1.5
†Mean eGFR (mL/min)	49.4 ± 12.9

†Normally distributed data, results expressed as mean ± standard deviation (SD). ‡Non-normally distributed data, results expressed as median (interquartile range, IQR).

Abbreviations: MFI-20=multi-dimensional fatigue inventory-20; DEXA=dual energy x-ray absorptiometry; LTM=lean tissue mass; Ht^2=height squared; LLTM=lower limb lean tissue mass; FM=fat mass; CMJ=single two-legged counter movement jump; VO_2max_{est}=estimated maximal oxygen consumption; O_2 pulse =oxygen pulse; BM=total body mass; RPE_{index}=rating of perceived exertion index; ICED=index of co-existing disease; HADS=hospital anxiety and depression scale; PSQI=Pittsburgh sleep quality index; hsCRP=high-sensitivity c-reactive protein; Hb=haemoglobin; eGFR=estimated glomerular filtration rate; NODAT=new onset diabetes after transplantation; Pre-DM=pre-existing diabetes mellitus.

5.4.1 Muscle Mass and Muscular Function

LTM from DEXA scanning adjusted to Ht^2 did not differ between KTRs (male = 18.7±2.4 kg/m^2, female = 15.8±1.6 kg/m^2) and control group A (male = 18.6±1.7 kg/m^2, female = 16.7±2.6 kg/m^2) in both male (p=0.81) and female (p=0.18), **Figure 5.3**. Similarly, LLTM adjusted to Ht^2 did not differ significantly between KTRs (male = 5.8±0.8 kg/m^2, female = 5.0±0.8 kg/m^2) and control group A (male = 6.1±0.7 kg/m^2, female = 5.4±1.1 kg/m^2) in both male (p=0.22) and female (p=0.21), **Figure 5.4**.

Figure 5.3. Comparison of Lean Tissue Mass (LTM) adjusted to Height Squared (Ht^2) between Kidney Transplant Recipients (KTRs) and Healthy Control Subjects (Control Group A)

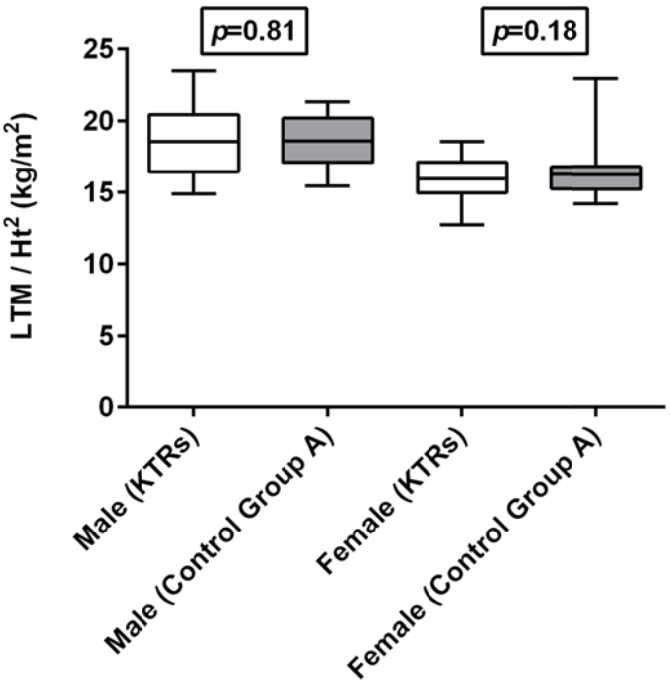
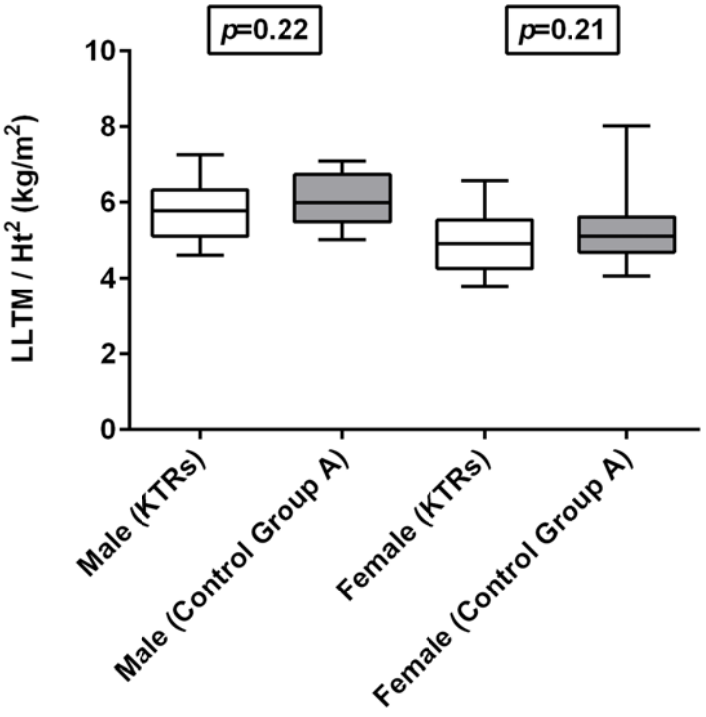
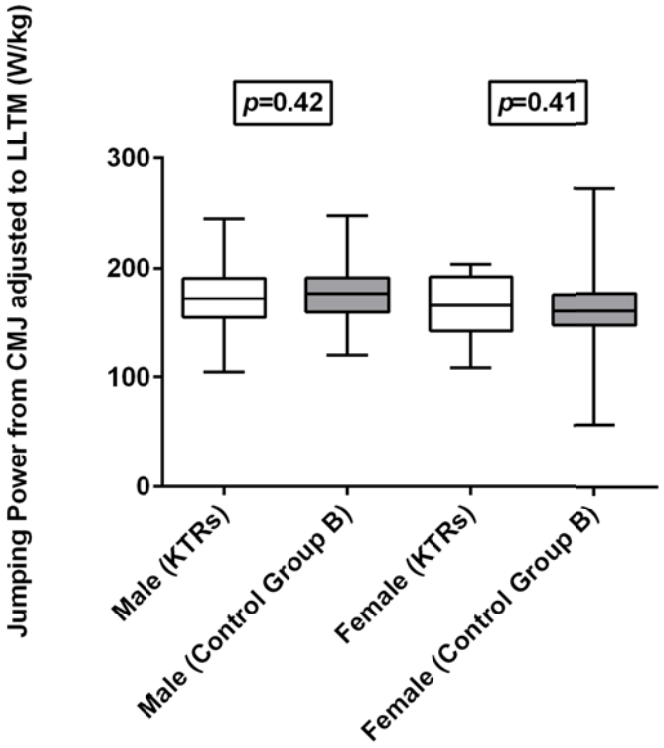


Figure 5.4. Comparison of Lower Limb Lean Tissue Mass (LLTM) adjusted to Height Squared (Ht^2) between Kidney Transplant Recipients (KTRs) and Healthy Control Subjects (Control Group A)



In terms of muscular function, jumping power from CMJ per kg BM did not differ significantly between female KTRs (32±6 W/kg) and female control group A (36±7 W/kg) (p=0.25). However, jumping power from CMJ per kg BM in male KTRs (37±8 W/kg) was significantly lower than male control group A (47±6 W/kg) (p=0.03). Additionally, jumping power from CMJ per kg LLTM did not differ significantly between KTRs (male = 172±33 W/kg, female = 166±29 W/kg) and control group B (male = 177±25 W/kg, female = 160±30 W/kg) in both male (p=0.42) and female (p=0.41), **Figure 5.5**.

Figure 5.5. Comparison of Jumping Power from Single 2-Legged Counter Movement Jump (CMJ) adjusted to Lower Limb Lean Tissue Mass (LLTM) between Kidney Transplant Recipients (KTRs) and Healthy Control Subjects (Control Group B)



In KTRs, no correlation was found between physical fatigue with LTM adjusted to Ht^2 (r=0.09, p=0.75, **Figure 5.6**), LLTM adjusted to Ht^2 (r=0.05, p=0.48, **Figure 5.7**), muscular power from CMJ adjusted to BM (r=0.19, p=0.31), and CMJ adjusted to LLTM (r=0.24, p=0.28, **Figure 5.8**).

Figure 5.6. Association between Physical Fatigue and Lean Tissue Mass (LTM) adjusted to Height Squared (Ht^2)

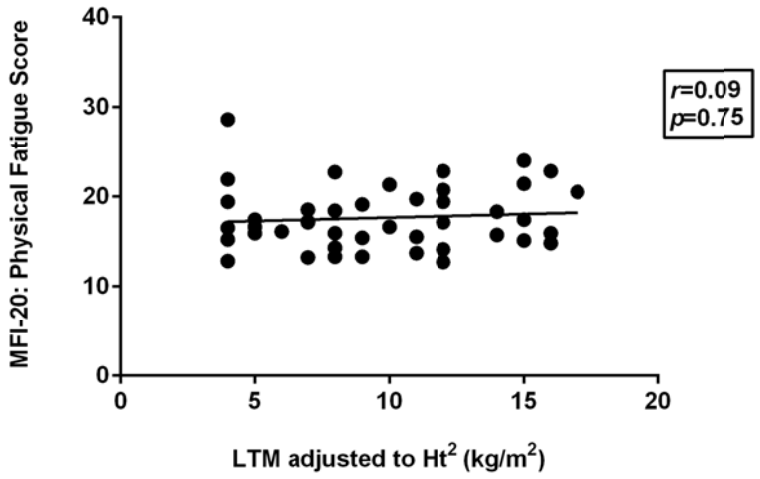


Figure 5.7. Association between Physical Fatigue and Lower Limb Lean Tissue Mass (LLTM) adjusted to Height Squared (Ht^2)

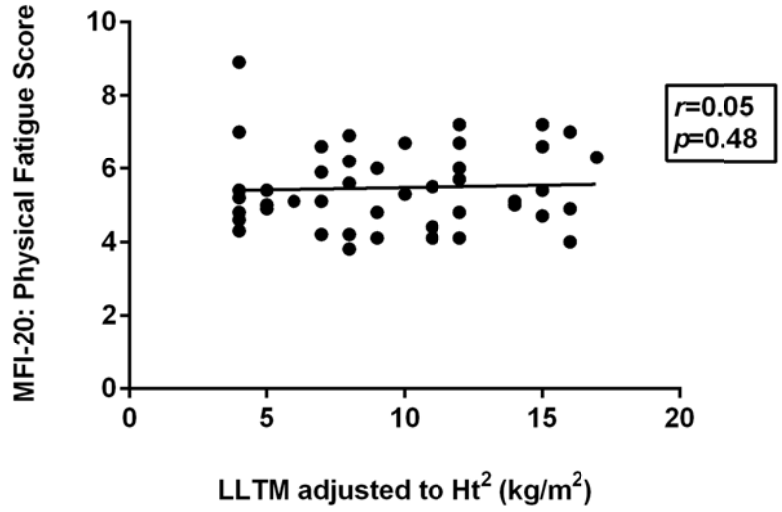
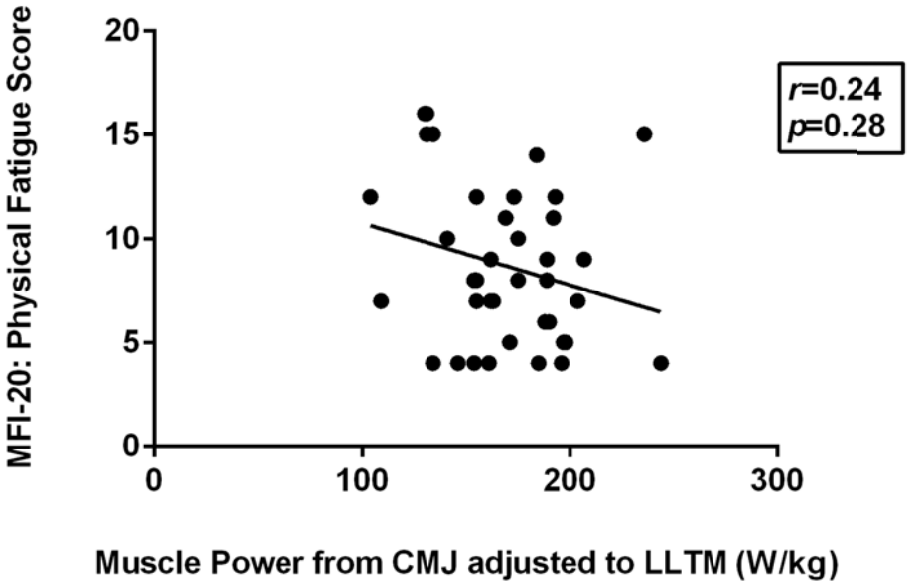


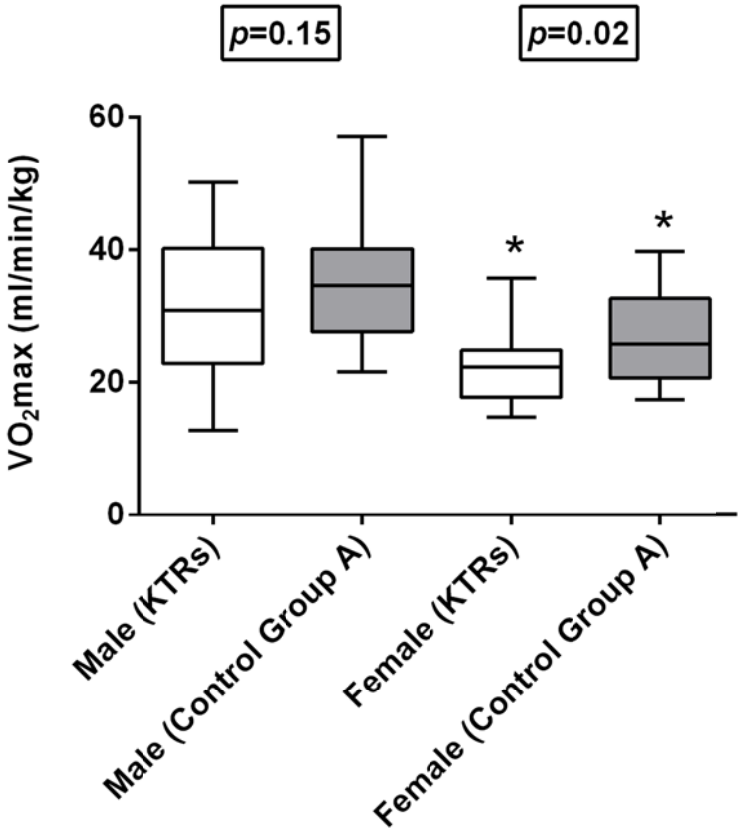
Figure 5.8. Association between Physical Fatigue and Muscle Power (Single 2-Legged Counter Movement Jump, CMJ, adjusted to Lower Limb Lean Tissue Mass, LLTM)



5.4.2 Cardiovascular Function

VO_2max for male and female KTRs were 31.1±10.7 and 22.4±5.5 ml/min/kg respectively. Although VO_2max in female was significantly lower in KTRs (p=0.02, **Figure 3a**) compared with control group A (27.2±6.7 ml/min/kg), it did not differ significantly between male KTRs and male control group A (35.0±8.2 ml/min/kg) (p=0.15, **Figure 3a**).

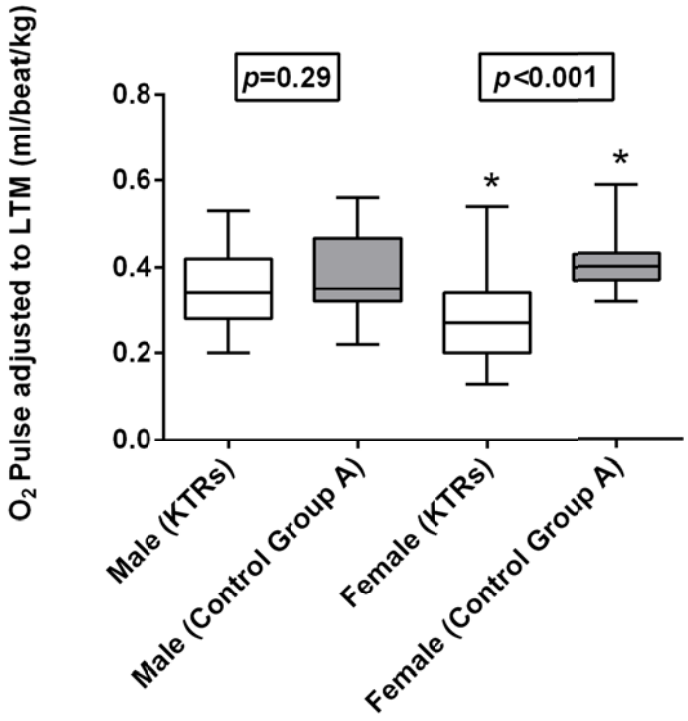
Figure 5.9. Comparison of Maximal Oxygen Consumption (VO_2max) between Kidney Transplant Recipients (KTRs) and Healthy Control Subjects (Control Group A)



Similarly, absolute O_2 pulse in in female KTRs (12.0 ± 4.2 ml/beat) was significantly lower than female control group A (17.2 ± 5.5 ml/beat) (p=0.03), while it did not differ significantly between male KTRs (21.6 ± 7.4 ml/beat) and male control group A (24.2 ± 5.5 ml/beat). When O_2 pulse was adjusted for BM, female KTRs displayed significantly lower O_2 pulse (0.18 ± 0.06 ml/beat/kg) compared to female control group A (0.24 ± 0.07 ml/beat/kg) (p=0.04), and there was no significant difference between male KTRs (0.26 ± 0.08 ml/beat/kg) and male control group A (0.30 ± 0.06 ml/beat/kg) (p=0.21). Finally, O_2 pulse adjusted for LTM in female KTRs (0.30±0.14 ml/beat/kg) was significantly lower

than female control group A (0.38±0.09 ml/beat/kg) (p<0.001, **Figure 5.10**). However, O_2 pulse adjusted for LTM in male did not differ significantly between KTRs (0.38±0.13 ml/beat/kg) and control group A (0.42±0.07 ml/beat/kg) (p=0.29, **Figure 3b**).

Figure 5.10. Comparison of Oxygen Pulse (O_2 Pulse) adjusted to Lean Tissue Mass (LTM) between Kidney Transplant Recipients (KTRs) and Healthy Control Subjects (Control Group A)



Of note, in KTRs, no significant correlations were seen between physical fatigue with VO_2max (r=0.23, p=0.09, **Figure 5.11**), absolute O_2 pulse (r=0.17, p=0.21), O_2 pulse adjusted for BM (r=0.21, p=0.20), and O_2 pulse adjusted for LTM (r=0.17, p=0.23, **Figure 5.12**).

Figure 5.11. Association between Physical Fatigue and Maximal Oxygen Consumption (VO_2max)

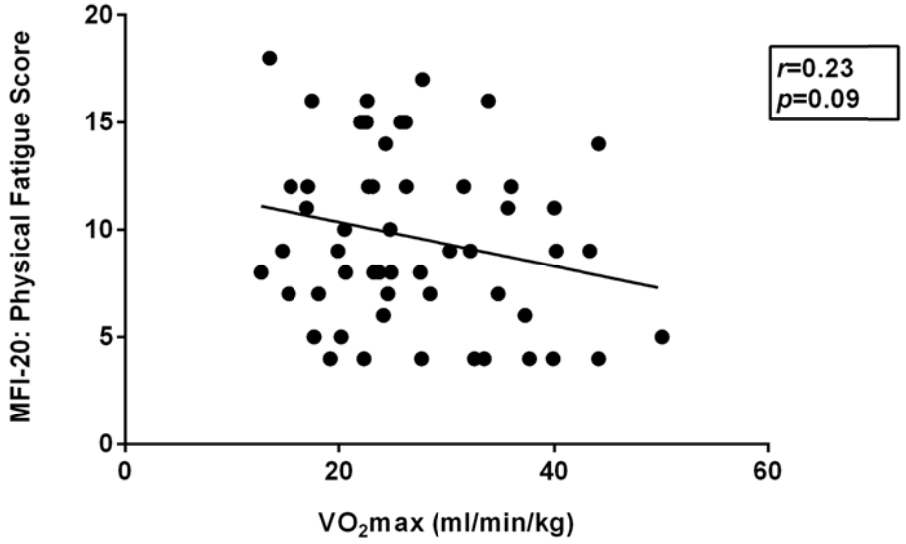
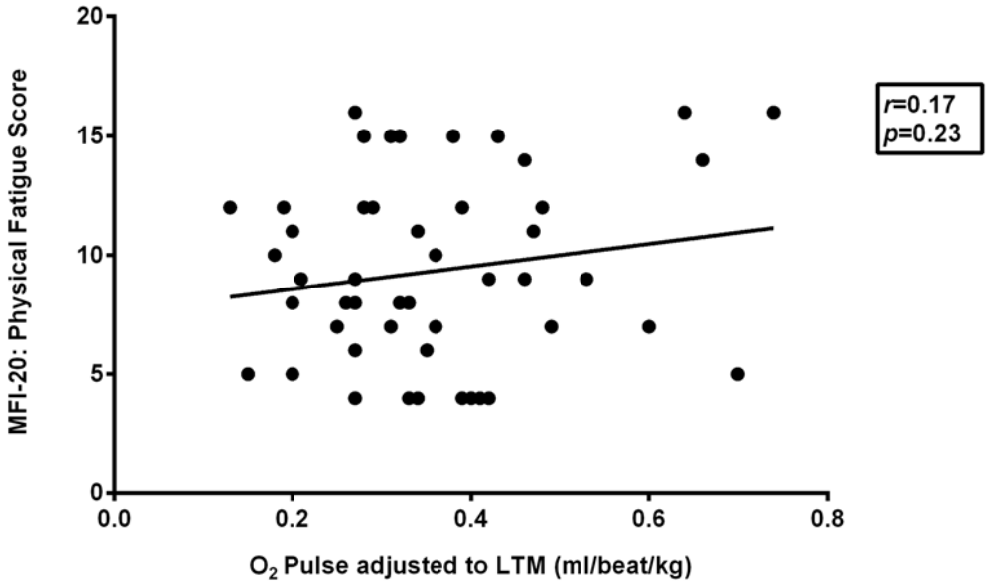


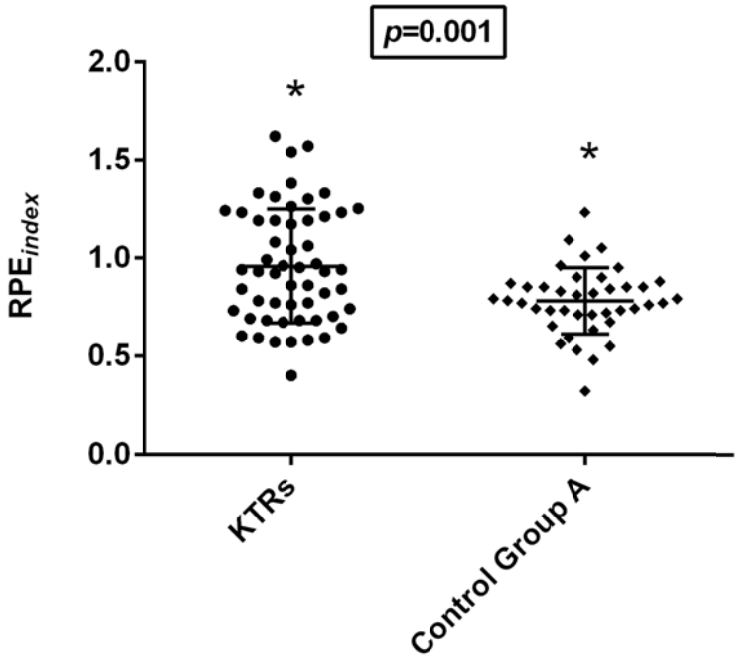
Figure 5.12. Association between Physical Fatigue and Oxygen Pulse (O_2 Pulse) adjusted to Lean Tissue Mass (LTM)



5.4.3 Perceived Exertion

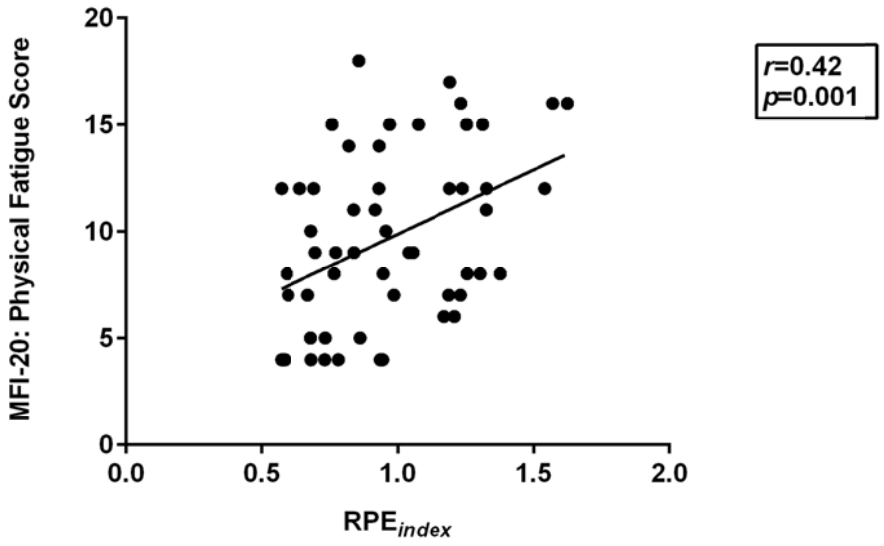
There was significant overlap of RPE_{index} between KTRs and control group A, **Figure 5.13**. Whether it was curtailed by volitional fatigue or the final minute of the highest work rate of the exercise protocol, RPE_{index} was significantly higher in KTRs (1.0±0.3) compared to control group A (0.8±0.2) (p=0.001, **Figure 5.13**).

Figure 5.13. Comparison of Rating of Perceived Exertion Index (RPEindex) between Kidney Transplant Recipients (KTRs) and Healthy Control Subjects (Control Group A)



Notably, in KTRs, RPE_{index} demonstrated significant correlation with physical fatigue (r=0.42, p=0.002, **Figure 5.14**).

Figure 5.14. Association between Physical Fatigue and Rating of Perceived Exertion Index (RPEindex)



5.4.4 Predictors of Physical Fatigue in Kidney Transplant Recipients

Table 5.4 shows the associations between physical fatigue and measures of muscle mass and function, VO_2max, O_2 pulse, and RPE_{index} in KTRs. On univariate analysis, the only significant predictor of increasing physical fatigue in KTRs were increased RPE_{index} (p=0.001). No significant associations were seen between physical fatigue and measures of muscle mass and strength. A trend towards an association between physical fatigue and VO_2max was evident on univariate analysis (p=0.09).

Table 5.4. Predictors for Mechanistic Aetiology of Physical Fatigue

	Univariate Analysis		Multivariate Analysis[a]	
	Regression Coefficient (95% CI[b])	***p*-value**	**Regression Coefficient (95% CI[b])**	***p*-value**
RPE_{index}	**5.7 (2.2, 9.2)**	**0.001**	**5.7 (2.2, 9.2)**	**0.001**
	[c]0.0 (-7.8, 7.9) *[d]0.0 (-0.2, 0.3)*	*[c]0.99* *[d]0.73*		
VO_2max (ml/min/kg)	**-0.1 (-0.2, 0.0)**	**0.09**		
	[c]-0.2 (-0.5, 0.2) *[d]0.0 (-0.0, 0.0)*	*[c]0.34* *[d]0.69*		
O_2 Pulse (ml/beat)	**3.5 (-4.1, 11.2)**	**0.21**		
	[c]-5.5 (-19.2, 12.1) *[d]0.1 (-0.8, 0.9)*	*[c]0.39* *[d]0.77*		
O_2 Pulse (ml/beat/kg BM)	**2.5 (-2.7, 11.4)**	**0.20**		
	[c]-5.7 (-16.2, 9.8) *[d]0.2 (-0.5, 0.4)*	*[c]0.22* *[d]0.68*		
O_2 Pulse (ml/beat/kg LTM)	**4.7 (-3.1, 12.6)**	**0.23**		
	[c]-6.5 (-23.1, 10.1) *[d]0.0 (-0.6, 0.6)*	*[c]0.43* *[d]0.88*		
LTM adjusted to Ht^2 (kg/m^2)	**0.1 (-0.4, 0.5)**	**0.75**		
	[c]0.8 (-0.4, 2.0) *[d]0.0 (-0.0, 0.1)*	*[c]0.29* *[d]0.51*		
LLTM adjusted to Ht^2 (kg/m^2)	**-0.4 (-1.6, 0.8)**	**0.48**		
	[c]0.8 (-1.9, 3.4) *[d]-0.0 (-0.1, 0.1)*	*[c]0.57* *[d]0.83*		
[‡]CMJ, absolute power (W)	**-0.1 (-0.2, 0.1)**	**0.33**		
	[c]0.0 (-0.3, 0.2) *[d]0.0 (-0.0, 0.0)*	*[c]0.91* *[d]0.94*		
[†]CMJ, power per BM (W/kg)	**-0.1 (-0.2, 0.1)**	**0.31**		
	[c]-1.1 (-5.2, 2.4) *[d]-0.0 (-0.1, 0.1)*	*[c]0.52* *[d]0.41*		
[†]CMJ, power per LLTM (W/kg)	**-0.1 (-0.3, 0.1)**	**0.28**		
	[c]-1.5 (-5.8, 2.8) *[d]-0.1 (-0.3, 0.1)*	*[c]0.47* *[d]0.24*		
R^2 value from final model			28%	

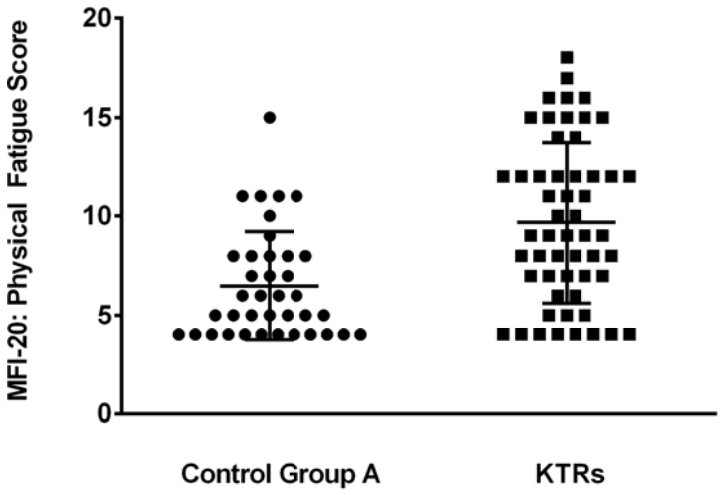
[a]Results in the final multivariate regression model were presented.
[b]CI = Confidence Interval.
[c]Results of interaction analysis moderated by the effect of gender.
[d]Results of interaction analysis moderated by the effect of age.
[†]Coefficients reported for a 10-unit increase in explanatory variable.
[‡]Coefficients reported for a 100-unit increase in explanatory variable.
Abbreviations: RPE_{index}=rating of perceived exertion index; VO_2max_{est}=estimated maximal oxygen consumption; O_2 pulse=oxygen pulse; BM=total body mass; LTM=lean tissue mass; Ht^2=height squared; LLTM=lower limb lean tissue mass; CMJ=single two-legged counter movement jump.

With RPE_{index} and VO_2max analysed in the multivariate model, VO_2max no longer retained significance (Beta coefficient, β = 0.0; 95% Confidence Interval, CI = -0.2, 0.1; p=0.54). In the adjusted multivariate model, RPE_{index} remained the single independent predictor of physical fatigue (β=5.7; 95% CI=2.2, 9.2; p=0.001). Of note, no significant age- and gender- interactions were found between physical fatigue and all the predicting variables (**Table 5.4**).

5.4.5 Prevalence of Physical Fatigue and Correlation with Quality of Life

The physical fatigue scores for both KTRs and control group A varied widely with considerable overlap, **Figure 5.15**. The mean score for physical fatigue in KTRs was 10±4, higher than reported by control group A (6±3; p<0.001; **Figure 5.15**). Based on the previously established definition of physical fatigue, with fatigue defined as ≥95th percentile for healthy subjects[4,19], the prevalence of physical fatigue was 22%.

Figure 5.15. Comparison of Physical Fatigue Scores between Healthy Control Subjects (Control Group A) and Kidney Transplant Recipients (KTRs)



In KTRs, physical fatigue correlated closely with SF-36 total score (r=-0.68, p<0.001), SF-36 physical health summary score (r=-0.74, p<0.001), and SF-36 mental health summary score (r=-0.60, p<0.001), **Figure 5.16**. To exclude the confounding effect of the SF-36 "energy and vitality" subscale, which is a general measure of fatigue within SF-36[46], results were reanalysed excluding this subscale, and the associations remained comparable after the exclusion, SF-36 total score (r=-0.65, p<0.001), SF-36 physical health summary score (r=-0.71, p<0.001), and SF-36 mental health summary score (r=-0.53, p<0.001), **Figure 5.17**.

Figure 5.16. Association between Physical Fatigue and Quality of Life (QoL) (All Subscales) in Kidney Transplant Recipients

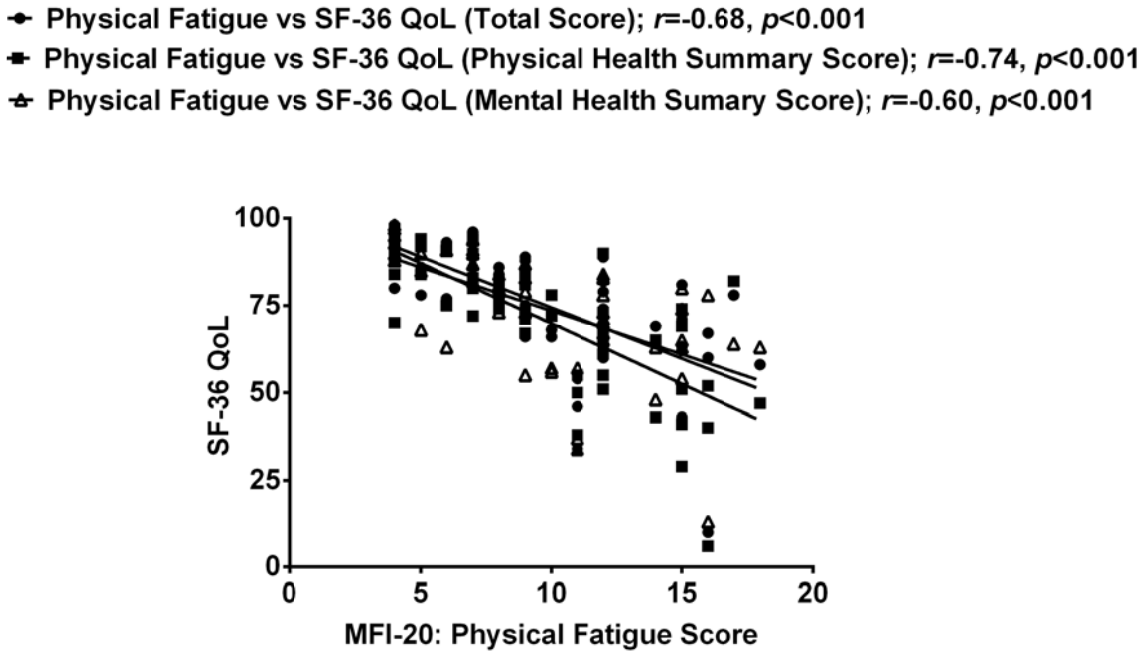
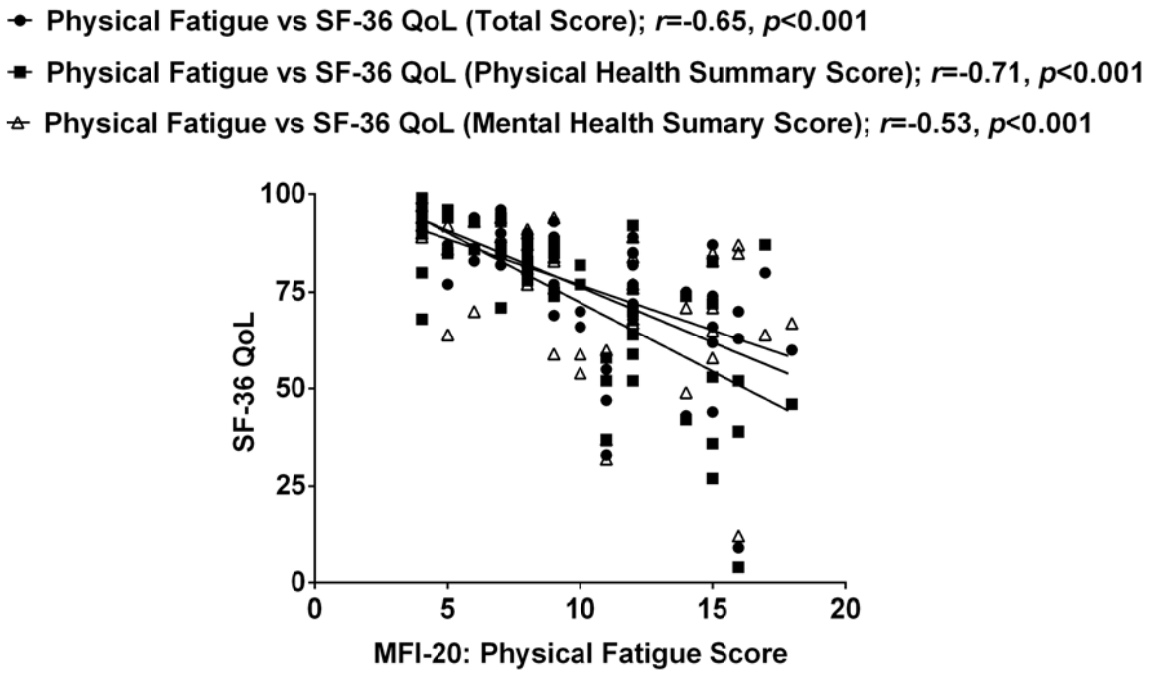


Figure 5.17. Association between Physical Fatigue and Quality of Life (QoL) (Excluding "Energy & Vitality Subscale) in Kidney Transplant Recipients



5.4.6 Predictors of Perceived Exertion in Kidney Transplant Recipients

In light of this association between RPE_{index} and physical fatigue in KTRs, and the previously described association between mental fatigue and perceived exertion in a non-transplant cohort [14], the impact of mental fatigue and other plausible predictors upon perceived exertion was examined in this cohort of KTRs.

As shown in **Table 5.5**, on univariate analysis, mental fatigue, NODAT, absence of cyclosporine, increasing age, low alcohol intake, anxiety, and depression were significantly associated with RPE_{index}.

Table 5.5. Predictors of Rating of Perceived Exertion Index (RPE_{index})

	Univariate Analysis		Multivariate Analysis[a]	
	Beta Coefficient, β (95% CI[b])	***p*-value**	**Beta Coefficient, β (95% CI[b])**	***p*-value**
Presence of diabetes				
Non-diabetic	0	0.02	0	0.04
NODAT	0.3 (0.0, 0.5)		0.2 (0.0, 0.5)	
Pre-DM	0.6 (-0.2, 1.4)		0.2 (-0.0, 0.5)	
Use of calcineurin inhibitor				
None	0	0.03	0	0.03
Cyclosporine	-0.3 (-0.5, -0.1)		-0.4 (-0.6, -0.2)	
Tacrolimus	-0.1 (-0.3, 0.2)		-0.0 (-0.3, 0.3)	
†Age (years)	0.1 (0.0, 0.2)	0.04		
†Mental fatigue (MFI-20 score)	0.3 (0.1, 0.5)	0.04	0.5 (0.1, 0.9)	0.03
†Alcohol intake (units per week)	-0.4 (-1.1, -0.0)	0.04	-0.6 (-1.1, -0.1)	0.03
†Anxiety (HADS score)	0.5 (0.1, 0.8)	0.04	0.4 (0.1, 0.7)	0.04
†Depression (HADS score)	0.5 (0.0, 0.1)	0.05		
Use of prednisolone				
No	0	0.10		
Yes	-0.4 (-0.9, 0.1)			
Co-morbidity (ICED score)	0.9 (-0.4, 2.2)	0.18		
Previous episodes of acute rejection				
No	0	0.20		
Yes	-0.4 (-1.1, 0.2)			
Time post transplantation (years)	0.1 (-0.2, 0.4)	0.35		
Marital status				
Married	0	0.46		
Single	0.2 (-0.6, 0.2)			
Divorced / Widowed	-0.0 (-0.2, 0.1)			
[c]**Ethnicity**				
Caucasian	0	0.48		
†Non-Caucasian	0.4 (-0.2, 3.8)			
Gender				
Female	0	0.51		
Male	0.1 (-0.2, 0.5)			
†FM (kg)	0.1 (-0.2, 0.3)	0.55		
Smoking status				
Never smoked	0	0.66		
†Ex-smoker	0.5 (-1.6, 2.4)			
Current smoker	0.1 (-7.3, 7.6)			
†Haemoglobin (g/dL)	0.3 (-0.9, 1.5)	0.66		
†PSQI (global score)	0.1 (-0.5, 0.6)	0.77		
†eGFR (mL/min)	0.0 (-0.1, 0.2)	0.82		
[f]hsCRP (mg/L)	0.0 (-0.3, 0.4)	0.94		
Use of adjunctive antiproliferative agents	0			
None	-0.2 (-4.3, 0.4)	0.98		
†Mycophenolate mofetil	0.0 (-0.3, 0.3)			
Azathioprine				
†LTM (kg)	0.00 (-0.2, 0.2)	0.99		
R^2 value from final model			38%	

[a]Results in the final multivariate regression model were presented. [b]CI = Confidence Interval.

[c]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 80% "Caucasian" versus 20% "Non-Caucasian".

† Coefficients reported for a 10-unit increase in explanatory variable. [f]Variable analysed on the log scale (base 10).

Abbreviations: HADS=hospital anxiety and depression scale; NODAT=new onset diabetes after transplantation; Pre-DM=pre-existing diabetes mellitus; MFI-20=multi-dimensional fatigue inventory-20; ICED=index of co-existing disease; FM=fat mass; PSQI=Pittsburgh sleep quality index; eGFR=estimated glomerular filtration rate; hsCRP=high-sensitivity C-reactive protein; LTM=lean tissue mass.

In the multivariate analysis, age and depression did not retain significance, but the remaining variables persisted as showing statistically significant relationships with RPE_{index} (**Table 5.5**). 38% of the variation in perceived exertion was explained by the variables in the final multivariate model (R^2: 38%).

5.5 Discussion

This is the first study to systematically investigate the potential aetiology of physical fatigue in KTRs, which may be mechanistically linked to symptoms of physical fatigue. This study reveals important findings. First, physical fatigue is unrelated to muscular and cardiovascular factors, but rather, it is driven by increased perception of exertion during exercise. The findings of the current study confirm physical fatigue as a common and disabling symptom among KTRs, negatively impacting on QoL[3-5]. In turn, mental fatigue significantly associated with such heightened perception of effort. Whilst novel to transplantation, these results resonate with findings from other populations, whereby heightened perception limits exercise capacity in healthy trained individuals[47] and diabetic patients[48], and mental fatigue impairs physical performance through increased perception of effort rather than limiting musculoenergetic or cardiorespiratory functions[14,15].

Similar to all aspects of fatigue assessment using the MFI-20 questionnaire, physical fatigue scores varied widely within both KTRs and control groups. Based on an established definition of physical fatigue ($\geq 95^{th}$ percentile for healthy control subjects)[4,19], 22% of KTRs experienced this symptom. It was somewhat lower than previously reported (38%)[4], possibly due to stringent exclusion criteria employed in this study for ethical and

safety issues. Also, KTRs who were eligible for entry into this study might have declined participation at enrolment due to the likely discomfort arising from the exercise test and the possibility of physical difficulty encountered with the vertical jump test. For these reasons, variations in the characteristics of the studied populations may explain the differences in the prevalence of physical fatigue. Indeed, the mean physical fatigue score for KTRs (10±4) was comparable to "chronically unwell" patients (10±4) reported by Lin[19]. This, together with the adverse associations on all aspects of QoL, indicates the severity of the problem.

Varied disease processes or lack of physical activity may result in muscle atrophy. In these circumstances, muscles work at a relatively high work-load and hence fatigue rapidly. However, in this cohort of KTRs, there was no association between physical fatigue with either whole body LTM or LLTM. Certainly, muscle mass *per se* may not be the crucial factor, the ability of musculature to generate force and movement arguably may be of greater importance. Interestingly, the results from the jumping mechanography studies showed no association between muscular power and physical fatigue. In support of these results in the KTRs group, muscle mass and power were similar to gender-specific healthy control subjects, with muscle mass data comparable to previous literature in this field[49,50].

Reduced aerobic fitness from disease or inactivity may lead to physical fatigue. VO_2max is the conventional measure of cardiovascular fitness, and its prognostic utility is well-established in research and clinical settings[51,52]. VO_2max is frequently estimated from a submaximal exercise test. However, there are 2 caveats to this approach. Firstly, estimation of maximum HR in relation to age may be unreliable[33,53]. Secondly, body

weight-adjusted VO_2max can be misleading due to inter-individuals' variability in body composition. An alternative, but complimentary measure of O_2 pulse has recently emerged[51,52], which, during exercise is predominately determined by cardiac stroke volume and peripheral oxygen extraction, thereby reflecting cardiovascular function more accurately[51,52,54]. Both male and female KTRs had numerically lower VO_2max and O_2 pulse compared to healthy controls, although only statistically significant in females. These results are comparable to findings from previous studies in this field[49,50,55,56]. Of relevance, however, neither VO_2max nor O_2 pulse were associated with physical fatigue in the analysis. The difference in cardiovascular fitness between KTRs and healthy subjects is perhaps unsurprising, but the underlying reasons for the variation were not the focus of the current study.

Using the Borg scale in healthy subjects, the RPE during exercise is linearly related to the actual work rate, measured by oxygen uptake or HR[36], ranging from a RPE of 6 at rest to 20 corresponding to age-predicted maximum HR. In this study, it was found that at the end of the exercise protocol, the RPE scores from KTRs were significantly higher than healthy control subjects, with both based on HR relative to age-adjusted maximum HR. This indicates that at the same relative work rate, KTRs had greater perception of exertion. Importantly, RPE_{index} in KTRs correlated significantly with physical fatigue, consistent with a heightened perception of exertion. The final multivariate analysis model suggests that this contribution explains 28% of physical fatigue experienced by KTRs.

The mechanisms by which perception of effort influences physical performance has been previously proposed by Marcora and colleagues using the Brehm's theory of motivation[14].

In this theory, individuals opt to withdraw from a task when it is perceived to be too difficult, or the effort required exceeds the individuals' willingness to perform[57]. During the decision-making process, individuals are suspected to have lowered their level of task difficulty for withdrawal[58,59]. Impaired physical performance is a common feature in KTRs[60-62], since physical fatigue represents a transient decrease in muscular performance, this may be seen as failure to generate and to maintain optimal physical performance. Therefore, the Brehm's theory of motivation may be extrapolated in this setting. In addition, there is evidence that disorders of the brainstem, dopaminergic systems and endogenous opiates may affect decision making[57-59,63,64]. In particular, increasing dopamine release in the brain through dopaminergic-modulating agent, is associated with reduced perceived fatigue and increased perceived QoL in chronic fatigue syndrome[65]. This may be applicable to KTRs with physical fatigue as an important-patient reported outcome.

Of importance, a caveat with the interpretation of the associations between self-report data, such as symptoms of physical and mental fatigue, perceived exertion, anxiety and depression, is that common method variance may partly drive the observed associations and may account for 25% of shared variance[66]. In common method variance, patients high in negative effect (i.e. negative mood) perceive, remember, and report more physical and psychological symptoms, and report those symptoms to be more severe than patients with less negative mood[67]. Although these would not render self-reports unimportant, potential interpretational difficulties may result.

Factors associated with heightened perceived exertion in the adjusted analysis included low alcohol intake, lack of cyclosporin prescription, NODAT, mental fatigue, and anxiety. Interestingly, other commonly studied clinical and demographic variables, including eGFR, haemoglobin and hsCRP, showed no association with perception of effort.

Overall, this study cohort consisted of very light drinkers (2 units a week), and previous studies have shown that light to moderate alcohol consumption is associated with improved cognitive function[68,69], with both social and physiological factors playing potential roles[68-71]. Absence of cyclosporin was also an independent predictor of perceived exertion. The exact mechanism remains unclear, although an animal study demonstrated that cyclosporine A preserves brain mitochondrial function that is associated with improved motor and cognitive behaviour[72]. Previous study have shown that higher cognition is closely associated with improved visual perception[73], however, it is unclear whether visual perception shares similar mechanisms as perceived exertion during exercise. Although these associations may be biologically plausible, at present, such correlations were only supported by weak rationale in the literature, possibly representing a type I statistical error.

Fatigue in diabetes is likely to be caused by the interplay of physiological, psychological and lifestyle-associated factors[74]. It is interesting that pre-DM was not associated with increased perceived exertion, a plausible explanation for the differences between NODAT and pre-DM is that KTRs with NODAT may experience exaggerated psychological distress having to cope with yet another disease state and requiring a novel diabetes treatment regimen.

KTRs displayed considerable mental fatigue, with an MFI-20 score of 10±5, comparable to "chronically unwell" patients reported by Lin[19] (11±4). Further, mental fatigue was an independent predictor of increased perception of exertion. This novel data in KTRs is reminiscent of that from Marcora in a non-transplant cohort[14] who showed mental fatigue decreases physical performance via increased perception of effort, without affecting conventional physiological variables such as stroke volume, oxygen uptake, blood pressure, or lactate levels[14]. However, it should be noted that mental fatigue was measured by self-report questionnaire in this study, whereas mental fatigue was induced experimentally by a 90-minute computer-based cognitive task in Marcora's study[14], it is unclear whether the two methodologies characterised equivalent effects. If common mechanisms exist, it is possible that increased perception of exertion is an aspect of mental fatigue in KTRs, contributing to symptoms of physical fatigue.

Mean anxiety score for KTRs in this study (8±5 on HADS) is considered mild anxiety[75], and was independently associated with increased perception of exertion. Depression also displayed a univariate association with perception. These observations have been noted previously in non-transplant studies[76,77], supporting the findings of this study. Anxious and depressed individuals are less attuned to interpret bodily sensations including fatigue during physical activity[77], with physiological responses to exercise "linked" inappropriately to catastrophic cognitions in such individuals.

This study has limitations that should be acknowledged. It is a pilot study that represents a single-centre experience, and validations of the findings are needed in larger cohorts. The observational and cross-sectional nature of the study design indicates that the direction of

the causality between predictor and outcome variables cannot be defined. However, the findings are intuitive and biologically plausible, and are in many aspects compatible with findings from other disease states and the general population. Despite this, there is a possibility that the large number of correlational analyses performed may lead to type I statistical error, especially where associations were supported by weak rationale in the literature. Similarly, absence of associations between certain variables should not be treated without reservations due to a pilot study with a small sample size, implicating an inherently high probability of type II statistical error. Finally, it is important to acknowledge that KTRs are often prescribed antihypertensive medications that exert cardioactive effects, specifically the negative chronotropic effect of beta adrenergic blockers and calcium-channel blockers which would have influenced measurements of HR in this study.

In conclusion, this study suggests that physical fatigue in KTRs is not affected by muscular and cardiovascular factors, but rather, it is caused by increased perception of exertion influenced by mental fatigue and anxiety. Improving physical fitness or strength *per se* is unlikely to improve physical fatigue, and other strategies such as cognitive behavioural therapy or centrally-acting pharmacological therapies may be more appropriate. Undoubtedly, physical fatigue represents a frequent and important patient-reported outcome. The findings of this study set the scene for future interventional research and therapeutic strategies.

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GENERAL DISCUSSION

Chapter 6

Effects of Body Composition on Clinical and Quality of Life Outcomes in Kidney Transplant Recipients

CHAPTER 6: GENERAL DISCUSSION

6.1 Overview of the Thesis

The studies presented in **Chapters 2** to **5** of this thesis explored the associations between different body composition compartments with morbidity (low haemoglobin, elevated blood pressure) and fatigue, the potential contributing factors to long-term patient- and graft- survival, as well as quality of life (QoL). There are significant gaps in the current literature on this area of research. A greater understanding of the relationships between different body composition compartments with morbidity, mortality, and QoL outcomes may provide insight into future interventional strategies, ultimately improving clinical and QoL outcomes in kidney transplant recipients (KTRs).

Chapters 2 and **3** focused on clinical outcomes in clinically stable kidney transplant recipients (KTRs). **Chapter 2** assessed the association between adiposity and inflammation, and its relationship with elevated hepcidin and reduced haemoglobin levels. **Chapter 3** determined the effects of hypervolemia on blood pressure and levels of N-terminal fragment of pro-hormone B-Type natriuretic peptide (NT-proBNP).

Chapters 4 and **5** attended to fatigue, an important QoL outcome in medically stable KTRs. Whilst **Chapter 4** explored the role of muscle mass and adiposity on post-transplantation fatigue; **Chapter 5** specifically examined the potential mechanisms of

physical fatigue by evaluation of muscle mass, muscular function, cardiovascular function, and fatigue perception.

6.2 Summary of the Major Findings Pertaining to Body Composition

The crucial finding of this thesis is that different body composition compartments exert varying effects on clinical and QoL outcomes in KTRs.

6.2.1 Associations between Adiposity with Inflammation, Hepcidin and Haemoglobin Levels in Kidney Transplant Recipients

As shown in **Chapter 2**, increased fat mass was independently and positively associated with inflammation. This is an important observation as previous studies in kidney transplantation yielded conflicting conclusions[1-3]. Further, a univariate association between fat mass and hepcidin level was found, but this association did not persist when adjusted for inflammation. This notion extends to the field of kidney transplantation, which supports the concept that adipose tissue may itself be a source of hepcidin, produced in response to the effect of inflammatory cytokines released by the fat tissue[4]. However, a relationship between adiposity and haemoglobin level was not established. Since independent correlations were observed between inflammation and raised hepcidin level, and between elevated hepcidin and reduced haemoglobin levels, it remains a possibility that adiposity-related inflammation in KTRs is associated with elevated hepcidin,

contributing to reduced haemoglobin in KTRs by dysregulation of iron homeostasis. The absence of such associations may suggest a type II statistical error. Though not evaluated in the current study, the proposed mechanism may potentially impact on patient- and graft-survivals[5-7].

6.2.2 Effects of Hypervolemia on Blood Pressure and Levels of N-Terminal Fragment of Pro-hormone B-Type Natriuretic Peptide in Kidney Transplant Recipients

As revealed by **Chapter 3**, hypervolemia was identified as an independent risk factor for elevated mean arterial, systolic and diastolic blood pressure, which has a recognised impact upon long-term patient- and graft- outcomes[8-10]. While the relationship between hypervolemia and elevated blood pressure resonates with findings in dialysis patients[11-13], it has not been previously demonstrated in KTRs and reflects novelty in this setting. In addition, the independent association between the objective measure of hypervolemia and raised NT-proBNP level is another novel and noteworthy observation of this study. Although the impact of elevated NT-proBNP level in KTRs remains undetermined, it is an independent predictor of mortality in patients with end-stage renal disease (ESRD)[14]. The relationship between hypervolemia and raised NT-proBNP level confirms and extends findings from the non-transplant populations, predominately patients undergoing dialysis[15-18].

6.2.3 The Role of Muscle Mass and Fat Mass on Post-transplantation Fatigue

As presented in **Chapter 4**, while significant univariate associations were observed between fat mass and different dimensions of fatigue, including general fatigue, physical fatigue, reduced activity, and reduced motivation; these relationships did not hold when adjusted for inflammation, suggesting that inflammation is a driver for fatigue rather than adiposity *per se*. This study advances understanding previously built upon from a study in this field[19], where raised body mass index (BMI), a proxy for fat mass, was identified as a predictor of fatigue, but detailed anthropometric and inflammatory evaluation was not undertaken. However, it is possible that the systemic low-grade inflammation present in obesity triggers adipocyte release of proinflammatory cytokines[20]; this in turn accelerates muscle catabolism[21], leading to muscle wasting[21]. Reduced muscle mass coupled with increased fat mass (i.e. sarcopenic obesity) is a common characteristic of body composition after kidney transplantation[22]. Such proposed mechanisms support another major finding in **Chapter 4**, where decreased muscle mass independently predicts two domains of fatigue, physical fatigue and reduced activity. In particular, the independent association between physical fatigue and reduced muscle mass is intuitively plausible, but not previously reported in KTRs. It replicates results from cancer-related fatigue[23], and fatigue associated with ESRD on haemodialysis[24,25]. Of important note, the negative associations between different domains of fatigue and all aspects of post-transplantation QoL shown in **Chapter 4** have important implications. In order to improve QoL in KTRs, fatigue, an important patient-reported outcome, and its potential determinants including reduced muscle mass, deserve more attention from clinical and research perspectives. This will be discussed under **Section 6.3.3.4**.

6.2.4 Potential Mechanistic Aetiology of Physical Fatigue in Kidney Transplant Recipients by Evaluation of Muscle Mass, Muscular and Cardiovascular Functions, and Fatigue Perception

Although **Chapter 4** showed that reduced muscle mass significantly correlated with fatigue in clinically stable KTRs, such an association did not persist in **Chapter 5** when the potential mechanisms of physical fatigue were evaluated by measurements of muscle mass, muscular strength, cardiovascular function and fatigue perception.

It is biologically plausible that varied disease processes or lack of physical activity may result in muscle atrophy[26-28]. In these circumstances, muscles work at relatively high work-load and hence fatigue rapidly. However, in the studied cohort of clinically stable KTRs, there was no association between physical fatigue with either whole body or lower limb lean tissue mass. Certainly, muscle mass *per se* may not be the crucial factor, the ability of musculature to generate force and movement may arguably be of greater importance. Interestingly, the results from the jumping mechanography studies showed no association between muscular power and physical fatigue. In support of these results, muscle mass and muscular power in KTRs were similar to that of age- and gender-matched healthy control subjects, with muscle mass data comparable to previous literature in this field[29,30].

Such discrepancy between **Chapter 4** and **Chapter 5** may be explained to a certain degree by selection effects. In contrast with **Chapter 4**, the study described in **Chapter 5**

employed stringent exclusion criteria. In addition to such criteria specified in **Chapter 4**, including episodes of acute rejection within the past six months, evidence of sepsis in the last 6 weeks, active malignancy or chronic infection, preceding diagnosis of psychiatric disorder or chronic fatigue syndrome, and history of thyroid disease or adrenal insufficiency; **Chapter 5** employed additional exclusion criteria due to ethical and safety reasons. **Chapter 5** excluded KTRs with evidence of unstable angina, acute coronary syndrome in the last 6 months, moderate or severe aortic stenosis, immobility and pregnancy. Furthermore, KTRs who were eligible for entry into the study reported in **Chapter 5** might have declined participation at enrolment, due to the likely discomfort arising from the exercise test and the possibility of physical difficulty encountered with the vertical jump test, although qualitative data would be needed to assess this speculation. For these reasons, variations in the characteristics of the studied cohorts between the two chapters may explain the differences in the findings.

6.3 Other Important Findings of the Thesis

In addition to body composition, the research studies presented in **Chapters 2** to **5** of this thesis yielded other valuable findings, these are summarised in the following sections.

6.3.1 Chapter 2: The Role of Hepcidin-25 in Kidney Transplantation

This study presented in **Chapter 2** represents the first evidence for an independent association between raised serum hepcidin and reduced haemoglobin levels in otherwise well and clinically stable KTRs, with hepcidin levels mostly driven by systemic inflammation and reduced renal function.

6.3.1.1 Association between Hepcidin and Haemoglobin Levels in Kidney Transplant Recipients

A progressive and clinically relevant reduction in haemoglobin level was observed with increasing hepcidin level. This association was independent of renal function and other potential confounding factors. Limited data exist from non-transplantation chronic kidney disease (CKD)[31], showing a positive association between hepcidin and haemoglobin levels[31], these observations now extend to the field of kidney transplantation.

6.3.1.2 Independent Predictors of Hepcidin Levels in Kidney Transplant Recipients

In addition to the positive and independent association between hepcidin and inflammation as discussed in **Section 6.2.1**, hepcidin levels were independently associated with increased transferrin saturation (the marker of iron storage), reduced renal function, and the use of marrow suppressive medication. Such associations are consistent with the prevailing

understanding of the determinants of hepcidin levels[32]. It raises the possibility that, at least partially, the identified risk factors may exert their effect on haemoglobin by means of elevating hepcidin levels.

6.3.1.2.1 Transferrin Saturation and Hepcidin Levels in Kidney Transplant Recipients

Although increased transferrin saturation was associated with raised hepcidin levels, no evidence was found for lower haemoglobin levels at the lower end of the spectrum of hepcidin levels, suggesting that iron deficiency was not in general a major mechanism for reduced haemoglobin levels in the current cohort. However, hepcidin may remain a valuable biomarker for identifying true iron deficiency, as suggested in studies of non-renal cohorts[33].

6.3.1.2.2 Allograft Function and Hepcidin Levels

The observed relationship between renal function and higher hepcidin levels in this study confirms and extends similar findings from non-transplantation cohorts[32,34-37], and a previous study in KTRs[38]. However, it contradicts with two recent studies in non-transplantation CKD[31,39], it is likely that differences in patient characteristics are responsible for these conflicting findings, in particular, with regard to levels of

haemoglobin and inflammation, the range of renal function studied, and the use of co-medication.

6.3.1.2.3 Marrow Suppressive Medications and Hepcidin Levels

An interesting and novel observation was the increase in hepcidin levels associated with the use of angiotensin-converting-enzyme inhibitor, angiotensin receptor blocker, mycophenolate, and azathioprine. These associations may be explained by the recognised effect of these medications on reducing bone marrow activity, possibly decreasing erythropoiesis, leading to reduced inhibition of hepcidin secretion, resulting in higher circulating levels. However, measurements of soluble transferrin receptor or reticulocyte count, and markers of erythropoietic activity, were not undertaken to support this hypothesis. Nevertheless, a recent study in the haemodialysis setting showed an association between renin-angiotensin system inhibitors and raised hepcidin levels[37], in keeping with the results of this study.

6.3.1.3 Chapter Summary, Clinical Implications and Future Directions of Chapter 2

In summary, **Chapter 2** highlighted the possible mechanisms of haemoglobin reduction in KTRs, and the therapeutic opportunities from understanding the role of hepcidin in this context. This finding suggests that targeting KTRs with raised hepcidin levels using therapies designed to antagonise hepcidin production or activity may be a useful strategy.

Currently, such agents remain in early phases of development, although preliminary clinical data appear encouraging[40,41].

Although **Chapter 2** did not establish a relationship between adiposity and haemoglobin level, as discussed, independent associations were observed between adiposity and inflammation, between inflammation and elevated hepcidin, and between raised hepcidin and reduced haemoglobin levels. It remains a possibility that adiposity-related inflammation in KTRs may be associated with elevated hepcidin, contributing to decreased haemoglobin by dysregulation of iron homeostasis. Further prospective longitudinal follow-up of this cross-sectional cohort may add further insight into these associations. Also, these findings require replication in larger independent cohorts.

6.3.2 Chapter 3: Hypervolemia and Blood Pressure in Prevalent Kidney Transplant Recipients

The study described in **Chapter 3** is the first to address in detail the prevalence, predictors, and consequences of hypervolemia in KTRs.

6.3.2.1 Prevalence of Hypervolemia in Prevalent Kidney Transplant Recipients

Based on the previously established definition of hypervolemia, 30% of KTRs were hypervolemic, of whom 5% suffered from severe hypervolemia. Despite a lower incidence when compared with continuous ambulatory peritoneal dialysis[12] or haemodialysis[42] populations, this degree of hypervolemia was unexpected, and is noteworthy in light of the specific selection of a clinically and biochemically stable cohort of KTRs for this study.

6.3.2.2 Dietary Sodium Intake and Hypervolemia in Kidney Transplant Recipients

Hypervolemia was associated with increasing sodium intake, highlighting an important target for intervention. Dietary sodium restriction has not been formally examined in KTRs, but has gained attention in other context[43]. The daily sodium intake in the current cohort of KTRs was 2,725 mg (118 mmol), lower than previously reported (3,588 mg or 156 mmol per day)[44], but well above the recommendation of Dietary Approach to Stop Hypertension (DASH) guideline (1,500 – 2,300 mg or 65 – 100 mmol per day)[45].

6.3.2.3 Dietary Sodium Intake and Blood Pressure in Kidney Transplant Recipients

A recent study in KTRs demonstrated a relationship between increased sodium intake and higher blood pressure, but the contribution of extracellular volume status was not evaluated therein[44]. Although the results of the current study confirmed a univariate association

between sodium intake and blood pressure, this relationship did not hold when the effect of extracellular volume status was taken into account.

6.3.2.4 Diuretic Usage and Hypervolemia in Kidney Transplant Recipients

It is important to note that, in the current study, the prevalence of diuretic usage was only 15%, with furosemide being the only diuretic prescription. No association between furosemide usage and volume status was observed, but this may be a reflection of "confounding by indication". Further, the median dosage of furosemide in this study was 40 mg, a dosage which may be insufficient to target hypervolemia in KTRs with a mean estimated glomerular filtration rate (eGFR) of 44 mL/min[46].

6.3.2.5 Fat Mass and Hypervolemia in Kidney Transplant Recipients

In regard to other determinants of extracellular volume status, an inverse association between fat mass and volume status was observed in the current study. This phenomenon has been demonstrated in non-transplanted population[47,48] which now extends to KTRs. The underlying mechanism remains unclear, further studies are necessary to delineate such observation. However, it is also possible that, in clinical practice, volume overload often accompanies obesity[49] and/or the physical appearance of obese patients may be clinically misclassified as volume overload[48]. Therefore, obese KTRs may be more likely to receive

adequate or surplus treatment of volume status. As a result, this finding may be confounded by clinical practice.

6.3.2.6 Hypervolemia and N-Terminal Fragment of Pro-hormone B-Type Natriuretic Peptide in Kidney Transplant Recipients

In addition to the independent association between hypervolemia and raised NT-proBNP as described in **Section 6.2.2**, reduced allograft function was another independent predictor of raised NT-proBNP levels. This is in keeping with findings from previous studies among KTRs[50,51], due to reduced renal clearance of NT-proBNP.[52] However, an important caveat is the high variability in the relationship between NT-proBNP levels with both percentage volume expansion and eGFR. This suggests that NT-proBNP may be a marker of volume expansion and renal dysfunction, it cannot yet be considered as an accurate surrogate for either. The utility of serial NT-proBNP measurements cannot be discerned by the current study.

6.3.2.7 Chapter Summary, Clinical Implications and Future Directions of Chapter 3

In summary, **Chapter 3** showed that hypervolemia is unexpectedly common among clinically stable KTRs, and is closely associated with elevated blood pressure and raised NT-proBNP levels. The findings from this study suggest that meticulous monitoring of both volume status and blood pressure should be in place to ensure optimal management of

hypertension in KTRs. It is hoped that the findings of this study will highlight the importance of extracellular volume status assessment in the management of hypertension, a tool yet to be incorporated into international guidelines from Kidney Disease: Improving Global Outcomes[53], European Renal Best Practice Work Group[54], and the United Kingdom Renal Association[55].

The relationship between increased sodium intake and hypervolemia signals potential nutritional focus. In addition, inadequate diuretics usage may contribute to the high prevalence of hypervolemia. Based on the findings from this study, a multi-modality approach involving the DASH diet and increased diuretic usage may be beneficial in the treatment of volume overload and hypertension in KTRs. Long-term longitudinal follow-up and experimental interventions, such as DASH diet and increased diuretic usage, are now required to evaluate its impact on extracellular volume status. Also, future studies should examine the impact of extracellular volume status on relevant end points in kidney transplantation.

6.3.3 Chapter 4: Predictors and Consequences of Fatigue in Prevalent Kidney Transplant Recipients

Chapter 4 revealed that, in clinically stable KTRs, fatigue in common, severe, and clinically under-appreciated. It has a close association with inferior QoL. In addition to reduced muscle mass already discussed in **Section 6.2.3**, other independent predictors of post-transplantation fatigue include depression, anxiety, inferior sleep quality,

inflammation, and renal dysfunction. These findings form the potential targets for future interventional studies.

6.3.3.1 Nature of Fatigue in Prevalent Kidney Transplant Recipients

Compared with healthy population[56,57], KTRs suffer from higher levels of fatigue on all dimensions, and were indeed similar to "chronically unwell" patients[56]. Further, severity in certain domains, such as physical fatigue, reduced activity, and mental fatigue, approached that of chronic fatigue syndrome[56], highlighting the burden of fatigue in KTRs. Of relevance, physical aspects of fatigue outweighed behavioural, emotional, and cognitive aspects, resembling findings in liver transplant recipients[58]. Also, the significant associations between different domains of fatigue suggest that treatment of behavioural, emotional, and cognitive aspects of fatigue may improve physical aspects of fatigue or vice versa.

6.3.3.2 Prevalence and Clinical Awareness of Fatigue in Prevalent Kidney Transplant Recipients

The prevalence of fatigue in the current cohort of KTRs is 59%, comparable with a single previous study in this field[19]. Despite the high prevalence, only 13% of patients had fatigue documented in medical records prior to participation in this study, suggesting that this symptom is either under-reported or under-acknowledged.

6.3.3.3 Independent Predictors of Fatigue in Prevalent Kidney Transplant Recipients

Depression was highlighted as the specific, independent predictor of four fatigue dimensions, including general fatigue, physical fatigue, reduced activity and reduced motivation. Anxiety was identified as a significant predictor of mental fatigue, similar to other chronic conditions such as multiple sclerosis[59]. KTRs are subjected to several mental challenges, including fears about transplant rejection and the necessity to adhere to a complex regimen of immunosuppression therapy that may generate distressing side effects[60].

Inferior sleep quality may intuitively be expected to have a pervasive and broad effect on multiple aspects of fatigue[19]. However, a significant association was only observed for the dimension of general fatigue.

The association between inflammation and fatigue is particularly notable as the studied cohort consisted of clinically stable KTRs, without overt evidence of ongoing acute or chronic inflammatory conditions. Evidence from studies of healthy volunteers, elderly populations, and other disease groups has shown that inflammatory cytokines possess potent neurological effects and are mediators of fatigue[19,61-64].

Although fatigue is a common and important symptom for patients on dialysis[65,66], the results from the current study showed, for the first time, a relationship between allograft dysfunction and physical fatigue in KTRs.

6.3.3.4 Chapter Summary, Clinical Implications and Future Directions of Chapter 4

In summary, **Chapter 4** showed that, in clinically stable KTRs, fatigue is common, severe, and clinically under-appreciated. It has a close association with inferior QoL.

The findings from this study suggest that psychological interventions addressing disease-related anxiety and depression *per se* may be beneficial in improving symptoms of fatigue[67]. Inferior sleep quality was associated with only one domain of fatigue, suggesting that mere sleep difficulties do not explain a large spectrum of fatigue complaints in KTRs, and interventions aiming to improve sleep quality may have limited effect on fatigue.

Reduced muscle mass coupled with increased fat mass (i.e. sarcopenic obesity) is a common characteristic of body composition in KTRs[22], and systemic low-grade inflammation is a hallmark of obesity[20]. The findings from **Chapter 4** suggest that lifestyle intervention focusing on increasing physical activity and dietary modification aiming to reverse this phenotype should be valuable for patients displaying symptoms of fatigue.

Additionally, clinical strategies exist to improve allograft function[68] and fatigue may represent an important patient-reported outcome in future interventional studies.

6.3.4 Chapter 5: Cardiovascular, Muscular and Perceptual Contributions to Physical Fatigue in Prevalent Kidney Transplant Recipients

The study described in **Chapter 5** is the first study to systematically investigate the potential aetiology of physical fatigue in KTRs, and reveal important findings. As already discussed in **Section 6.2.4**, physical fatigue is unrelated to muscular and cardiovascular factors, but rather, it is driven by increased perception of exertion during exercise. The findings of **Chapter 5** confirm physical fatigue as a common and disabling symptom among KTRs, occurring in 22% in the studied cohort, negatively impacting on QoL[19,69,70].

6.3.4.1 Perceived Exertion and Physical Fatigue in Kidney Transplant Recipients

Physical fatigue in KTRs is driven by increased perception of exertion during exercise. Such findings arising from the earlier part of this study led to the further investigation of the plausible predictors of heightened perception. In turn, mental fatigue significantly associated with such heightened perception of effort. Whilst novel to transplantation, these results resonate with findings from other populations, whereby heightened perception limits exercise capacity in healthy trained individuals[71] and diabetic patients[72], and mental

fatigue impairs physical performance through increased perception of effort rather than limiting musculoenergetic and cardiorespiratory functions[73,74].

The mechanisms by which perception of exertion influences physical performance has been previously proposed by Marcora and colleagues using the Brehm's theory of motivation[73]. In this theory, individuals opt to withdraw from a task when it is perceived to be too difficult, or the effort required exceeds the individuals' willingness to perform[75]. During the decision-making process, individuals are suspected to have lowered their level of task difficulty for withdrawal[76,77]. Impaired physical performance is a common feature in KTRs[78-80], since physical fatigue represents a transient decrease in muscular performance, this may be seen as failure to generate and to maintain optimal physical performance. Therefore, the Brehm's theory of motivation may be extrapolated in this setting. In addition, there is evidence that disorders of the brainstem, dopaminergic systems and endogenous opiates may affect decision making[75-77,81,82]. In particular, increasing dopamine release in the brain through dopaminergic-modulating agent, is associated with reduced perceived fatigue and increased perceived QoL in chronic fatigue syndrome[83]. This may be applicable to KTRs with physical fatigue as an important patient-reported outcome.

Of importance, a caveat with the interpretation of the associations between self-report data, such as symptoms of physical and mental fatigue, perceived exertion, anxiety and depression, is that common method variance may partly drive the observed associations and may account for 25% of shared variance[84]. In common method variance, patients high

in negative effect (i.e. negative mood) perceived, remember, and report more physical and psychological symptoms, and report those symptoms to be more severe than patients with less negative mood[85]. Although these would not render self-reports unimportant, potential interpretational difficulties may result.

6.3.4.2 Predictors of Perceived Exertion in Kidney Transplant Recipients

In addition to mental fatigue as already discussed in **Section 6.3.4.1**, other factors associated with heightened perceived exertion included new onset diabetes after transplantation (NODAT), low alcohol intake, lack of cyclosporin prescription, and anxiety. Interestingly, other commonly studied clinical and demographic variables, including estimated glomerular filtration rate, Hb and inflammation, showed no association with perception of effort.

Fatigue in diabetes is likely to be caused by the interplay of physiological, psychological and lifestyle-associated factors[86]. It is interesting to note that pre-existing diabetes was not associated with perceived exertion. A plausible explanation for the differences between NODAT and pre-existing diabetes is that KTRs with NODAT may experience exaggerated psychological distress having to cope with yet another disease state and requiring a novel diabetes treatment.

The associations between raised perceived exertion with low alcohol intake and lack of cyclosporin prescription may be biologically plausible. However, at present, such correlations were supported by weak rationale in the literature[87-92], possibly representing a type I statistical error.

Finally, anxiety independently associated with increased perception of exertion; and depression displayed a univariate association with perception. These observations have been noted previously in non-transplant studies[93,94], supporting the findings of this study. Anxious and depressed individuals are less attuned to interpret bodily sensations including fatigue during physical activity[94], possibly with physiological response linked inappropriately to catastrophic cognitions in such individuals.

6.3.4.3 Chapter Summary, Clinical Implications and Future Directions of Chapter 5

In summary, **Chapter 5** suggested that physical fatigue in KTRs is caused by perceived exertion influenced by mental fatigue and anxiety. The findings from this chapter suggest that improving physical fitness or strength *per se* is unlikely to improve physical fatigue. Other strategies such as cognitive behavioural therapy or centrally-acting pharmacological therapies may be appropriate. Undoubtedly, physical fatigue represents a frequent and important patient-reported outcome. The results from this chapter set the scene for future interventional research and therapeutic strategies.

6.4 Limitations of the Thesis

This thesis has limitations that should be acknowledged. All studies described in **Chapters 2** to **5** represent single-centre experience. The cross-sectional nature of the study design in these chapters inherently means that the direction of the causality between predictor and outcome variables cannot be defined.

In addition, all studies described in **Chapters 2** to **5** were pilot observational studies in nature. As such, power calculations were not conducted. Various associations have been established, supported by explanations which are biologically plausible, and are in many aspects compatible with findings from other disease states and the general population. These associations were further reinforced by statistically significant *p*-values, and hence, in general, the probability of type 1 statistical error remains low. Nevertheless, type I statistical errors may still exist especially in associations supported by weak rationale in the literature. Of importance, the absence of associations between certain variables should not be treated without reservations due to studies with small sample sizes, implicating an inherently high probability of type II statistical error. Future studies with larger cohorts are necessary to validate the conclusions drawn from the current pilot studies, in addition to minimising any type II or type I statistical errors.

6.5 Conclusion

To conclude, the studies presented in this thesis identified potential predictors of post-transplantation morbidity and fatigue, the potential contributing factors to long-term patient- and graft- survival, as well as QoL. In particular, different body composition compartments exert varying effects on inflammation, blood pressure, NT-proBNP level, and fatigue. The findings from this thesis set the scene for future interventional research and therapeutic strategies.

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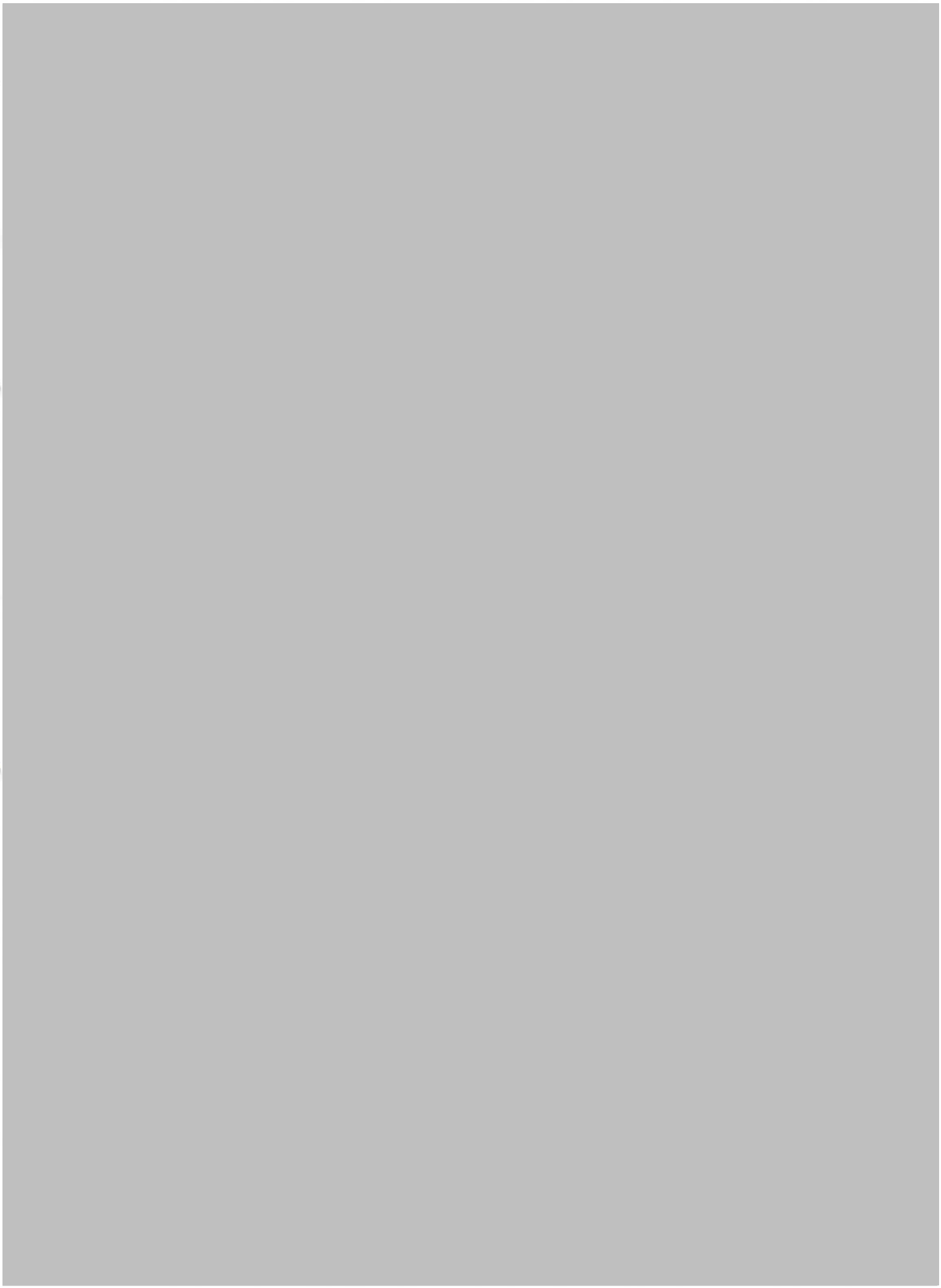
APPENDICES

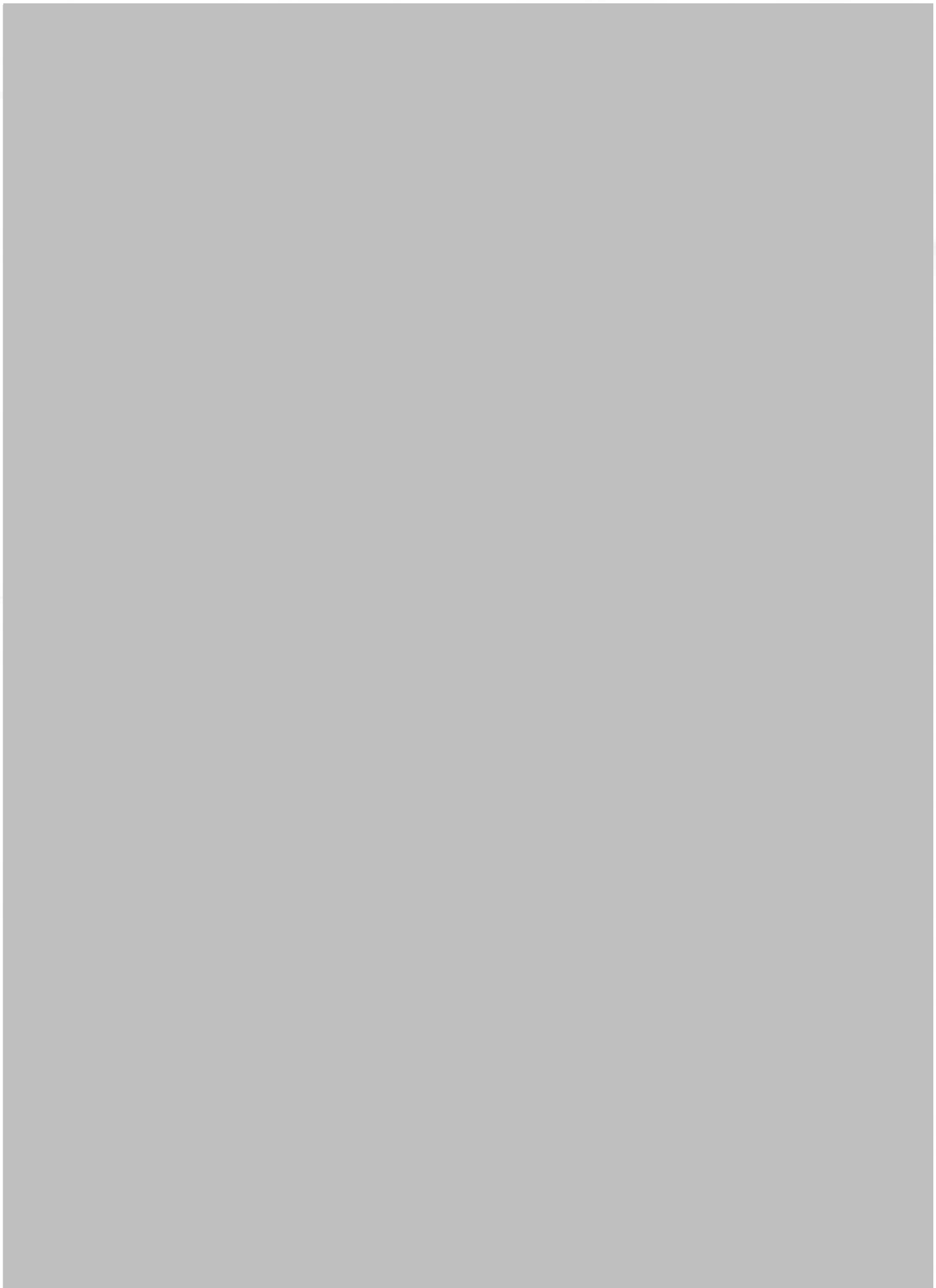
Chapter 7

Effects of Body Composition on Clinical and Quality of Life Outcomes in Kidney Transplant Recipients

7.1 Original Ethical Approval:

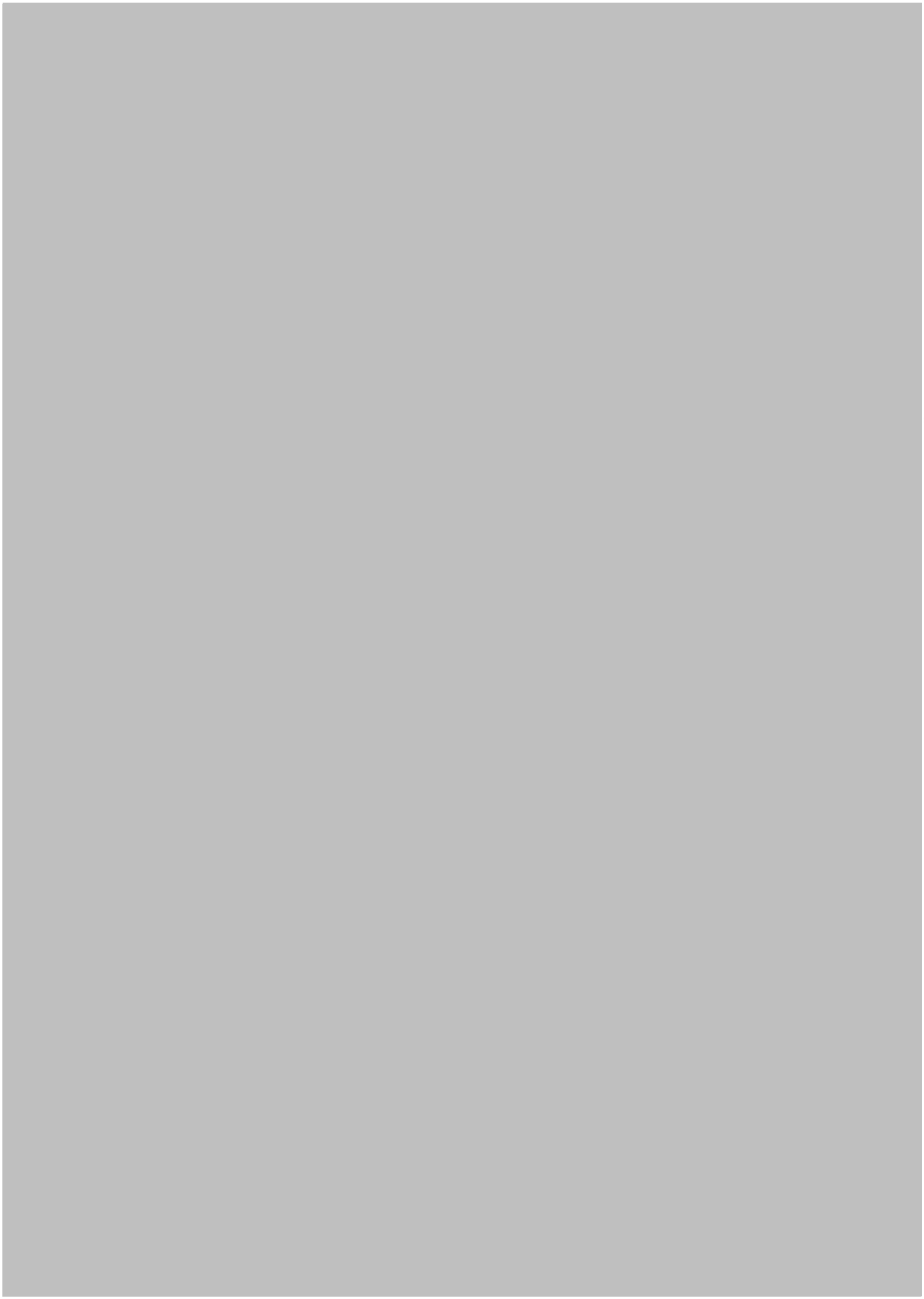
Confirmation of Favourable Ethical Opinion on 12th July 2010





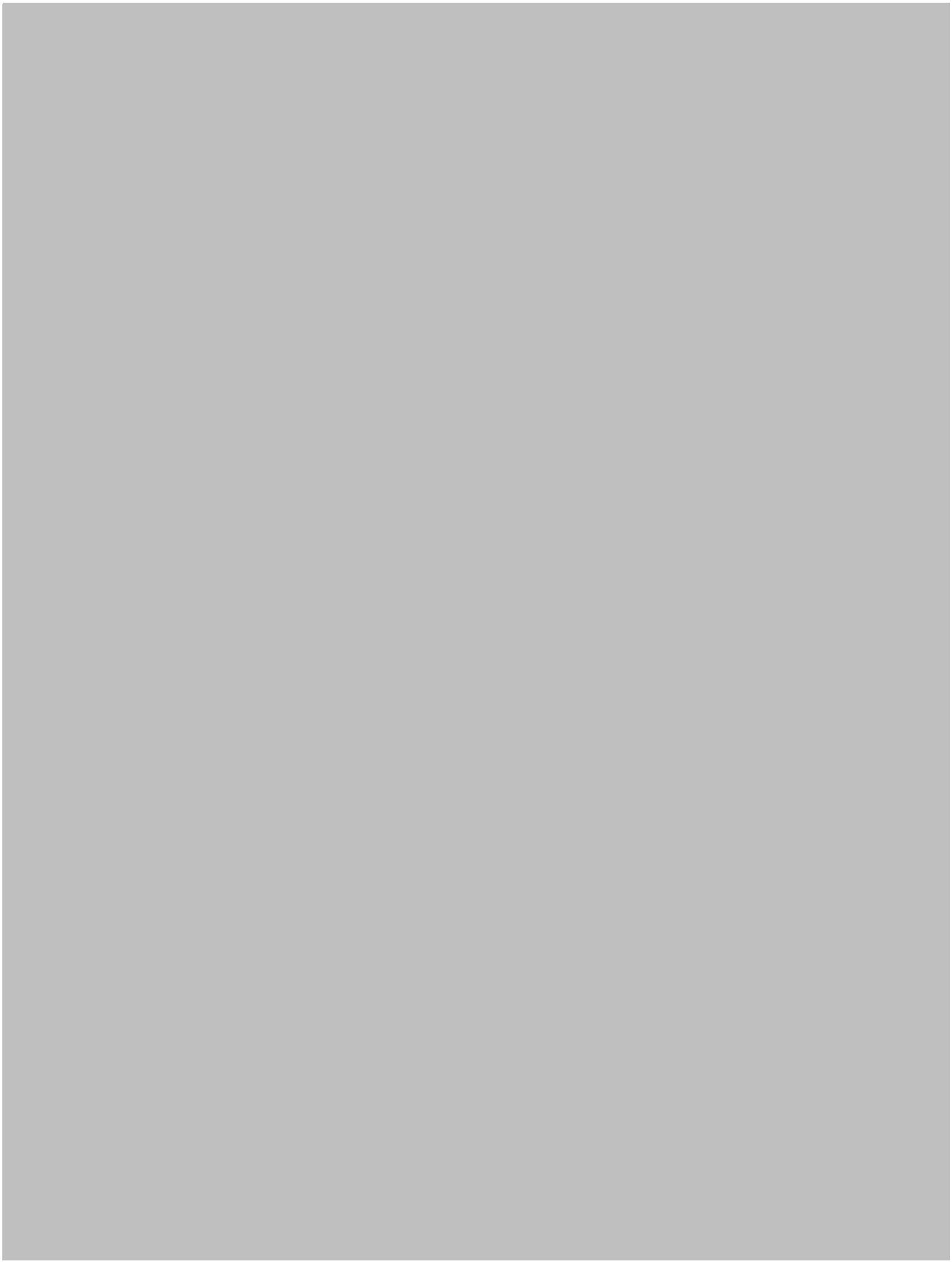
7.2 Amendment 01 Ethical Approval:

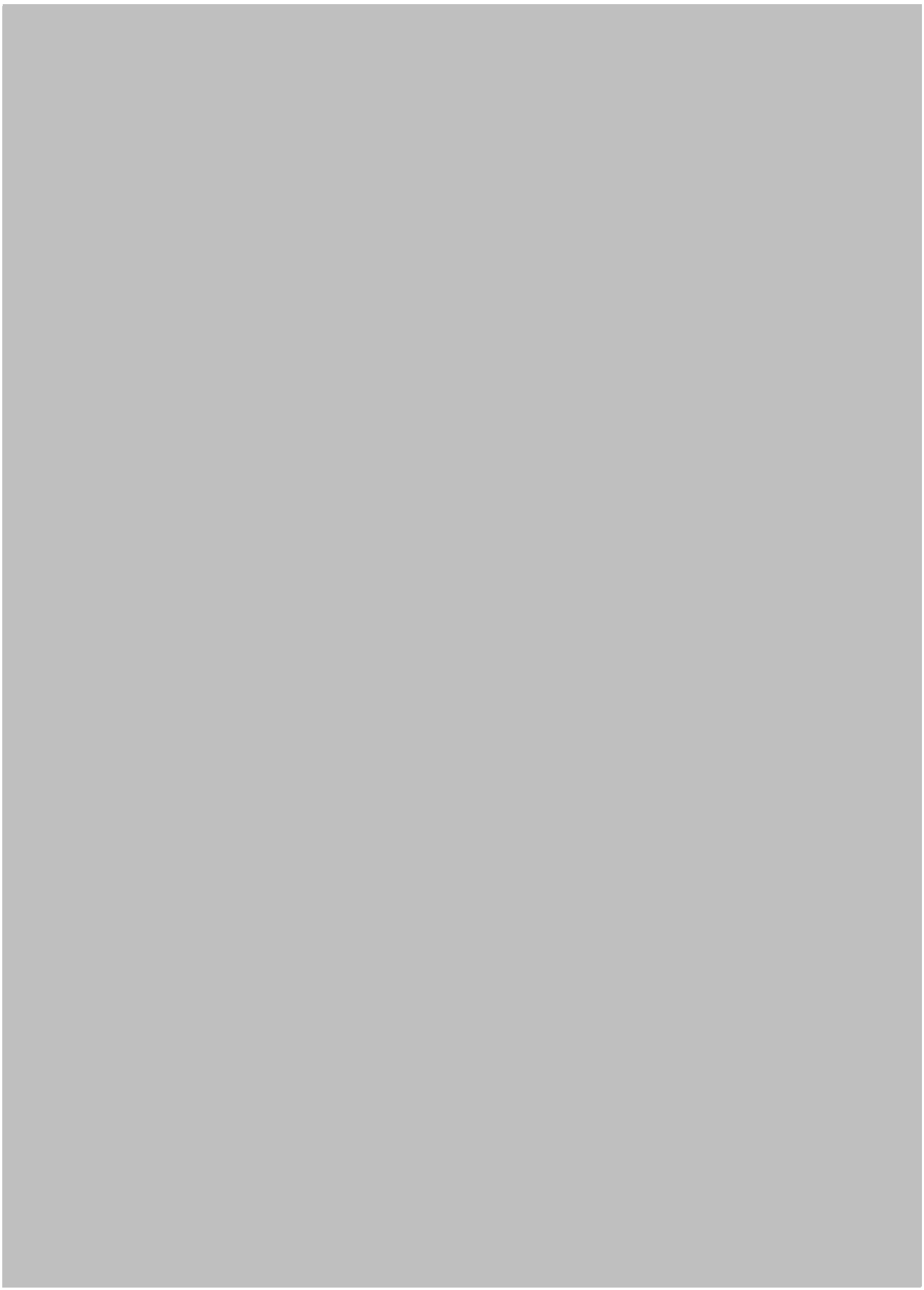
Confirmation of Favourable Ethical Opinion on 26th October 2010



7.3 Amendment 02 Ethical Approval:

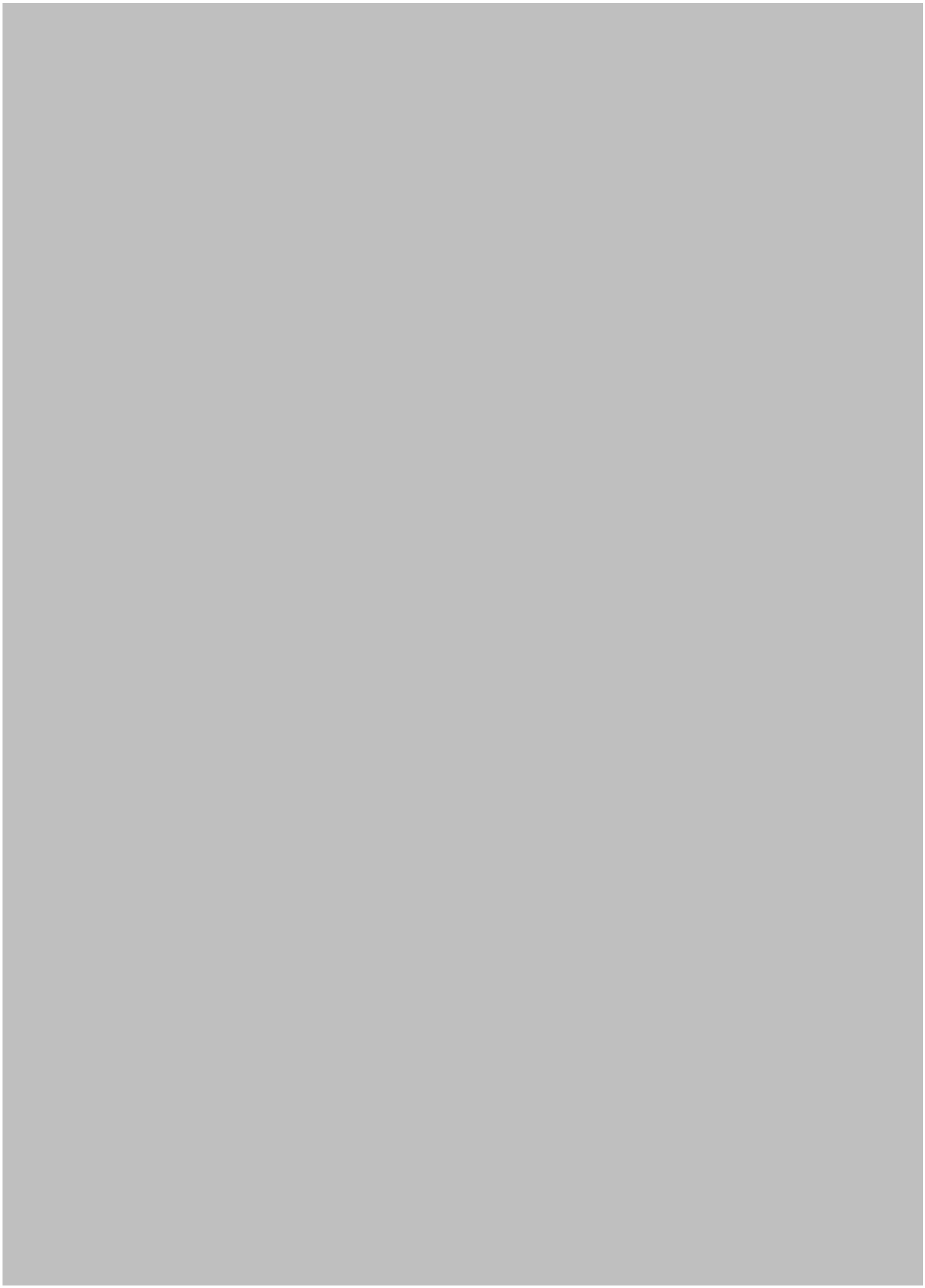
Confirmation of Favourable Ethical Opinion on 15th February 2011

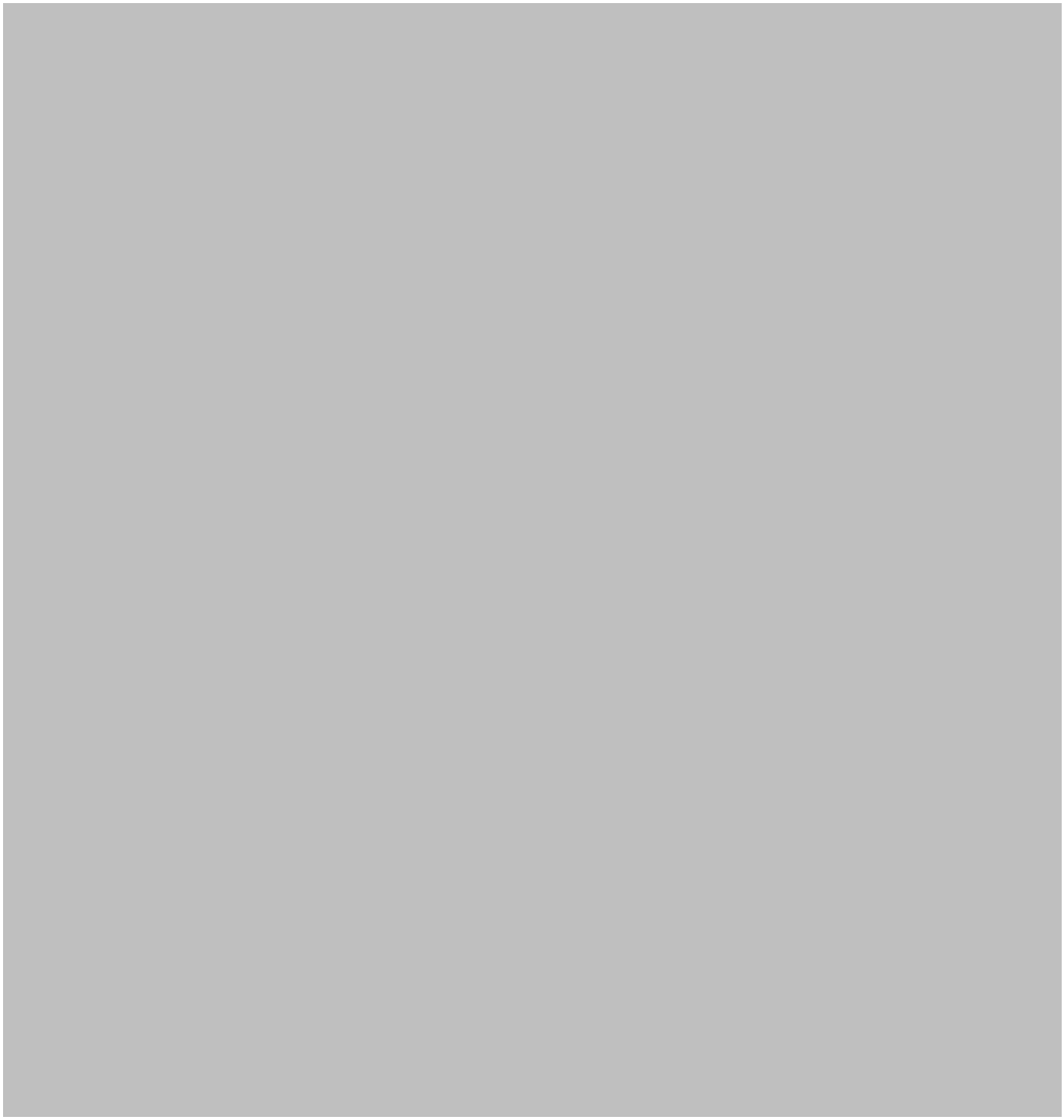




7.4 Amendment 03 Ethical Approval:

Confirmation of Favourable Ethical Opinion on 22nd July 2013





7.5 Section 1: General Questions about You

Section 1: General Questions about You

1 What is your date of birth?

Day	Month	Year

2 Are you a male or a female? ***(Please tick)***

Male		Female	

3 What is your current marital status? ***(Please tick)***

Married / living with partner	
Widowed	
Divorced	
Separated	
Single	

4 Which of these ethnic groups do you consider you belong to?
(Please tick)

White British		Pakistani	
White Irish		Bangladeshi	
Black Caribbean		Chinese	
Black African		Mixed	
Indian		Other	

If other, please define: .....

5 Which of the following best describes your working status?
(Pleas tick)

Working full time	
Working part time	
Semi-retired	
Retired	
Working in the home	
Not working (due to ill health or disability)	
Unemployed but seeking work	
Student	

If you have already left full time education or your training scheme:

6 How old were you when you left full time education or your training scheme (whichever was later)?

Years of age

7 Do you have any of the following qualifications? ***(Please tick all that apply)***

School leaving certificate	
CSE	
GCE 'O' Level or GCSE	
Technical College Exams / City and Guilds	
Completed Apprenticeship	
Higher National Diploma (HND)	
'A' Level, Highers	
Trade Certificates	
Teaching Diploma, NHC	
University Degree	

8 Which of the following best describes your use of tobacco products?
(Please tick)

Never smoked	
Currently smoking	
Former smoker	

If you are a current or a former smoker:

9 How many of the following tobacco products do you smoke / did you smoke a day? ***(If none, please put zero "0" in the box.)***

	Cigarettes
	Cigars
	Pipe

If you are a current or a former smoker:

10 At what age did you start to smoke?

	Years of age

If you are a former smoker:

11 At what age did you stop?

	Years of age

With regards to caffeine intake, how many cups of each of the following drinks might you have in a normal week? (If none, please put zero "0" in the box.)

		How Many?	
12	Coffee		Cups
13	Tea		Cups

With regards to alcohol intake, how many measures of each of the following drinks might you have in a normal week? (If none, please put zero "0" in the box.)

		How Many?	
14	Wine		Small Glasses
15	Fortified Wine (e.g. port or sherry)		Small Glasses
16	Beer (e.g. lager, stout, bitter)		Pints
17	Cider		Pints
18	Spirits		Pub measure (25cl)
19	Liqueurs (e.g. Tia Maria, Baileys)		Pub measure (25cl)

During a normal day of the week, how much time do you usually spend sitting? (Please tick)

20	Never	
21	0 to 4 hours a day	
22	5 to 9 hours a day	
23	10 to 14 hours a day	
24	All day	

With regards to physical activity, how often during the last year did you do the physical activities listed below?

(Please tick)

		Never	0 to 2 hours a week	3 to 4 hours a week	5 to 9 hours a week	10 to 14 hours a week	More than 15 hours a week
25	Walking						
26	Jogging or running						
27	Swimming						
28	Cycling						
29	Exercise or dance classes						
30	Housework						
31	Gardening						
32	Other, please specify ……..						

7.6 Section 2: Medical Outcomes Study Short Form-36 (SF-36)

Section 2: Questions about your General Health (SF-36 QoL)

1	In general, would you say your health is: ***(Please tick)***	
	Excellent	
	Very good	
	Good	
	Fair	
	Poor	

2	Compared to one year ago, how would you rate your health in general now: ***(Please tick)***	
	Much better than one year ago	
	Somewhat better than one year ago	
	About the same	
	Somewhat worse now than one year ago	
	Much worse than one year ago	

The following questions are about activities you might do during a typical day.

DOES YOUR HEALTH NOW LIMIT YOU IN THESE ACTIVITIES?

If so, how much does it limit you? *(Please tick)*

		Yes, limited a lot.	Yes, limited a little.	No, not limited at all.
3	Vigorous activities, such as running, lifting heaving objects, participating in strenuous sports such as playing golf.			
4	Moderated activities, such as moving a table, pushing a vacuum cleaner, or bowling.			

The following questions are about activities you might do during a typical day.

DOES YOUR HEALTH NOW LIMIT YOU IN THESE ACTIVITIES?

If so, how much does it limit you? *(Please tick)*

		Yes, limited a lot.	Yes, limited a little.	No, not limited at all.
5	Lifting or carrying groceries.			
6	Climbing several flights of stairs.			
7	Climbing one flight of stairs.			
8	Bending, kneeing or stopping.			
9	Walking more than a mile.			
10	Walking half a mile.			
11	Walking 100 yards.			
12	Bathing and dressing yourself.			

During the past 4 weeks, have you had any of the following problems with your work or other daily activities as a result of your physical health? *(Please tick)*

		Yes	No
13	Cut down on the amount of time you spent on work or other activities.		
14	Accomplished less than you would like.		
15	Were limited in the kind of work or other activities.		
16	Had difficulty performing the work or other activities (e.g. it took extra effort)		

During the past 4 weeks, have you had any of the following problems with your work or other daily activities *as a result of any emotional problems (such as feeling depressed or anxious)*? *(Please tick)*

		Yes	No
17	Cut down on the amount of time you spent on work or other activities.		
18	Accomplished less than you would like.		
19	Didn't do work or other activities as carefully as usual.		

20 During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups? ***(Please tick)***

Not at all	
Slightly	
Moderately	
Quite a bit	
Extremely	

21 How much bodily pain have you had during the past 4 weeks?
(Please tick)

None	
Very mild	
Mild	
Moderate	
Severe	
Very severe	

22 During the past 4 weeks, how much did pain interfere with your normal work (including work both outside the home and housework)?
(Please tick)

Not at all	
A little bit	
Moderately	
Quite a bit	
Extremely	

These questions are about how you feel and how things have been with you during the past 4 weeks. Please indicate the answer that closest describe the way you have been feeling. How much time during the past 4 weeks:
(Please tick)

		All the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23	Have you felt full of life?						
24	Have you been a nervous person?						
25	Have you felt so down in the dumps that nothing could cheer you up?						
26	Have you felt calm and peaceful?						
27	Have you had a lot of energy?						
28	Have you felt downhearted and low?						
29	Have you felt worn out?						
30	Have you been a happy person?						

These questions are about how you feel and how things have been with you during the past 4 weeks. Please indicate the answer closest describe the way you have been feeling.

How much time during the past 4 weeks:

(Please tick)

		All the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
31	Have you felt tired?						
32	Have your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?						

How TRUE or FALSE is each of the following statements for you? *(Please tick)*

		Definitely true	Mostly true	Not sure	Mostly false	Definitely false
33	I seem to get ill more easily than other people.					
34	I am as healthy as anybody I know.					
35	I expect my health to get worse.					
36	My health is excellent.					

7.7 Section 3: Multi-Dimensional Fatigue Inventory-20 (MFI-20)

Section 3: Questions about how you have been feeling over the past few days (MFI-20)

We would like to get an idea of how you have been feeling **over the last few days**. If you think any of these statements are entirely true, please ***tick the box*** for "1" on the extreme left. The more you disagree with the statement, the more you can tick the box in the direction of "no, that is not true". Please do not miss out a statement.

		Yes, that is true.				**No, that is not true.**
		1	**2**	**3**	**4**	**5**
1	I feel fit.					
2	Physically I feel only able to do a little.					
3	I feel very active.					
4	I feel like doing all sort of nice things.					
5	I feel tired.					
6	I think I do a lot in a day.					
7	When I am doing something, I can keep my thoughts on it.					
8	Physically I can take on a lot.					
9	I dread having to do things.					

		Yes, that is true.				**No, that is not true.**
		1	**2**	**3**	**4**	**5**
10	I think I do very little in a day.					
11	I can concentrate well.					
12	I am rested.					
13	It takes a lot of effort to concentrate on things.					
14	Physically I feel I am in a bad condition.					
15	I have a lot of plans.					
16	I tire easily.					
17	I get little done.					
18	I don’t feel like doing anything.					
19	My thoughts easily wander.					
20	Physically I feel I am in excellent condition.					

7.8 Section 4: Hospital Anxiety and Depression Scale (HADS)

Section 4: Questions about how you have been feeling during the past week? (HADS)

The next questions are designed to help us know how you feel in further detail. Please ***tick the reply which comes closest to how you have been feeling in the past week***?

1	I feel tense or wound up.

Most of the time	☐
A lot of the time	☐
Time to time, occasionally	☐
Not at all	☐

2	I still enjoy things I used to enjoy.

Definitely as much	☐
Not quite as much	☐
Only a little	☐
Hardly at all	☐

3	I get a sort of frightened feeling as if something awful is about to happen.

Very definitely and quite badly	☐
Yes, but not too badly	☐
A little, but doesn't worry me	☐
Not at all	☐

4	I can laugh and see the funny side of things.

As much as I always could	
Not quite as much now	
Definitely not so much now	
Not at all	

5	Worrying thoughts go through my mind.

A great deal of the time	
A lot of the time	
From time to time but not too often	
Only occasionally	

6	I feel cheerful.

Not at all	
Not often	
Sometimes	
Most of the time	

7	I can sit at ease and feel relaxed.

Definitely	
Usually	
Not often	
Not at all	

8	I feel as if I am slowed down.

Nearly all the time	
Very often	
Sometimes	
Not at all	

9	I get a sort of frightened feeling like “butterflies” in my stomach.

Not at all	
Occasionally	
Quite often	
Very often	

10	I have lost interest in my appearance.

Definitely	
I don’t take so much care as I should	
I may not take quite as much care	
I take just as much care as ever	

11	I feel restless as if I have to be on the move.

Very much indeed	
Quite a lot	
Not very much	
Not at all	

12 I look forward with enjoyment to things.

As much as ever I did	☐
Rather less than I used to	☐
Definitely less than I use to	☐
Hardly at all	☐

13 I get sudden feelings of panic.

Very often indeed	☐
Quite often	☐
Not very often	☐
Not at all	☐

14 I can enjoy a good book or radio or TV programme.

Often	☐
Sometimes	☐
Not often	☐
Very seldom	☐

7.9 Section 5: Pittsburgh Sleep Quality Index (PSQI)

Section 5: Questions about sleep (PSQI)

We would like to ask you questions about sleep.

During the <u>past month</u>, did you:

(Please tick the appropriate box)

		Not at all	**Yes**				
			1-3 days	4-7 days	8-14 days	15-21 days	22-31 days
1	Have trouble falling asleep?						
2	Wake up several times at night?						
3	Having trouble staying asleep (including waking far too early)?						
4	Wake up after your normal amount of sleep feeling tired and worn out?						

5	During the past month, what time have you usually gone to bed?

BED TIME ☐

6	During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

NUMBER OF MINUTES ☐

7	During the past month, what time have you usually gotten up in the morning?

GETTING UP TIME ☐

8	During the past month, how many hours of **actual sleep** did you get at night? (This may be different than the number of hours you spent in bed.)

HOURS OF SLEEP PER NIGHT

For the following questions, tick the one best response. During the past month, how often have you had trouble sleeping because you… *(Please tick)*

		Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
9	Could not get to sleep within 30 minutes.				
10	Woke up in the middle of the night or early in the morning.				
11	Had to get up to use the bathroom.				
12	Could not breathe comfortably.				
13	Coughed or snored loudly.				
14	Felt too cold.				
15	Felt too hot.				
16	Had bad dreams.				
17	Had pain.				
18	Other reason(s), please describe …….....				

19 During the past month, how would you rate your sleep quality overall? ***(Please tick)***

☐ Very good	☐ Fairly good	☐ Fairly bad	☐ Very bad

20 During the past month, how often have you taken medicine to help you sleep? (prescribed or "over the counter")? ***(Please tick)***

☐ Not during the last month	☐ Less than once a week	☐ Once or twice a week	☐ Three or more times a week

21 During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity? ***(Please tick)***

☐ Not during the past month	☐ Less than once a week	☐ Once or twice a week	☐ Three or more times a week

22 During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done? ***(Please tick)***

☐ No problem at all	☐ Only a very slight problem	☐ Somewhat of a problem	☐ A very big problem

23	Do you have a bed partner or roommate? ***(Please tick)***

No bed partner or roommate	Partner / roommate in the other room	In same room, but not same bed	Partner in same bed

If you have a roommate or partner, ask him / her how often in the past month you have had …….

(Please tick)

		Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
24	Loud snoring.				
25	Long pauses between breaths when asleep.				
26	Legs twitching or jerking while you sleep.				
27	Episodes of disorientation or confusion during sleep.				
28	Other restlessness while you sleep, please describe ……				