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Exercise and Lifestyle Intervention and High Intensity Interval Training in Patients with Chronic Kidney Disease

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Abstract

Chronic kidney disease (CKD) patients have a shorter life expectancy than healthy individuals, from the premature development of cardiovascular disease due to traditional and non-traditional risk factors. It is therefore pertinent that management of CKD be aimed at modifying these cardiovascular disease risk factors. Exercise therapy, in particular aerobic exercise, in the end stage kidney disease population has been shown to decrease cardiovascular disease risk factors and subsequent cardiovascular disease. However, randomized exercise training trials are scarce in the stage 3-4 CKD cohort. High intensity interval training (HIIT) is a promising exercise training approach that has been shown to elicit greater improvements in cardiovascular disease risk factors than moderate intensity continuous training (MICT) in other chronic disease populations. However, HIIT has not yet been investigated in patients with CKD. This thesis comprises two sections, each containing a randomised control trial (RCT) investigating the effects of exercise training in CKD patients.

Section 1 contains data from the Landmark III (LM3) trial, which investigated the effects of a nurse-led, multi-disciplinary lifestyle intervention (LI) in 187 CKD patients. Testing of both LI and standard care (control) groups occurred every 6 months for 3 years. The exercise training in the LI consisted of a combination of individualized aerobic and resistance training and encouraged patients to participate in higher intensity exercise if possible. The main study in Section 1 assesses the change in various outcome measures after 3 years of LI (Chapter 6). Section 1 also includes baseline cross-sectional analyses (Chapter 3) and 12 month sub-studies (Chapters 4 and 5).

Section 2 contains the second trial that investigated the feasibility and efficacy of a 12 week HIIT program in CKD patients. Recruitment for the HIIT study was through participants who had completed the LM3 study.

Chapter 1 provides an introduction to the studies, including the aims of the thesis. Topics reviewed include; pathophysiology of CKD, exercise capacity, autonomic dysfunction, haemoglobin, muscle atrophy, oxidative stress, inflammation, blood lipids and HIIT. Section 1 starts with the detailed methods of the LM3 study (Chapter 2). Further methods applicable to specific studies are reported in the relevant chapter.

Chapter 3 investigates the determinants of reduced muscle strength at baseline through a cross-sectional analysis of the LM3 study. This study identified the association of oxidative

stress with low muscle strength and reduced lean mass. The relationship was independent of known influences on muscle mass and strength.

The feasibility and determinants of the LI group participating in higher intensity exercise training in the first 12 months of the LM3 study was investigated in Chapter 4. The findings identified that higher intensity exercise training was indeed feasible in the CKD cohort, with the number of participants reporting higher intensity exercise increasing from 20% to 43%. Reported higher intensity exercise during the LI was also associated with the greatest exercise capacity at 12 months. Furthermore, the analysis identified haemoglobin to be the greatest predictor of individuals reporting higher intensity exercise.

Chapter 5 investigates the agreement between creatinine and cystatin-C estimated glomerular filtration rate (eGFR) measurements in patients completing 12 months of the LM3 study. This chapter explores whether discrepancies in the CKD Epidemiology Collaboration (CKD-EPI)cystatin-C, CKD-EPIcreatinine and modification of diet in renal disease (MDRD) eGFR equations occur after an exercise intervention. It was identified that cystatin-C based eGFR measurements were considerably lower than creatinine based eGFR measurements. Despite this discrepancy at baseline, the agreement between the different GFR estimates did not change after exercise training.

Chapter 6 contains the main study from LM3, which investigates the changes in cardiorespiratory fitness, exercise capacity, functional capacity, blood biochemistry, anthropometry measures and other cardiovascular disease risk factors after 3 years of LI compared to the control group. The primary finding from this study was the ability of a long-term LI to significantly increase physical activity levels, exercise capacity and autonomic function and decrease arterial stiffness in patients with CKD.

Section 2 includes a systematic review and meta-analysis of HIIT in patients with cardiometabolic diseases (Chapter 7). This review investigated the efficacy and safety of HIIT compared to MICT in individuals with a population sample of chronic disease, where poor lifestyle was considered a main contributor to the disease. The meta-analysis was performed using oxygen uptake (VO_2peak or VO_2maximum) as the primary outcome measure, however the influence of HIIT on all cardiovascular disease risk factors was explored. It was identified that HIIT provokes nearly twice the increase in VO_2peak compared to MICT.

Chapter 8 in Section 2 investigates the feasibility of HIIT and the efficacy of HIIT compared to MICT in improving cardiovascular disease risk factors. It was identified that HIIT was a feasible option for patients with CKD. However, the improvements in cardiorespiratory fitness and exercise capacity were slightly greater in the MICT than the HIIT group, although the changes were not statistically significant. Participants from the HIIT group also reported higher levels of enjoyment from the intervention, as measured by the Physical Activity Enjoyment Scale.

Chapter 9 provides a summary of the outcomes from all studies. This chapter discusses how this thesis has extensively contributed to the current knowledge in exercise training in CKD patients. Based on the findings, it is recommended that a combination of aerobic and resistance training, with some higher intensity exercise if possible, be prescribed to patients with CKD to elicit the greatest health outcomes. Important factors to consider with exercise training and testing of CKD patients, such as the effects of certain medications and hydration status on physiological responses, are also explored. Further, this chapter suggests future directions for research which will extend the findings from this thesis.

Declaration by author

This thesis **is composed of my original work, and contains** no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted **to qualify for the award of any** other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Rossi M, Campbell KL, Johnson DW, Stanton T, Vesey DA, Coombes JS, **Weston KS**, Hawley CM, McWhinney BC, Ungerer JPJ, Isbel N. Uremic toxins, inflammation and oxidative stress: a cross-sectional study in stage 3-4 CKD. *Archives of Medical Research*. May 2014; 45(4):309-17. doi: 10.1016/j.arcmed.2014.04.002.

Howden EJ, **Weston KS**, Leano R, Sharman JE, Marwick TH, Isbel NM, Coombes JS. Cardiorespiratory fitness and cardiovascular burden in CKD. *Journal of Science and Medicine in Sport*. January 2014; in press.

Ramos JS, Dalleck LC, **Beetham KS**, Coombes JS. The effect of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. *Sports Medicine.* February 2015; in press.

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Weston KS, Howden EJ, Isbel NM, Coombes JS. Three-year Randomized Control Lifestyle Intervention in Patients with CKD. *Medicine and Science in Sports and Exercise*. May 2014, 45:5S.

Publications included in the thesis

Two first author manuscript have been published in international, peer-reviewed journals. Both are included in whole in Chapters 3 and 7, respectively.

Weston KS, Wisloff U, Coombes JS. High intensity interval training in patients with lifestyle-induced chronic disease: a systematic review and meta-analysis. *British Journal of Sports Medicine*. October 2013; 48(16);1227-34.doi: 10.1136/bjsports-2013-092576.

Contributor	**Statement of contribution**
Kassia Beetham (Candidate)	Designed search terms and criteria (80%) Critically reviewed the papers (70%) Compiled the manuscript (100%) Performed statistical analysis (70%)
Ulrik Wisloff	Critically revised the manuscript (20%)
Jeff Coombes	Designed search terms and criteria (20%) Critically reviewed the papers (30%) Critically revised the manuscript (80%) Assisted in statistical analysis (30%)

Beetham KS, Howden EJ, Small DM, Briskey DR, Rossi M, Isbel NM, Coombes JS. Oxidative stress contributes to muscle atrophy in CKD patients. *Redox Report*. November 2014; in press.

Contributor	**Statement of contribution**
Kassia Beetham (Candidate)	Conceived and designed the study (70%) Collected the data (50%) Analysed the data (20%) Interpreted the data (60%) Compiled the manuscript (100%)

Erin Howden	Collected the data (50%) Analysed the data (20%) Assisted with interpretation of the data (10%) Revised the manuscript (20%)
David Small	Analysed the data (20%) Revised the manuscript (10%)
David Briskey	Analysed the data (20%) Revised the manuscript (10%)
Megan Rossi	Analysed the data (20%) Revised the manuscript (10%)
Nicole Isbel	Assisted with interpretation of the data (10%) Revised the manuscript (20%)
Jeff Coombes	Assisted in designing the study (30%) Assisted with interpretation of the data (20%) Revised the manuscript (30%)

Contributions by others to the thesis

Professor Jeff Coombes assisted in design of the thesis, as well as conception, design, interpretation and critical revision of all chapters from Sections 1 and 2. Associate Professor Nicole Isbel advised on conceptualisation of chapters from Section 1 as well as critical revision and interpretation of all works from Section 1. Dr Erin Howden assisted in critical revision and interpretation of all chapters from Section 1 and Chapter 8. Dr Rathika Krishnasamy revised Chapter 4. Professor Rob Fassett revised Chapters 1 and 8. The School

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Individual contribution to the studies

Study 1 from this thesis is a large scale multi-disciplinary trial with contributions from a number of different health professionals and researchers. It was conducted for 6.5 years and I am the second Exercise Physiologist to complete their PhD from this study. The first PhD student reported the effects of the first 12 months of the study on cardiac function as well as cross-sectional analyses of cardiorespiratory fitness and cardiovascular risk factors. My role within the project was to provide exercise training and collect data until completion of the study (3.5 years). The first PhD candidate completed the same roles and tasks in the project for the first 3 years of the study. In particular, I collected all of the fitness and exercise measures, as well as conducting and analyzing the DEXA scans, pulse wave velocity, pulse wave analysis and heart rate variability measures. I also independently performed the laboratory analysis of the cystatin-C measures. The oxidative stress and inflammation measures were performed by a PhD candidate and Physician, respectively, external to the study. The changes in oxidative stress will be reported by the external PhD candidate. The remaining biochemistry measures were completed by the Pathology Department at the Princess Alexandra Hospital. The echocardiography and endothelial function measures were performed by qualified Sonographers at the Princess Alexandra Hospital. The patient history, venipuncture and basic anthropometry were performed by the study's research nurse. The 3 year echocardiography and endothelial function data will be reported by a Nephrologist PhD candidate who was involved in recruitment and supervision of the exercise stress tests, as well as the Chief Investigator of the study (also a Nephrologist). Concurrent to my role in Study 1, I also designed the protocol, recruited patients, provided exercise training and collected and analysed all of the outcome measures for Study 2. The muscle biopsy was performed by a Nephrologist with my assistance. Due to the intensive time commitment of completing both studies concurrently, the muscle tissue collected from Study 2 will be analysed by Victoria University and I will publish the findings at a later date.

Statement of parts of the thesis submitted to qualify for the award of another degree

None

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I am so proud of finishing this thesis in such an important area of health research. Through the many opportunities that were afforded to me throughout this PhD I have grown as both a researcher and an Exercise Physiologist. I feel fortunate to have been involved in both a large scale multi-disciplinary study as well as a smaller self-designed study. There are a number of people who have played a significant part in my life in the past 4 years which have helped bring this thesis to fruition.

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I doubt I would have been able to make it to the end of this PhD without the unwavering support of my family and friends. To all my friends at UQ, it was so great to have you all to go through this journey with together. When I was facing a research problem it was always reassuring to talk to someone else going through the same thing. Likewise, it was so

wonderful to have people in my life who I could celebrate the triumphs of research with. I loved working with you all and I really valued how we put aside our own pressing deadlines to help each other out. I look forward to catching up with you all at future conferences around the world.

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Keywords

High intensity exercise, chronic diseases, exercise, kidney diseases, muscular atrophy, muscle strength, oxidative stress, haemoglobin, glomerular filtration rate, creatinine, cystatin C, oxygen consumption

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 110602 Exercise Physiology (100%)

Fields of Research (FoR) Classification

FoR code: 1106 Human Movement and Sports Science (100%)

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List of abbreviations and acronyms

ACSM= American College of Sports Medicine

AIx= augmentation index

ANOVA= analysis of variance

BMI= body mass index

CI= confidence interval

CKD= chronic kidney disease

CKD-EPI= Chronic Kidney Disease Epidemiology Collaboration

Cr= creatinine

Cys= cystatin-C

CRP= C-reactive protein

DEXA= dual-energy x-ray absorptiometry

ECG= electrocardiogram

eGFR= estimated glomerular filtration rate

EPO= erythropoietin

ESSA= Exercise and Sports Science Australia

GEE= generalized estimating equation

GFR= glomerular filtration rate

GPX= glutathione peroxidase

HDL= high-density lipoproteins

HF= high frequency

HIIT= high intensity interval training

HOMA-IR= homeostatic model assessment of insulin resistance

HRR= heart rate reserve

HRV= heart rate variability

IGF= insulin growth factor

IL-6= interleukin-6

IL-10= interleukin-10

INF-γ= interferon-γ

IQR= interquartile range

KDIGO=Kidney Disease- Improving Global Outcomes

LDL= low-density lipoproteins

LF= low frequency

LI= lifestyle intervention

LM3= Landmark III

MDRD= modification of diet in renal disease

MD= mean difference

MeSH= medical subject heading

METs= metabolic equivalent tasks

MHR= maximum heart rate

MICT= moderate intensity continuous training

NADPH= nucleotinamide adenine dinucleotide phosphate

PEDro= physiotherapy evidences database

PHR= peak heart rate

PWA= pulse wave analysis

PWR= peak work rate

PWV= pulse wave velocity

RMSSD= root mean square of the standard deviation of N-N interval

RM= repetition maximum

ROS= reactive oxidative species

RPE= rating of perceived exertion

SD= standard deviation

SEM= standard error of mean

SDNN= standard deviation of the N-N interval

SIT= sprint interval training

TAC= total anti-oxidant capacity

TC= total cholesterol

TG= triglycerides

TNF-α= tumour necrosis factor-α

TP= total power

VLF= very low frequency

VO_2= maximal oxygen uptake

Chapter 1.
Introduction and general review of the literature

1.1 Introduction

Chronic kidney disease (CKD) is a debilitating public health problem, reported to affect approximately 16% of Australian adults.(1) Moreover, CKD patients often have a shorter life expectancy than that of healthy individuals due to the premature development of cardiovascular disease.(2) This is partly due to the association of CKD with lifestyle related traditional cardiovascular disease risk factors such as hypertension, diabetes, hypercholesterolemia and left ventricular hypertrophy. It has also been reported that non-traditional risk factors such as anaemia, abnormal phosphate-calcium metabolism, inflammation and oxidative stress may contribute to the accelerated burden of cardiovascular disease.(3)

The economic burden of kidney disease in the years of 2004 and 2005 has been estimated at almost $900 million, which is equivalent to 1.7% of total health care expenditure (Australian Institute of Health and Welfare, 2013). Dialysis is the major contributor to this cost, accounting for 2/3 of the total expenditure. Many of the adverse events associated with CKD, such as kidney failure and cardiovascular disease, can be delayed with effective treatment. As such, management of CKD should be aimed at modifying these cardiovascular disease risk factors.(4)

1.1.1 Classification of CKD

CKD can be defined and classified according to abnormalities of kidney structure or function, present for > 3 months, with implications for health.(5) In particular, the criteria for CKD diagnosis is: duration of >3 months, based on documentation or inference, of GFR <60 ml/min/1.73m^2) (GFR categories G3a-G5 [Table 1.2])(5), or kidney damage as defined by structural abnormalities or functional abnormalities other than decreased GFR (Table 1.3).(6) Commonly, initial screening of kidney dysfunction occurs through general practitioners by detection of urinary sediment and assessment of blood biochemistry. Urinalysis allows detection of albuminuria (Table 1.2), proteinuria, creatinine and urea, all of which can provide indications of kidney damage.(7) The prognosis of CKD by GFR and albuminuria has been reported by KDIGO 2012 (Table 1.2).(5)

Table 1.1 Criteria for CKD (either of the following present for >3 months) (KDIGO, 2012)

Markers of kidney damage (one or more)	Albuminuria (AER ≥30mg/24 hours; ACR ≥30mg/g [≥3 mg/mmol])
	Urine sediment abnormalities
	Electrolyte and other abnormalities due to tubular disorders
	Abnormalities detected by histology
	Structural abnormalities detected by imaging
	History of kidney transplantation
Decreased GFR	GFR <60 ml/min/1.73m^2 (GFR categories G3a-G5)

AER= albumin excretion rate; ACR=albumin creatinine ratio; GFR= glomerular filtration rate

Table 1.2 Prognosis of CKD by GFR and albuminuria categories (KDIGO, 2012)

				Persistent albuminuria categories		
				A1	**A2**	**A3**
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
GFR categories (ml/min/1.73m^2). Description and range	**G1**	Normal or high	≥90	Low risk	Moderately increased risk	High risk
	G2	Mildly decreased	60-89	Low risk	Moderately increased risk	High risk
	G3a	Mildly to moderately decreased	45-59	Moderately increased risk	High risk	Very high risk
	G3b	Moderately to severely decreased	30-44	High risk	Very high risk	Very high risk
	G4	Severely decreased	15-29	Very high risk	Very high risk	Very high risk
	G5	Kidney failure	<15	Very high risk	Very high risk	Very high risk

1.1.2 Measurement of kidney function

There have been a number of proposed ways to measure kidney function. Quantifying the clearance of an exogenous marker such as inulin, is accepted to be the reference method for measuring renal function,(8) however it is not used routinely due to its complexity in testing. More commonly, indirect assessment of endogenous markers to provide an eGFR is used for routine clinical measurements.(9) These formulas use biomarkers of either creatinine, or cystatin-C in serum, plasma or urine, alongside age, sex and race to estimate filtration rates. The four most frequently used eGFR equations are: modification of diet in renal disease (MDRD),(10) CKD-EPI creatinine (CKD-EPIcr)(11), CKD-EPI cystatin-C (CKD-EPIcys)(12) and CKD-EPI creatinine-cystatin-C (CKD-EPIcr-cys)(12). The original MDRD equation used a 6 variable estimate (serum creatinine, age, sex, race, serum urea nitrogen and albumin concentrations),(13) however this was later simplified to 4 variables (creatinine, age, sex and race).(14) The 4-variable MDRD equation was shortly after re-expressed for use with standardized serum creatinine assays (isotope-dilution mass spectrometry).(10) The MDRD equation was shown to be more simplistic than the Cockcroft-Gault formula, as it did not require a patient's weight for the equation.(15) More recently it was proposed that CKD-EPIcr provided a more precise estimate of GFR and categorization for risk of mortality compared to the MDRD study equation.(16) CKD-EPIcr was developed using the same variables as MDRD but with different coefficients, which created a moderate improvement in overall accuracy.(17) Furthermore, the MDRD equation has only been validated in eGFR <60 ml/min/1.73m^2 whereas the CKD-EPI equation has been validated to eGFR 90 ml/min/1.73m^2.(5) It has been suggested that standardized serum creatinine assays be used as a first test for assessing eGFR in adults and that CKD-EPIcys be used a confirmatory test if a below normal creatinine eGFR is detected.(18) The combination of both creatinine and cystatin-C used in the CKD-EPIcr-cys equation may provide the most accurate assessment of kidney function.(18-20)

1.1.3 Causes of CKD

Diabetes, hypertension and glomerulonephritis, are the leading causes of end stage kidney disease in all developed and many developing countries.(21) Indeed, the rising prevalence of CKD(22) is likely attributable to the increase in diabetes and hypertension in the aging population. Diabetic nephropathy occurs as a consequence of prolonged exposure of the

microvasculature to elevated blood glucose levels. Glomerulonephritis refers to a range of primary conditions which cause inflammation and damage to the glomerulus.(23) Managing hypertension and glycaemic control are the most important early interventions in slowing the progression of diabetic nephropathy if albuminuria is present. Specifically, first line medical management for early stage CKD guidelines are treatment of hypertension and proteinuria through angiotensin converting enzyme inhibitors or, angiotensin receptor blockers and lifestyle changes. Second and third line anti-hypertension therapies include β-blockers, diuretics and calcium channel blockers.(24)

1.1.4 Exercise as therapy

Cardiorespiratory fitness is a known predictor of morbidity and mortality outcomes both in healthy and chronic disease populations.(25-28) The increase in cardiovascular mortality in CKD patients is hypothesised to occur as a result of the myriad of metabolic co-morbidities which are associated with this population. However, the resistance to improvement in cardiovascular disease risk factors with traditional strategies, suggests that other mechanisms may be at play. Consequently, the use of exercise therapy may provide an additional treatment to reducing cardiovascular disease risk factors. Indeed, it has been reported in 6213 men aged 59.0±11.2 years, that every 1-MET increase in exercise capacity results in a 12% improvement in survival.(29)

According to Exercise and Sports Science Australia, exercise training for patients with CKD should include aerobic, resistance and flexibility exercises and the contribution of each should be based on clinical judgement.(30) Furthermore, it is recommended that resistance training be performed twice weekly on non-consecutive days. It is also advised that appropriately trained and qualified personnel, such as an Accredited Exercise Physiologist, should design and deliver exercise programs, taking into account individual patient needs. Individual recommendations by stage of CKD or treatment plan do not currently exist.(31) The Kidney Disease- Improving Global Outcomes group (KDIGO) recommends encouraging lifestyle modification in patients with CKD to lower blood pressure and improve long-term cardiovascular outcomes.(32) In particular, they recommend undertaking an exercise program compatible with cardiovascular health and tolerance for at least 30 minutes, 5 days/week. Despite not having recommendations specific to CKD, the American College of Sports Medicine suggests older adults, or younger individuals with chronic conditions, undertake

either moderate intensity aerobic exercise for at least 30 minutes on most days of the week, or vigorous intensity exercise for 20 minutes on a least 3 days/week.(33) They also recommend whole body resistance training on at least 2 non-consecutive days/week and flexibility training for at least 10 minutes, 2 days/week. The Swedish exercise guidelines by Heiwe et al. for CKD and kidney transplant are slightly different to the guidelines mentioned above. Specifically, they recommend moderate intensity aerobic exercise 3x/week for 60 minutes, resistance training for 3x/week (80% 1RM), muscular endurance training 3x/week (50% 1RM) and functional training 3x/week (ie. walking, balance and coordination training).(34)

1.1.5 Thesis introduction

The American College of Sports Medicine (ACSM) guidelines for older adults/clinical populations suggests that to achieve health enhancing benefits, a combination of resistance and aerobic exercise training should be utilized.(35) The primary study in this thesis, the Landmark III (LM3) trial, investigated the effects of a nurse-led, multi-disciplinary lifestyle intervention (LI) in 187 CKD patients for 3 years. The exercise training in the LI consisted of a combination of individualized aerobic and resistance training, in order to elicit the greatest health outcomes possible. The multi-disciplinary nature of LM3 was designed to target the complex and co-morbid nature of this disease. The objective of the 3 year intervention was aimed at not only testing the magnitude of health improvements but also the feasibility of such programs in this representative cohort of CKD patients. It was the goal of the study to identify whether this type of multi-disciplinary practice could target sustainable behaviour changes and be integrated as standard clinical management in the future. Once the effectiveness of this style of intervention in improving health outcomes is identified, the next step forward in exercise and kidney research is to ascertain a generalized optimal exercise prescription. This thesis includes a comparison of two different types of exercise training styles; the combination aerobic and resistance training in a long-term predominantly home-based program in the LM3 study (Section 1) and a comparison of 12 weeks of supervised high intensity interval training (HIIT) and moderate intensity continuous training (MICT) (Section 2). However, an optimal dose-response for exercise prescription in CKD patients still warrants considerably more research.

The overall aim of the LM3 study was to identify whether a nurse-led exercise and LI can improve cardiovascular disease risk factors and clinical outcomes. This study comprises a

representative cohort of CKD patients from the Nephrology Department at the Princess Alexandra Hospital in Brisbane, Australia. As such, the participants in the study are representative of the general CKD population, with a high number of co-morbidities and incidence of cardiovascular disease. This alone makes this study unique to other exercise studies in kidney disease patients which have excluded patients with significant cardiovascular disease. Therefore, these findings can be extrapolated to the general CKD population. The goal of the study was to investigate if a multi-disciplinary co-ordinated clinical care approach was effective in improving cardiovascular disease risk factors over an extended period.

HIIT has recently gained popular interest due to its success in improving a number of health outcomes in chronic disease populations, however this is yet to be studied in patients with kidney disease. Therefore, Section 2 aims to explore whether HIIT is a feasible option for CKD patients. The goal of this study was to provide further evidence on the most effective type of aerobic exercise prescription, which may influence future exercise guidelines for the CKD population.

1.2 Thesis aims

1) To review the literature on all topics covered in the thesis, including pathophysiology of CKD, exercise training in CKD patients and specific effects on- autonomic function, muscle atrophy and blood biochemistry.

2) To investigate the determinants and predictors of reduced strength and lean mass in patients with CKD.

3) To investigate the feasibility and determinants of CKD patients reporting higher intensity exercise in a 12 month exercise program.

4) To assess the agreement of creatinine and cystatin-C based eGFR measures in CKD patients completing a 12 month exercise program.

5) To determine the effects of a 3 year lifestyle modification on cardiovascular disease risk factors in patients with CKD.

6) To systematically review and meta-analyse HIIT in cardiometabolic lifestyle diseases.

7) To compare the feasibility and efficacy of HIIT versus MICT in patients with CKD.

1.3 General review of the literature

This thesis encompasses studies involving the effects of different approaches to exercise training on numerous physiological systems and biochemical processes. Therefore, the review of the literature has been written to give the necessary background for the reader to understand the current literature on each topic. Further summarised backgrounds are provided in each study chapter as all studies have, or will be, submitted to peer-reviewed journals.

1.3.1 Aerobic exercise

Ten published studies have investigated the effects of aerobic exercise training on a variety of outcomes in patients with CKD (Table 1.4). The study by Eidemak et al. (1997) (n=30) was a randomized control trial with participants undertaking 3 exercise sessions per week for 18 months.(36) The findings from the study indicate an increase in VO_2peak of 8% in the intervention group and a decrease of 10% in the control group.(36) Likewise, Toyama et al. (2010) (n=19) used a non-randomized selection of intervention and control groups and found similar improvements in VO_2peak in the intervention group (10%) and similar decline in the control group (14%).(37) In this study Toyama et al. (2010) used a combination of 3 supervised and unsupervised sessions per week for 12 weeks. Boyce et al. (1997) (n=8) performed a supervised cross-over design trial for four months with participants attending three sessions per week.(38) The intensity of the training sessions were progressively increased from 50-70% of heart rate reserve. The investigators of this study found a significant increase in VO_2peak after the training period with a subsequent decrease after 2 months of de-training. The findings by Eidemark et al, (1997), Toyama et al. (2010) and Boyce et al. (1997) suggest that non-exercising CKD patients experience a decline in cardiorespiratory fitness which can be successfully ameliorated with aerobic exercise training.

Pechter et al. (2003) (n=26) used a non-randomized selection of intervention and control groups who trained for 12 weeks and Leehey et al. (2009) (n=11) conducted an RCT for 24 weeks.(39, 40) However in contrast to the above studies, Pechter et al. (2003) and Leehey et

al. (2009) found no significant change in VO_2peak in the intervention group compared to the control group. The study by Pechter et al. (2003) comprised two supervised sessions per week for 12 weeks. The study was considered low intensity by the investigators and although was monitored by a heart rate monitor the exact intensity was not reported. The exercise training protocol was described as vertical exercises in the pool, which may indicate that this modality of training is not sufficient to improve cardiorespiratory fitness. Furthermore, as cardiorespiratory testing occurred on a bicycle ergometer, the specificity of the aquatic training may have limited the transferrable improvements in fitness to the post-test. However, the sessions were only performed twice per week, which may also have contributed to the lack of significant progression in VO_2peak. Although, this study did find an improvement in peak load in the intervention group, which may indicate health enhancing physiological adaptations, such as intrinsic changes in skeletal muscle,(41) which are occurring independently of changes in cardiorespiratory fitness. The lack of change in cardiorespiratory fitness in the study by Leehey et al. (2009) may be due to the high dropout rate experienced in the study. After 20 participants were recruited and consented, 7 subjects were not randomized due to: not meeting screening laboratory tests (n=1), a positive stress test (n=2), investigator decision (n=2), patient wish (n=1) and commencement of haemodialysis (n=1). A further two patients dropped out of the control group due to investigator decision and patient wish after randomization, leaving seven subjects in the intervention group and only four in the control group. The high drop-out rate may mean the remaining sample is biased and not representative of the CKD population. This issue highlights the difficulty in performing exercise studies in this population. Indeed, the limitation of reduced sample size in diminishing statistically significant cardiorespiratory fitness changes is likely, considering the study by Mustata et al. (2010), which also had both supervised and unsupervised sessions, had a significant improvement in VO_2peak in the intervention group with a sample population nearly twice the size (n=20). The RCT by Mustata et al. (2010) showed an increase in VO_2peak, despite an adherence of 80% in the supervised period and only 20% adherence reported in the home sessions.

Kosmadakis et al. (2011), Watson et al. (2013) and Viana et al. (2014) published papers from the same trial investigating a non-randomized walking intervention performed 5x/week for 6 months.(42-44) The first 20 participants were allocated to the exercise group and the following 20 participants were allocated to the control group. In addition, the 40 participants were randomized to four groups of bicarbonate supplementation of 24 mmol/l (exercise vs.

control) or 29 mmol/l (exercise vs. control). Watson et al. (2013) identified that exercise training depleted amino acid concentrations in participants receiving standard oral bicarbonate supplementation (24 mmol/l). However, participants randomized to receive additional oral bicarbonate supplementation (29 mmol/l) found that this depletion in amino acid concentrations was avoided. Significant improvements in exercise tolerance, weight loss and improvements in quality of life were reported in the publication by Kosmadakis et al. (2011) of the same study, however there were no significant changes in lean mass.(42) Furthermore, there was no net decrease in myofibrillar proteolysis.(44) These findings may suggest that despite additional blood buffering, aerobic exercise isn't sufficient in stimulating hypertrophy in CKD patients. Viana et al. (2014) found a reduction in the ratio of plasma IL-6 to interleukin-10 (IL-10) levels, suggesting an anti-inflammatory effect of regular aerobic exercise.(43) Indeed, frequency of exercise training may be important to anti-inflammatory response, as the authors also identified significant changes in IL-6 and IL-10 after an acute bout of exercise.

Baria et al. (2014) randomized 27 sedentary, obese men to either aerobic exercise or control group in a 2:1 ratio.(45) Participants randomized to the aerobic exercise were able to choose between exercising in a supervised gym based setting (n=10) or at home (n=8), both for three sessions per week for 12 weeks. The modality of aerobic exercise was unspecified for both gym and home based settings. Baria et al. (2014) showed that 12 weeks of mild to moderate intensity (ventilatory threshold equivalent to 40-60% of maximum VO_2) gym based aerobic exercise training can significantly reduce visceral fat, as measured by abdominal computed tomography and waist circumference.(45) Furthermore, when a home-based exercise group was given the same exercise instructions, diminutions in visceral fat and waist circumference were trending towards significance. This study also identified a significant increase in leg lean mass in the gym based group only. Furthermore, the investigators from this study found similar enhancements in both VO_2peak, six minute walk time and sit to stand test in both supervised gym based and home based exercise.(45) Centre based *and* home based improvements inVO_2peak and sit to stand were statistically significant from baseline, however they weren't significantly different from each other. These findings provide support for economical home-based programs which can provide similar benefits to supervised gym-based sessions.

Table 1.3 Summary of studies investigating the effects of aerobic exercise training

Author (year)	Exercise:control	Design	Intervention	Duration	Exercise group outcomes
Eidemak et al. (1997)	15:15	RCT	Unsupervised, home based 30 minutes/day	Exercise: 18 months (range 8-20 months) Control: 20 months (range 10-30 months)	⇑ VO_2peak ⇔ eGFR ⇔ Lipids
Boyce et al. (1997)	8	Cross-over	Supervised, 60 minutes, 3x/week	4 months	⇑ VO_2peak ⇑ Blood pressure ⇔ LV function ⇑ Muscle strength ⇓ Creatinine clearance ⇓ eGFR ⇔ Haemoglobin ⇔ Lipids
Pechter et al. (2003)	17:9	Non-random self select	Supervised, 30 minutes, 2x/week	12 weeks	⇔ VO_2peak ⇑ Peak O_2 pulse ⇑ Peak ventilation

					⇑ Peak load ⇑ Proteinuria ⇑ Cystatin-C ⇑ eGFR ⇑ Body mass index ⇑ Total cholesterol ⇑ Oxidative stress ⇔ Blood pressure
Leehey et al. (2009)	7:4	RCT	Supervised + home based, 30-40 minutes, 3x/week	6 weeks supervised + 18 weeks home based	⇔ VO_2max ⇔ Exercise duration ⇔ Proteinuria ⇔ eGFR ⇔ Glycated haemoglobin ⇔ Lipids ⇔ Haemoglobin ⇔ CRP ⇔ Body composition ⇔ Blood pressure
Mustata et al. (2010)	10:10	RCT	Supervised + home based, progressed	1 month supervised + 11	⇑ VO_2peak ⇑ Endurance time

			from 5-60 minutes, 3x/week	months supervised and home based	⇑ AIx ⇔ Quality of Life
Toyama et al. (2010)	10:9	Non-random	Supervised + home based, 30 minutes, 2x/week	12 weeks	⇑ AT-VO_2 ⇑ Lipids ⇑ eGFR
Kosmadakis et al. (2011)	20:20	Non-random for exercise program. Randomized to usual or additional bicarbonate supplementation.	Unsupervised, home based, 30 minutes, 5x/week	6 months	⇑ Exercise tolerance ⇑ Quality of life ⇑ reported uraemic symptoms ⇑ BMI ⇔ Blood pressure ⇔ Cardiovascular function ⇔ Lean mass or fat mass
Watson et al. (2013)	20:20	Non-random for exercise program. Randomized to usual or additional	Unsupervised, home based, 30 minutes, 5x/week	6 months	Standard bicarbonate: ⇔ Blood lactate ⇓ Intramuscular free amino acids ⇔ Anabolic indicators ⇔ Myofibrillar proteolysis Additional bicarbonate:

		bicarbonate supplementation.			⇑ Blood lactate ⇑ Intramuscular free amino acids ⇑ transcription MuRF1 ⇔ Other anabolic indicators ⇔ Myofibrillar proteolysis
Viana et al. (2014)	20:20	for exercise program. Randomized to usual or additional bicarbonate supplementation.	Unsupervised home based, 30 minutes, 5x/week	6 months	⇑ Anti-inflammatory effects ⇑ T-lymphocyte and monocyte activation ⇔ Immune cell numbers ⇔ Neutrophil degranulation responses ⇔ Proteinuria ⇔ Blood pressure
Baria et al. (2014)	Centre-based:home-based:control: 10:8:9	RCT	Supervised or unsupervised, 30 minutes, 3x/week	12 weeks	Centre: ⇑ Speed achieved at VO_2peak ⇑ Sit to stand ⇑ Six minute walk test ⇑ Visceral fat, waist circumference and total body fat ⇑ Leg lean mass ⇑ eGFR

			⇑ Blood pressure Home: ⇑ Speed achieved at VO_2peak ⇑ Sit to stand ⇑ Six minute walk test ⇔ Visceral fat, waist circumference and total body fat ⇔ Leg lean mass ⇑ Blood pressure ⇔ eGFR

eGFR= estimated glomerular filtration rate; RCT= randomized control trial; AT= anaerobic threshold; AIx= augmentation index. ⇑ = positive change; ⇓ negative change; ⇔ no change.

1.3.2 Resistance exercise

Six published studies from three trials have investigated the effects of resistance exercise training in patients with CKD (Table 1.4). Castaneda et al. (2001 and 2004) (n=26), Balakrishnan et al. (2010) (n=23) and Watson et al. (2014) (n=38) conducted RCTs,(17, 46-48) whereas Heiwe et al. (2001 and 2005) studied self-selected groups of CKD patients in an exercise (n=12), non-exercise control, (n=5) and healthy exercise group (n=6).(49, 50) All exercise sessions were performed in a supervised environment three times per week for 12 weeks. The trial by Castaneda et al. and Balakrishnan et al. performed 3x 8 repetitions at 60% 1-repetition maximum (1RM) and the trial by Heiwe et al. performed 3x 20 repetitions at 80% 1RM respectively. The trial reported by Castaneda et al. (2001 and 2004) and Balakrishnan et al. (2010) found significant enlargements in both type 1 and type 2 muscle fibre cross-sectional area of the vastus lateralis and upper and lower body strength.(17, 46, 47) Balakrishnan et al. (2010) also found significant increases in skeletal muscle mitochondrial content DNA copy number, supporting the anabolic benefit of resistance training in CKD patients. This improvement occurred despite a low-protein diet prescribed to participants. On the other hand, Heiwe et al. (2005) found no statistical differences in muscle fibre type proportion between CKD patients and healthy controls at baseline, with no change after exercise training.(49) However, the study did identify a significant increase in muscular strength measured by 1RM. The lack of morphometrical changes in this study may indicate neural adaptations instigated the improved muscle function. Heiwe et al. (2001) found the resistance training intervention group had significant increases in walking distance compared to the control group.(50) Watson et al. (2014) conducted a parallel randomized control feasibility study in where intervention participants underwent 8 weeks of progressive resistance exercise training. Participants attended the supervised sessions 3x/week and performed 3 sets of 10-12 leg extensions at 70% of estimated 1RM.(48) Watson et al. (2014) found that 8 weeks of progressive resistance training increased muscle anatomical cross-sectional area, muscle volume and knee extensor strength.(48)

Table 1.4 Summary of studies investigating the effects of resistance exercise training

Author (year)	Exercise:control	Design	Intervention	Duration	Outcomes of exercise
Castaneda et al. (2001)	14:12	RCT	Supervised, 45 minutes, 3x/week	12 weeks	⇔ Thigh computed tomography ⇑ Type I and II cross-sectional area ⇔ BMI ⇓ GFR
Castaneda et al. (2004)	14:12	RCT	Supervised, 45 minutes, 3x/week	12 weeks	⇓ IL-6 and CRP ⇑ Type I and II cross-sectional area ⇔ BMI ⇑ muscle strength
Balakrishnan et al. (2010)	13:12	RCT	Supervised, 45 minutes, 3x/week	12 weeks	⇑ Type I and II cross-sectional area ⇑ Muscle strength ⇑ mtDNA copy number
Heiwe et al. (2001)	Uraemic: 16:9 Healthy: 18:5	Non-random self select	Supervised, 30 minutes, 3x/week	12 weeks	Both exercise groups: ⇑ Muscle strength ⇑ Six minute walk time ⇑ Timed up and go ⇔ Muscle endurance ⇔ Quality of life
Heiwe et al. (2005)	7:5 + healthy	Non-	Supervised, 30	12 weeks	⇑ Muscle strength

	exercising control:6	random self select	minutes, 3x/week		⇔ Muscle endurance ⇔ Type I, IIA, IIB cross sectional area ⇔ Type I, IIA, IIB proportion
Watson et al. (2014)	20:18	RCT	Supervised, 3x/week	8 weeks	⇑ Quadriceps cross-sectional area ⇑ Quadriceps volume ⇑ Knee extensor strength ⇑ Endurance capacity ⇔ Exercise tolerance

BMI= body mass index; eGFR= estimated glomerular filtration rate; RCT= randomized control trial. ⇑ = positive change; ⇓ negative change; ⇔ no change.

1.3.3 Combination training

Five published studies from four trials (three non-randomized and 1 RCT) investigated the effects of combination exercise training (aerobic and resistance) in patients with CKD (Table 1.5). Clyne et al. (1991) (n=19) conducted three supervised training sessions per week for 12 weeks.(51) Participants were instructed to exercise at 60-70% of their maximal capacity. Whereas, the intervention in the study by Cook et al. (2008) (n=54) included three *un*supervised sessions per week for 12 months in obese patients with stage 2-5 CKD.(52) The intervention was a combination of aerobic, thera-band and hand weight exercises which also included a calorie restricted diet with weight loss agent (Orlistat). Both Clyne et al. (1991) and Cook et al. (2008) found significant improvements in exercise capacity and 6 minute walk time with combination training, respectively.(51, 52)

Greenwood et al. (2012) (n=77) provided a 12 week renal rehabilitation program to patients with stage 3-4 CKD, maintenance haemodialysis and post kidney transplant.(53) Training consisted of two supervised sessions per week and one home session and the training programme included aerobic exercise (rating of perceived exertion [RPE] 13-15), strength conditioning, muscular endurance and balance training. Greenwood et al. (2012) also identified significant improvements in exercise capacity (shuttle walk) and functional ability (Duke's activity status index, timed up and go, stair climb and sit to stand test) in stage 3-4 CKD, haemodialysis and post-transplant patients after 12 weeks of combination training.(53)

Gregory et al. (2011) and Headley et al. (2012) conducted a 48 week randomized supervised combination exercise training program on 21 patients with stage 2-4 CKD.(54, 55) The exercise group completed 48 weeks of aerobic exercise at 50-60% of VO_2peak. From weeks 24 to 48 participants were allowed to include 1-2 sets of 10-15 repetitions of six resistance exercises. Gregory et al. (2011) identified no significant changes in immunoreactive IGF-1, IGF-II, bioactive IGF-1, IGFbinding protein-1 or IGFbinding protein-2 after 48 weeks of combination exercise training.(54) Headley et al. (2012) found a significant time interaction between the exercise and usual care group for VO_2peak.(55) The exercise group showed modest improvements in VO_2peak, however it is interesting to note the decline of 2.8 ml/kg/min at 48 weeks in the usual care group.(55) This decline in fitness in the control group is consistent with the findings from aerobic training studies by Boyce et al. (1997) and Toyama et al. (2010).(37, 38)

Table 1.5 Summary of studies investigating the effects of combination (aerobic and resistance) exercise training

Author (year)	Exercise:control	Design	Intervention	Duration	Exercise group outcomes
Clyne et al. (1991)	10:9	Allocated based on proximity to gym	Supervised, 60 minutes, 3x/week	3 months	⇑ Exercise capacity ⇑ Muscle strength ⇔ Total haemoglobin ⇔ Blood volume ⇔ eGFR ⇔ Blood pressure ⇔ Echocardiographic variables
Cook et al. (2008)	32:22	Non-random self select	Unsupervised, 20-30 minutes, 3x/week	12 months	⇑ Weight ⇑ BMI ⇑ Waist circumference ⇑ Six minute walk time ⇑ Timed get up and go ⇑ Duke's activity status index
Greenwood et al. (2012)	77	Non-random	Supervised, 2x/week, 60 minutes, Unsupervised 1x/week, 30	12 weeks	⇑ Exercise capacity ⇑ Functional ability ⇑ Anxiety and depression

			minutes		
Gregory et al. (2011)	10:11	RCT	Supervised, 10-45 minutes, 3x/week	48 weeks	⇑ VO_2peak ⇑ Total treadmill time ⇔ IGF system ⇔ eGFR
Headley et al. (2012)	10:11	RCT	Supervised, 10-45 minutes, 3x/week	48 weeks	⇑ VO_2peak ⇑ Resting heart rate ⇑ Ambulatory heart rate ⇓ LDL ⇓ Triglycerides ⇔ eGFR

eGFR= estimated glomerular filtration rate; RCT= randomized control trial; LDL= low-density lipoprotein; IGF= insulin-like growth factor. ⇑ = positive change; ⇓ negative change; ⇔ no change.

1.3.4 High intensity interval training

Exercise therapy, in particular aerobic exercise, in the CKD population has been shown to decrease cardiovascular disease risk factors and subsequent cardiovascular disease,(38) however there has been little work comparing the effectiveness of different exercise prescriptions. HIIT has been shown to be superior to MICT in improving fitness and decreasing cardiovascular disease risk in both healthy populations and other chronic diseases, however is yet to be studied in the CKD population. HIIT involves alternating short bursts of high intensity exercise with rest periods or light exercise. Numerous studies in athletes and the general population have shown that increasing the intensity of exercise augments the training stimulus and associated adaptations such as increased oxygen uptake and anaerobic capacity.(56, 57) This implies that the same health enhancing benefits could be gained in a lesser amount of time; a more efficient option for those citing lack of time as a barrier to exercise.(58, 59) Moreover, short bursts of activity may address another common limiting factor, lack of motivation. It may be a more enticing option than the prospect of continuously exercising for an extended period of time. HIIT may also be a more suitable way for people with poor exercise capacity to reach intensity levels that are capable of achieving health-enhancing benefits. Short work periods at a higher intensity allow a reduction in ventilatory response and resultant dyspneoa which in many chronic disease patients would be a limiting factor to continuous exercise.(60-62) Although the Cochrane review by Heiwe et al. indicates that vigorous intensity exercise is more effective than moderate intensity in improving a number of health outcomes in CKD patients, the definition in this paper of vigorous intensity exercise is >60% (whether it is a percentage of maximum heart rate (MHR) or VO_2peak is not defined) which is not in line with the current Australian position statement on exercise intensity terminology.(31, 63) This statement defines vigorous intensity exercise as >70<90% MHR, with high intensity exercise defined as >90%. This thesis investigates the feasibility and determinants of vigorous and high intensity exercise.

The feasibility of HIIT in other clinical populations has been investigated. Askim et al. (2014) found that it was feasible for recent stroke patients to participate in 4x4 minutes of HIIT. Although there was no significant change in VO_2peak there was a significant improvement in six minute walk time.(64) The study by Keteyian et al. (2014) found significant improvements in VO_2peak in HIIT compared to MICT (3.6±3.1 vs. 1.7±1.7 ml/kg/min^2) in patients undergoing cardiac rehabilitation, using the 4x4 approach.(65) HIIT has also been shown to improve VO_2peak in chronotropically incompetent heart transplant

recipients without improvements in cardiac function, suggesting improvements in autonomic control.(66) On the other hand, Currie et al. (2013) found similar improvements in VO_2peak and flow mediated dilatation between HIIT and MICT groups in patients with coronary artery disease.(67) In this study, the HIIT group performed 1 minute intervals with 1 minute recovery. These findings provide insight into a dose-response relationship which provides a time efficient alternative to traditional, moderate intensity activity with similar benefits. Koufaki et al. (2014) compared the effectiveness of HIIT and MICT in patients with chronic heart failure.(68) Participants performed a work to recovery ratio of 1:2, with 30 second work intervals, three times a week for 24 weeks. The patients in this study had particularly low levels of cardiorespiratory fitness (15.3±4.7 ml/kg/min^2). They found that HIIT was well tolerated and despite a considerably shorter training time, had the same benefits in physical function as MICT. As suggested by the authors, the potential for using HIIT in deconditioned, cachetic patients with low exercise tolerance is of value in the context of energy preservation and time efficiency. This is particularly relevant for CKD patients in the more severe stages of renal disease, as these patients often experience significant muscle wasting and the reduced strength is likely contributing to the low exercise capacity in this population.(69)

A study by Keating et al. (2014) in overweight adults found that sprint intervals of 30-45 seconds at 120% VO_2peak could elicit the same improvements in 50-60% of the time taken for the same gains from MICT.(70) However, it was identified that MICT and not the sprint intervals reduced total body and android fat. The difference between high intensity aerobic intervals and sprint intervals on weight loss should be further investigated.

There are a number of different factors to consider when prescribing HIIT, which may influence the magnitude of change. A common approach in measuring exercise intensity is to achieve a percentage of MHR. A study by Aamot et al. (2014) assessed whether RPE is a valid method for achieving target exercise intensity during HIIT.(71) They found when patients were told to aim for an RPE corresponding to 85% peak heart rate (PHR), the actual blinded heart rate was 82% PHR. Therefore, using heart rate monitors encourages a slightly higher intensity of exercise. However, if a patient is on β-blockers than it seems reasonable to suggest that a 3% discrepancy is preferable to exclusion from this type of activity all together. Given the high prevalence of cardiovascular disease in the CKD population, there are a number of CKD patients who are prescribed β-blocker medication.(72) Ensuring the right protocol for measuring exercise intensity in the CKD population specifically on this

medication is important for maximising the outcomes of exercise training. Therefore, the HIIT study in Chapter 8 utilizes both heart rate and RPE for assessing appropriate intensities.

1.3.5 Cardiorespiratory fitness and exercise capacity

Cardiorespiratory fitness is highly associated with mortality,(73) and is found to be significantly lower in CKD patients when referenced against healthy normative values.(74) Specifically, patients with stage 3-5 CKD are reported to have VO_2peak levels 50-80% of the VO_2peak of healthy individuals.(75) Changes in body composition, specifically an increase in adiposity and a decrease in lean mass, are common in the pathogenesis of kidney disease and has likely influences on exercise capacity.(76) A limited number of studies have looked at the effects of exercise training in improving cardiorespiratory fitness in CKD patients, with mixed protocols and subsequently varied results.(36-40, 77) It is not known whether the increase in cardiorespiratory fitness is influencing the development of cardiovascular disease, morbidity or mortality rates. Nonetheless, considering a 1 ml/kg/min increase in VO_2peak has been associated with an approximate 15% decrease in risk of death in patients it seems likely that the improvements in cardiorespiratory fitness in the reviewed studies in CKD patients are influencing mortality rates.(78) However, in order to influence future health outcomes, it is important to assess whether these improvements in cardiorespiratory fitness and exercise capacity can be maintained with a long-term exercise training program.

Due to the limited research on the effects of exercise training in improving cardiorespiratory fitness in CKD patients, cardiorespiratory fitness is an important outcome of this thesis. The LM3 study investigates the effects of cardiorespiratory fitness and exercise capacity on aspects of cardiovascular health over 3 years.

1.3.6 Oxidative stress

One of the aims of this thesis is to investigate the contribution of oxidative stress to reduced strength and lean mass. Elevated oxidative stress levels have been reported in CKD and are associated with poor clinical outcomes.(79) Oxidative stress occurs when the reactive oxidative species (ROS) outbalances total antioxidant capacity.(80) The increase in oxidative stress in CKD patients is due in part to two reasons. Firstly, CKD patients have impaired endogenous production of antioxidants, which disturbs the homeostatic balance of anti and

pro-oxidants.(81) Secondly, there is an increase in ROS. One of the ways ROS is formed, is as a byproduct of leaked electrons in the electron transport system of the mitochondria. Nicotinamide adenine dinucleotide phosphate, is an electron carrier (Nox-4 in particular) and is most abundantly expressed in the kidneys.(82) Upregulation of Nox-4 by uraemia from kidney dysfunction results in electron leaking in mitochondria and increased ROS production.(83)

Although it is likely that the uraemic environment is promoting oxidative stress, in the reverse situation high levels of oxidative stress may also be advancing kidney dysfunction. As elevated oxidative stress levels contributes to mitochondrial dysfunction, and as the kidneys rely heavily on aerobic metabolism, a decrease in the adenosine triphosphate produced may result in renal cell apoptosis. It is also likely that mitochondrial dysfunction would contribute to the reduced exercise capacity common in this population. The contribution of oxidative stress to the reduced strength which is common in CKD patients is investigated in Chapter 3. It has been reported that exercise training can improve anti-oxidant status (as measured by glutathione peroxidase) and lipid peroxidation (as measured by malondialdehyde and 4-hydroxalkenals) in patients with CKD.(39) Whether this also improves the gold standard oxidative stress biomarker, F2-isoprostanes, is yet to be established. Our group is currently examining the change in oxidative stress parameters after 12 months of the LM3 study in patients identified as having elevated levels of F2-isoprostanes, however these findings are not published in this thesis. Also, the contribution of oxidative stress to autonomic dysfunction is discussed in Appendix 11.8.

1.3.7 Haemoglobin

An aim of this thesis is to identify determinants of participating in higher intensity exercise. It seems likely that the fatigue that occurs from low haemoglobin may limit the motivation to participate in high intensity exercise. Anaemia is prevalent in CKD and may also contribute to the reduced exercise capacity that is connected with this population. Reduced haemoglobin can limit VO_2max, through the reduced oxygen-carrying capacity of the blood.(84, 85) The kidneys synthesise and secrete most of the body's endogenous erythropoietin (EPO)- the hormone that regulates red blood cell production.(86) EPO travels to the bone marrow to stimulate the differentiation and proliferation of erythroid progenitor cells. As a result EPO causes an increase in circulating red blood cell concentration. In CKD patients, the release

and synthesis of EPO is impaired by the damaged kidneys- leading to a decrease in red blood cell production, with the low haemoglobin resulting in subsequent anaemia.(87) The degree of renal impairment affects the likelihood of a CKD patient developing anaemia. The prevalence of anaemia in patients with CKD increases as the GFR progressively falls. McClellan et al. (2004) identified the common occurrence of anaemia in 5222 males and females with CKD, with 47.7% of patients with haemoglobin <12 g/dL.(88) It has been reported that in 5 European countries (n=4591), 60% of haemodialysis patients were administered intravenous iron and 75% were administered EPO.(89)

The fatigue that occurs in lower haemoglobin levels may influence the inclination to participate in higher intensity exercise. A few studies have indirectly assessed the influence of haemoglobin on physical performance. Leikis et al. (2006), followed 12 stage 3-4 CKD patients over two years and identified that VO_2peak declined, alongside renal function, despite maintenance of haemoglobin levels.(90) This suggests that anaemia is not the only factor influencing exercise performance. However, the haemoglobin levels were considerably high in this study for a CKD cohort (12.9±9.0 g/dL). Likewise, Kaysen et al. (2011) found haemoglobin to be inversely related with physical function questionnaire but not Short Physical Performance Battery.(91) Similarly, studies by Painter et al. (1994) and Marrades et al. (1996) found minimal improvements in aerobic exercise capacity despite significant increases in haemoglobin concentration with EPO therapy.(92, 93) Furthermore, the study by Marrades et al. found an increase in arterial O_2 content of 59%, with only 33% increase in VO_2 in the leg at peak exercise, due to a fall in femoral venous blood flow. This may indicate an alteration in the microvasculature of renal patients, which is reducing the ability to increase exercise capacity even after EPO therapy. Nonetheless, exercise training may augment the uptake of oxygen in the muscle, thus complementing EPO therapy.(92) On the other hand, McMahon et al. (1999) identified that by normalizing haemoglobin levels in end stage renal failure patients with lower than normal exercise capacity, they were able to substantially improve exercise performance.(94) Although it was identified by the authors that there was a distinct advantage in achieving the target haemoglobin, values were still below age-matched sedentary controls. Mancini et al. (2003) also found a significant increase in VO_2peak after 3 months of EPO therapy compared to a control group.(95) It is unclear whether low haemoglobin levels limit the likelihood of a patient with CKD performing vigorous or high intensity exercise training.

It has been suggested that exercise training can increase total haemoglobin and red cell mass by stimulating erythropoiesis with hyperplasia of the haematopoietic bone marrow.(96) However, the effects of exercise training on counteracting haemoglobin have been controversial.(96) Furthermore, it is not clear whether exercise can upregulate EPO in patients with kidney damage. Indeed, only two exercise studies in CKD patients have reported haemoglobin levels and both found no change after exercise training.(38, 51) Chapter 4 identifies the influence of lower haemoglobin on the ability to undertake high intensity exercise. Chapter 6 also explores the change in haemoglobin over the 36 month exercise training period.

1.3.8 Other blood biochemistry

Dyslipidaemia is a risk factor for kidney disease progression suggested to occur by increased ROS induced activation of nuclear kappa factor B.(97) This subsequently causes elevation of interleukin-6 (IL-6), which causes tissue injury through glomerulosclerosis.(98) A meta-analysis on ten aerobic exercise training studies (n=1 260) in patients with cardiovascular disease reported increases in high-density lipoproteins (HDL) and decreases in triglycerides (TG), with no change in total cholesterol (TC) and low density lipoprotein (LDL).(99) Toyama et al. (2010) found an increase in HDL and a decrease in TG and LDL after aerobic exercise.(37) Conversely, Headley et al. (2012) found that LDL and TG were actually increased after 48 weeks of combination exercise training, despite no difference in dietary intake between the exercise and control groups.(55) Eidemak et al. (1997) also identified a significant increase in TC in the exercise group.(36) However, this study doesn't report change in body weight, which may have influenced the findings. On the other hand, the aerobic exercise studies by Boyce et al. (1997), Leehey et al. (2009) and Pechter et al. (2003) found no change in blood lipids.(38-40) Chapter 6 of this thesis explores the changes in the blood lipid profile over 36 months.

Progression of kidney dysfunction appears to cause elevated inflammatory cytokines, which can contribute to a number of metabolic disease processes.(100) Indeed, our group has previously shown a positive association between uraemic toxins and elevated inflammation markers.(101) There is a potential for an increase in insulin growth factor (IGF)-1 to provide an anabolic effect in CKD patients.(102) However, there is a variable response of IGF-1 to exercise training in other populations.(103) Chapter 6 explores the change in inflammation as

measured by CRP over the 36 month exercise training period. Inflammation has also been suggested to contribute to muscle wasting in the CKD population. Chapter 3 investigates the contribution of inflammation markers (IL-6, tumour necrosis factor- α [TNF-α], interferon- γ [IFN-γ] and CRP) alongside other variables, to reduced strength in CKD patients.

1.3.9 Body composition

Body composition has been suggested to be a significant contributor in the reduction of exercise capacity in CKD.(104) A decline in kidney function is often associated with impaired muscle protein leading to skeletal muscle atrophy and a resultant loss in strength. (105, 106) Decreases in strength can greatly impact on functionality and the ability to perform daily tasks with ease. The findings from Chapter 6 highlight the influence of strength on reduced exercise capacity. Reduced functional strength may be a significant contributor to the low quality of life and depression that is consistently reported in this population. (107) Furthermore, skeletal muscle atrophy is associated with a 3-fold increase in mortality over a 4-6 year period in dialysis patients.(108) Suggested factors in the development of muscle wasting in CKD are metabolic acidosis, excess angiotensin II and inflammation.(106) As previously mentioned, one mechanistic pathway of muscle atrophy may be through increased oxidative stress. The increase ROS inhibits the phosphorylation of Akt (a protein kinase)(109) which in turn reduces the phosphorylation of the forkhead transcription factors. This increases the expression of the E3 ligase, atrogin-1/muscle atrophy F-box.(105) The increase in atrogin-1/muscle atrophy F-box recognises specific muscle proteins to increase their degradation by the UPS.(110, 111) In turn, this increase in degradation results in muscle atrophy.(112)

Increases in body mass can also contribute to the progression of kidney dysfunction through a rise in metabolic waste. Consequently there is hyperfiltration of the nephrons, which ultimately leads to nephron loss. However, this hyperfiltration of the glomerulus has been shown to improve with weight loss.(113) The change in body composition over 3 years in CKD is an important outcome in this thesis, particularly identifying the effects of an exercise intervention on lean mass and body fat percentage.

1.3.10 Autonomic function

An aim of this thesis is to investigate the change in autonomic function after an exercise training intervention. It has been suggested that a significant contributor to sudden cardiac death in the kidney disease population may be autonomic dysfunction.(114) Heart rate variability (HRV) can be used to non-invasively measure autonomic function. Autonomic dysfunction is thought to be more prevalent in the CKD population through damage caused by uraemic neuropathy.(115) The combination of uraemic neuropathy in CKD and glycaemic neuropathy in diabetes has been identified by lower HRV in patients with CKD and diabetes compared to patients with just CKD. (Appendix 11.7).(116) Whether or not the combination of uraemic and glycaemic neuropathy in patients with both CKD *and* diabetes is more resistant to improvements with exercise training is yet to be studied. A study looking at the initiation of haemodialysis in improving HRV in patients with and without diabetes found significant changes in CKD patients without diabetes, with no changes found in CKD patients with diabetes.(116) Exercise studies have shown significant increases in HRV, and therefore autonomic function, in both uraemic haemodialysis patients (117) and diabetes (118). Exercise training may reduce sympathetic dominance. As sympathetic dominance is associated with increased ventricular ectopic frequency, the shift in sympathovagal balance with regular exercise training may inhibit dangerous arrhythmias.(119) Headley et al. (2012) identified that 48 weeks of combination exercise preserved heart rate recovery, whereas the usual care group showed a significant decline in the same measure.(55) The authors also identified a significant reduction in resting and ambulatory heart rate after the exercise intervention which was not identified in the usual care group. These findings suggest a favourable influence of exercise training on parasympathetic tone. Chapter 6 looks at the change in HRV time parameters Standard deviation of the N-N interval (SDNN) and Root mean square of the standard deviation (RMSSD) and frequency parameters total power (TP), very low frequency (VLF), low frequency (LF) and high frequency (HF) after 36 months of exercise training. Appendix 11.9 looks at the longitudinal effects of an exercise intervention on HRV, heart rate recovery and chronotropic incompetence in intervention and control patients.

1.3.11 Arterial stiffness and hypertension

Whether a change in arterial stiffness occurs after both three years of combination exercise training and 12 weeks of HIIT is another aim of this thesis. The increased risk of mortality in

CKD patients may be explained by the predisposition of patients to vascular calcification. The prevalence and severity of vascular calcification is reported to increase with progressive worsening of kidney function.(120) Disordered bone mineral metabolism occurs in kidney dysfunction and results in hypercalcaemia, hyperphosphatemia, and hyperparathyroidism.(121, 122) If plaque exists through atherosclerosis then hypercalcaemia will promote its calcification.(121) Vascular calcification thickens the arterial wall and causes a loss of elasticity, or, arterial stiffness. Arterial stiffness is an important prognostic marker of mortality as it influences left ventricular hypertrophy, coronary artery disease and increases the likelihood of a cardiac event occurring.(123) The translation of vascular calcification into arterial stiffness can be measured by pulse wave velocity (PWV) and augmentation index (AIx) by pulse wave analysis (PWA).(124) Mustata et al. (2011) found a 12 month exercise intervention to improve AIx.(77) Although, PWV was not measured in this study (and is considered the gold standard measure of non-invasive arterial stiffness measures), this reduction in AIx is a promising indicator of the benefits of exercise training on arterial stiffness.

Hypertension has a bi-directional involvement in the pathophysiology of CKD. Hypertension subsequent to kidney dysfunction occurs as a result of a positive sodium balance causing elevated extracellular fluid volume. The increased extracellular volume tracks with a decrease in eGFR.(125). Arguably, one of the most important cardiovascular modifications in treating CKD is controlling blood pressure. PWA is a technique which allows calculation of central blood pressure at the ascending aorta by using applanation tonometry of the radial artery. It has previously been shown that 20 weeks of progressive resistance training is effective in reducing central blood pressure in older adults.(126) However, the effects of exercise training on central blood pressure in CKD patients has not been investigated. Measurement of arterial stiffness (PWV and AIx) and central blood pressure (PWA) was assessed every year in the 3 year LI study (Chapter 6) as well as before and after 12 weeks of HIIT (Chapter 8), to determine if long term exercise training improves these ‘non- traditional’ cardiovascular disease risk factors.

1.3.12 Safety of exercise training

As this thesis contains a focus on high intensity exercise training and this has previously been suggested to be unsafe for certain individuals,(127) it is important to investigate the safety of

this form of training in CKD patients (Chapter 4, 7 and 8). In a position stand by Exercise and Sports Science Australia (ESSA), it is reported that the weight of evidence suggests it is safe for people with CKD to exercise and that the risk associated with remaining inactive is far greater.(30) Indeed, Heiwe and Jacobson et al. (2011) report that no fatal or serious exercise related adverse events have been reported by exercise studies.(31) Although, this finding may be attributable to many exercise studies excluding CKD patients with cardiovascular disease. Before commencing an exercise program it is suggested that patients first undergo a thorough medical review including assessment of: blood pressure, medication usage, biochemistry, haematology and 12-lead electrocardiogram (ECG) exercise testing. It is suggested by ESSA that in addition to the American College of Cardiology Foundation/ American Heart Association(128) reported contraindications to exercise, specific contraindications to CKD should be: electrolyte abnormalities, in particular hypo/hyperkalaemia, pulmonary congestion and peripheral oedema.

1.3.13 Multi-disciplinary treatment of CKD

There are a number of cardiovascular risk factors which can progress renal damage. Indeed, risk factors such as obesity, poor blood glucose control, sarcopenia and low cardiorespiratory fitness are difficult for Nephrologists or Physicians to effectively treat alone. Individualized multi-disciplinary care, often nurse-led, can promote self-care and modify negative lifestyle habits, which in turn ameliorates renal function decline and reduces the risk of future cardiovascular disease.(129) Studies on multi-disciplinary care in patients with CKD have had varied results. Three studies have shown no improvement in renal function, mortality or cardiovascular risk factors.(130-132) However, only one of these multi-disciplinary teams included a dietician and none included an exercise physiologist. On the other hand, two randomized control trials have shown improved survival (133) and slower decline in eGFR with multi-disciplinary management.(134) Again, neither of these trials included exercise training support. Furthermore, a questionnaire completed by 142 multi-disciplinary renal care professionals indicated that only 42% of professionals discussed and encouraged physical activity and only 18% actually facilitated physical activity implementation.(135) A multi-disciplinary team including professionals such as a nurse practitioner, dietitian, exercise physiologist, diabetic educator, psychologist and social worker, has the potential to

specifically target many of the risk factors associated with decline in renal function and progression of cardiovascular disease in CKD patients, rather than by treatment from a Nephrologist alone. However, the literature indicates inclusion of an exercise professional in these multi-disciplinary teams has previously been omitted. Thus, the addition of exercise support within multi-disciplinary care in this thesis is novel in patients with CKD. This thesis focusses specifically on the exercise training prescription and fitness related outcomes of the presented study.

1.3.14 Summary of exercise training in CKD patients

The effects of aerobic training, resistance training and a combination of the both have been investigated in 21 published studies from 14 trials with 453 participants with CKD. Of these 14 trials, 50% are RCT's. Despite exercise guidelines for CKD patients recommending a combination of aerobic and resistance exercise for the greatest health benefits,(30) only four trials have used this type of training, with only one RCT.(54, 55) The majority of studies excluded patients with cardiovascular disease or did not report inclusion or exclusion criteria. Due to the high prevalence of cardiovascular disease in this population, this significantly limits the generalizability of the findings. The heterogeneity of the protocols of the reviewed studies makes it difficult to compare the mean differences between groups. However, the weight of evidence suggests that both aerobic and a combination of aerobic and resistance exercise improves cardiorespiratory fitness and exercise capacity. Furthermore, the two studies which did not show increases in VO_2peak had methodological limitations. Considering the well-recognized correlation between cardiorespiratory fitness and mortality in other populations,(78) the robust increases in VO_2peak would suggest that the exercise-induced adaptations are providing significant health benefits to CKD patients.

The majority of studies which utilized resistance training interventions indicated an improvement in muscle strength, function and size. These findings suggest that resistance training does have the potential to ameliorate the muscle atrophy which is common in this population. Although the research is limited, it seems reasonable to propose that exercise training has the potential to improve other cardiovascular disease risk factors such as arterial stiffness, blood pressure, blood lipids, oxidative stress and inflammation. There also seems to be a benefit to quality of life and functional ability. However, the effects of exercise training on kidney function, the progression and development of cardiovascular disease, morbidity

and mortality are still unknown. The majority of findings suggest that kidney function is not affected by exercise training, although the sample size needed (n>1000)(136) to adequately assess this change has not been studied. Although the benefits of exercise training in CKD reported in the literature are promising, significantly more research is needed to delineate the effects of exercise on the above outcomes.

The diverse protocols in the studies reviewed make it difficult to ascertain an optimal exercise prescription for CKD patients. However, in line with current guidelines and the above studies, a study investigating exercise training in CKD patients should consist of a combination of aerobic and resistance exercise to elicit the greatest health outcomes.

1.3.15 Chapter summary

The review of the literature in this chapter has identified the high prevalence of cardiovascular disease risk factors in CKD. Considering the high rate of cardiovascular events in this population, it seems that the current therapeutic targets to improve the prognosis of CKD are ineffectual. Exercise therapy is a currently underutilized strategy in improving health outcomes in CKD patients, in particular compared to other chronic diseases. As such, exercise training has great potential to play a significant role in standard clinical treatment. Large randomized control trials are lacking in the CKD population and thus exercise guidelines in these patients are based on limited evidence. Many CKD clinical guidelines suggest that regular exercise training should be included in standard patient management. However it seems that specific advice and recommendations are deficient, which is likely contributing to the high sedentary rates of CKD patients. This chapter explored risk factors of both kidney disease progression and cardiovascular disease and the potential benefit exercise training may have in ameliorating these risks. The chapter also identified previous exercise trials in CKD patients, and how the current thesis can fill the gaps of missing research areas.

SECTION 1- Landmark III

Chapter 2.
Methods

2.1 Introduction

This chapter describes the protocols and outcome measures from Section 1, the Landmark III study (**L**ongitudinal **A**ssessment of **N**umerous **D**iscrete **M**odifications of **A**therosclerotic **R**isk in **K**idney disease). All measures were performed according to the standard operating procedures at the Cardiovascular Imaging Research Group at the Princess Alexandra Hospital. The general methods are described in detail below, and methods specific to individual chapters are described in each relevant chapter. The methods specific to the HIIT study only are described in Chapter 8.

2.1.1 Study 1

The LM3 study is described herein as Study 1. The study was registered at the Australian and New Zealand Clinical Trials Registry- www.anzctr.org.au (Registration Number ANZCTR12608000337370). The primary outcomes of the study were to limit the progression of cardiovascular disease by targeting cardiovascular risk factors with exercise training and lifestyle modification. The study was powered to assess change in atherosclerotic burden, measured by carotid intima media thickness. This was assessed by ultrasound imaging in three planes. Secondary outcomes were development of new areas of poor heart flow measured by stress echocardiography and endothelial function measured by brachial artery reactivity. These measures are not included in this thesis. The primary outcome for this thesis is the change in VO_2peak over three years.

2.1.2 Study population

Participants were recruited from the Nephrology Department at the Princess Alexandra Hospital, and Logan Hospital, Brisbane, Australia. The patient information and consent form is attached in Appendix 11.1. Unlike previous exercise studies in CKD patients, participants from this study were not excluded if they had cardiovascular co-morbidities, thereby ensuring the studied cohort is generalizable to the CKD population. At baseline the cohort was generalizable to the stage 3-4 CKD population. Although no population data exists for stage 3-4 CKD patients in Australia, a cohort study in Spain had similar baseline values for age (Spain vs. Australia 68±13 vs. 60.4±8.7 years), BMI (28.4±4.9 vs. 31.9[7] m/kg^2), diabetes (40.8 vs. 40.5%), eGFR MDRD (28±8 vs. 39.3±8.2 ml/min/1.73m^2), hypertension (92.7 vs.

94.8%), haemoglobin (128±1.6 vs. 133.8±15.3 g/L), resting systolic blood pressure (141±19 vs. 138.4[29.7] mmHg) and resting diastolic blood pressure (76±11 vs. 80.2[14.5] mmHg).

From both the Princess Alexandra Hospital and the Logan Hospital, 2 231 CKD patients were screened for eligibility for the LM3 study. From the 2 231 patients screened; 1 392 were considered ineligible based on the below inclusion and exclusion criteria; 416 were monitored for potential future participation; 236 declined participation and 187 were ultimately randomized to the study. Therefore, from the initial screening, 8.4% of participants were randomized to participate in LM3. The final participation numbers for each analysis is outlined in each chapter in Section 1.

2.1.3 Study design

LM3 was a three-year prospective open-labelled trial assessing nurse-led multi-disciplinary care versus standard nephrological care. It was a multi-centre trial, with the LI taking place at either the Princess Alexandra Hospital or Logan Hospital. After obtaining written informed consent, participants attended the Princess Alexandra Hospital for baseline testing.

2.1.4 Randomization

Participants were randomized into one of two arms after baseline testing- the lifestyle intervention (LI) or standard care in a ratio of 1:1 by the Clinical Trial Co-ordinator using a computer random assignment program. A person external to the study also witnessed the randomization of all participants. Randomization contained 3 levels of stratification: renal function (eGFR high [>44 ml/min/1.73m^2] or low [≤44]), sex and diabetes status (yes or no). Eligibility for inclusion was confirmed through baseline testing. Any participants with cardiac abnormalities identified at baseline testing were referred to a Cardiologist for a clinical decision regarding his/her appropriateness to be included before randomization. The Exercise Physiologists in the program were not blinded, as they provided the exercise delivery and the majority of data collection.

2.1.5 Inclusion criteria

Subjects with stage 3 or 4 CKD (modification of diet in renal disease [MDRD] eGFR 25-60 ml/min/1.73m^2) were included. Participants were aged 18 to 75 years and had at least one of the following risk factors at the time of enrolment – blood pressure or lipids not at target; overweight (body mass index [BMI] >25 kg/m^2); and poor diabetic control (haemoglobin A1c >7%).

2.1.6 Exclusion criteria

Intervention for, or, symptomatic coronary artery disease (within 3 months), current heart failure (New York Heart Association class III and IV), significant valvular heart disease, Cardiologist deemed not suitable, pregnant or planning to become pregnant and life expectancy or anticipated time to dialysis or organ transplant <6 months.

2.1.7 Ethics

The study protocol was approved by the Princess Alexandra Human Research Ethics Committee (HREC 2007/190). All patients gave written, informed consent to participate in this study.

2.1.8 Lifestyle Intervention

In addition to the nephrological and general practitioner care provided to CKD patients, intervention participants were also treated by a multidisciplinary team consisting of Exercise Physiologist, Nurse Practitioner, Dietician, Psychologist and Diabetic Educator. A Social Worker was also available if needed. Participants trained for eight weeks for a minimum of two times per week with an Accredited Exercise Physiologist. After the eight weeks of gym based training, patients completed a home program for 34 months with regular gym refresher sessions, and regular follow-up via telephone phone calls and/or email exchange.

Exercise prescription included a combination of aerobic and resistance training and was individualised (based on co-morbidities, motivation and interests) so as to promote the best adherence to the program and to optimise fitness adaptations. In line with ACSM exercise

training guidelines for older adults, it was the goal of the program for patients to achieve at least 150 minutes of moderate intensity exercise and two sessions of resistance training per week.(33) Additionally, higher intensity exercise was encouraged where possible. The initial gym session with the Exercise Physiologist included goal setting, establishing previous exercise experience and enjoyed activities, and identifying any potential barriers to exercise. A typical gym session consisted of 20-30 minutes of aerobic training on the treadmill or bike, 20 minutes of whole body resistance training followed by another ten minutes of aerobic training on the bike or rower. The first four weeks of the program was focussed on building confidence with a variety of different exercises, improving fitness and educating patients on the benefits of exercise training. The following two weeks were concentrated on exercises that could be incorporated into a home program based on any equipment the patient may already have, or useful fixtures around the house (ie. steps or benches). The final two weeks of the gym based sessions were predominantly patient led, to ensure their clarity and understanding of the correct technique for the home-program. During the gym based sessions, resistance exercises were based on theraband and swiss ball exercises, so familiarity with this equipment could be attained before use in the home program. On completion of the eight week gym-based training, participants were provided with the necessary tools (swiss ball and theraband) and education (a home program and an exercise booklet with different exercise, tips and goals) to become as physically active as possible. A typical home program comprised of whole body resistance training exercises, utilizing the swiss ball and theraband, for approximately 20-30 minutes, 2-3 days per week, with moderate to high intensity aerobic exercise on most days of the week. The use of the thera-band and swiss ball decreases the need for expensive resistance training equipment and allows home-based training. Again, the exercises as well as the intensity, was individualized according to each patient's condition, home equipment and interests. The telephone follow-up calls were completed approximately every week for the first three months, every fortnight from month 6-8 and once a month thereafter, or as needed. Regular gym refresher sessions were also offered to participants to utilize as needed ie. (a range from once a week to once every 6 months, depending on the participants needs and inclination). The telephone follow-up calls and gym refresher sessions were an opportunity to increase motivation to exercise by modifying the exercise prescription as appropriate as well as encouraging accountability through the regular check-ins. Furthermore, the telephone follow-ups calls allowed an opportunity to address any relevant disruptions or barriers to exercising and provide suggestions to increase adherence.

In the initial four weeks, patients also attended weekly Dietician and Psychologist group sessions. The focus of these sessions were on behaviour and lifestyle change to assist weight loss. The weekly topics were- 1) goal setting, guide to a healthy diet and self-monitoring, 2) cholesterol, fats, sugars, salt and healthy meal plans, 3) motivation to change, 4) carbohydrate, glycaemic index, label reading, recipe modification and shopping trolley. A workbook was also provided to the participants with the discussed topics and self-monitoring exercises to assist with evaluation and goal setting. Subsequent to the 4 week program, regular contact with the dietician was made both in person and on the telephone as necessary. Furthermore, quarterly appointments with the nurse practitioner occurred for the remainder of the intervention. Patients also had access to a social worker, diabetes educator and psychologist as required.

2.1.9 Control

Patients randomized to the control group underwent standard nephrological care according to clinical guidelines. Medications were prescribed by physicians as needed and patients were referred to other health professions or specialists as required.

2.1.10 Trial visits

Participants were required to attend 7 trial visits for testing throughout the research program (Figure 2.1). At baseline, 12 months, 24 months and 36 months participants underwent a full battery of tests. These were: blood analyses, anthropometric measures, dual-energy x-ray absorptiometry (DEXA), heart rate variability (HRV), pulse wave velocity (PWV), pulse wave analysis (PWA), resting echocardiography, stress echocardiography, endothelial structure and function (carotid intima media thickness and brachial artery reactivity), peak oxygen uptake (VO_2peak), physical activity questionnaire (Appendix 11.2) and functional tests. The echocardiography and endothelial function measures are not included in this thesis. Functional tests consisted of hand grip strength, the get up and go test and 6 minute walk test. Functional tests were completed every six months, so in addition to the four above mentioned visits, they were also performed at 6 months, 18 months and 30 months. Likewise, blood analyses, physical activity questionnaire, blood pressure and anthropometric measures were also performed at every six month visit. Tests were performed in the same order for every participant at each visit.

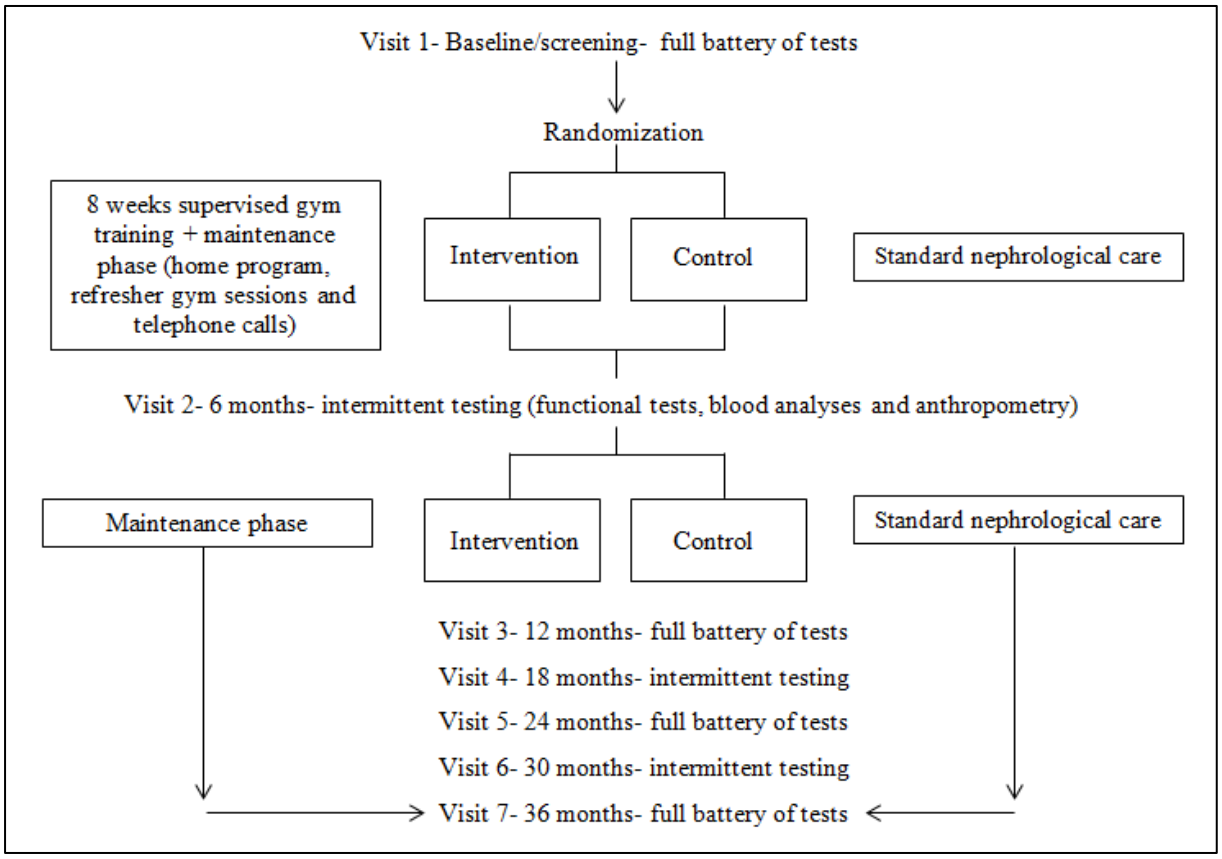

Figure 2.1 Timeline of trial visits for study 1

2.1.11 Patient history

Previous history of cardiovascular disease risk factors such as diabetes status, hyperlipidaemia and hypertension was based on patient's hospital files. The presence of a prior cardiac event was defined as being either: 1) non-fatal myocardial infarction, defined as elevated cardiac enzymes such as creatine kinase and troponin-1 alongside either, typical symptoms, development of pathological Q waves, acute ischaemic changes evident on ECG, or coronary intervention, 2) angina, 3) coronary artery bypass graft or 4) percutaneous coronary intervention. Peripheral vascular disease was defined as typical claudication pain, history of angioplasty, bypass or amputation. Ischaemic heart disease (IHD) was defined as previous MI or angina.

2.1.12 Medications

Medication usage was recorded at each visit, however dosage was not documented.

2.1.13 Physical Activity

The self- reported Active Australia questionnaire (Appendix 11.2) was used to assess average weekly physical activity levels from the preceding 6 months.(137) Questions in the Active Australia used in the data analysis are; average time weekly, in the past 6 months, for time spent walking (for at least 10 minutes without stopping), time spent doing moderate intensity activity and time spent doing vigorous activity. Questions on the previous weeks activity were also asked, however due to the possibility of reporting a non-representative week, the average week in the past 6 months was used for analysis. The questions were asked by an Accredited Exercise Physiologist to avoid any misunderstanding and clarity was checked on each question. Participants were required to provide details of the activity that they reported in each of the three categories (walking, moderate and/or vigorous) in order to monitor any over-representation of an activity. Standardized examples for each category (eg. moderate = gentle swimming/social tennis/golf; vigorous = jogging/cycling/aerobics/competitive tennis) were provided to the participant as suggested on the Active Australia questionnaire. The total physical activity time is the sum of walking time, moderate intensity exercise and vigorous intensity exercise. According to the International Physical Activity Questionnaire, metabolic equivalent tasks (MET) intensities were calculated by time in each intensity multiplied by either 8 (vigorous), 4 (moderate) or 3.3 (walking), respectively (www.ipaq.ki.se). Total MET minutes is calculated by the addition of vigorous MET minutes, moderate MET minutes and walking MET minutes. The Active Australia questionnaire has been reported to have good validity and reliability in population studies,(138) overweight men and women,(139) middle aged women(140) and community-dwelling older adults.(137) However, this measure has not been validated in the CKD population.

2.1.14 Exercise capacity

The test protocol was determined by the Duke Activity Status Index (Appendix 11.3), which was completed by the participants.(141) The Duke Activity Status Index has previously been established as a reliable estimate of exercise capacity in patients with stage 3-4 CKD, particularly when eGFR is <35 ml/min/1.73m^2.(142) Based on participant's responses to this

questionnaire they performed either the Bruce (estimated VO_2peak >34.66 ml/kg/min), Balke (estimated VO_2peak 18.38-34.65 ml/kg/min) or Naughton (estimated VO_2peak <18.37 ml/kg/min) protocols (Table 2.1). The determination of the appropriate protocol was based on an estimated time on the treadmill of approximately 10 minutes.(143) The same protocol was then used for each subsequent visit.

Table 2.1 Bruce, Balke and Naughton treadmill protocols

Stage	Time (mins)	Speed (km/hr)	Grade (%)
Bruce (144)			
1	3	2.7	10
2	3	4	12
3	3	5.5	14
4	3	6.8	16
5	3	8	18
6	3	8.9	20
Balke (145)			
1	2	5.5	2
2	1	5.5	3
3	1	5.5	4
4	1	5.5	5
5	1	5.5	6
6	1	5.5	7
7	1	5.5	8
8	1	5.5	9
9	1	5.5	10
10	1	5.5	11
11	1	5.5	12
12	1	5.5	13
Naughton (146)			
1	2	1.6	0
2	2	3.2	0
3	2	3.2	3.5
4	2	3.2	7

5	2	3.2	10.5
6	2	3.2	14
7	2	3.2	17.5
8	2	3.2	22
9	2	3.2	25.5
10	2	3.2	29

A graded maximal treadmill test was used to assess exercise capacity (estimated METs) with concurrent 12-lead ECG monitoring. METs were provided by the treadmill software (CASE V6.51, GE Medical Systems, Milwaukee, WI, USA) from the treadmill speed and incline at completion of the test. The final MET value was calculated by linear interpolation of the preceeding and proceeding MET stage values. The formula used to evaluate estimated METs was (147):

$$\text{Estimated METs} = \frac{(\text{speed} \times 26.8 \times 0.1) + (\text{grade}/100 \times 1.8 \times \text{speed} \times 26.8) + 3.5}{3.5}$$

Where: Speed=miles/hour

Grade=percent

2.1.15 Maximal oxygen uptake

Cardiorespiratory fitness was assessed by maximal oxygen uptake (VO_2peak) by breath by breath indirect calorimetry (Vmax29c, SensorMedics, Yorba Linda, CA, USA) using the peak 20 second average of the final minute of exercise. Gas analysers and the flow meter were calibrated before each use. Achieved VO_2peak was compared to predicted normative data for women, [VO_2 peak = (14.7 – (0.13 * age))*3.5],(148) and men [VO_2 peak = (18.4 – (0.16 * age))*3.5].(29)

2.1.16 Six minute walk test

The six minute walk test provided a measure of functional capacity. The six minute walk test is a quick and inexpensive performance based measure and reflects the ability to undertake day to day activities.(149) Specifically, it was assessed by the total distance walked in six

minutes over a 20m track. Participants were required to cross the 20m line with both feet before turning around to complete the next lap. If necessary, a trundle wheel was used to measure the additional distance between laps on cessation of the test at six minutes. The use of walking aids were allowed if essential to completion of the test and it was ensured that the same conditions were adhered to at each visit.

2.1.17 Get up and Go test

Muscular power and agility was assessed using the get up and go test, as per the instructions outlined in Hruda et al. (2003).(150) Participants were timed as to how long it took them to rise from an un-armed chair (without using their arms to push up from their legs) and walk three metres, cross a line with both feet, turn around and sit back down again. The test was performed three times and the fastest time was used for future analyses.

2.1.18 Grip strength

Grip strength was measured using a hand grip dynamometer (Jamar 5030 J1, Bolingbrook, IL, US). Each participant received the same instructions after a demonstration by the tester, and the hand grip was adjusted accordingly for the participants comfort. Participants undertook the test 6 times, alternating between each hand, with an approximate 30 second rest between each trial. The maximum grip strength was attained from the highest reading of either hand. Age predicted grip strength values were calculated by a predictive equation for males and females reported by Desrosiers et al. (1994).(151)

2.1.19 Anthropometry

Anthropometric measures were made at each 6 month visit. Height (centimetres) and weight (kilograms) were recorded according to standard techniques. Waist and hip measurements were attained, to the nearest 0.5 centimetres. The waist was identified as the midpoint between the bottom of the lowest rib and the iliac crest. The hip measurement was taken standing with feet together at the greatest protuberance of the buttocks.

2.1.20 Lean Mass

DEXA, using whole body composition analysis was used to assess body fat percentage and lean mass (Hologic QDR 4500A Version 12.6, Massachusetts, USA). Percentage of lean mass was calculated as the proportion of lean mass to total mass. Appendicular lean mass was calculated from the average of the 4 limbs. The DEXA underwent verification using a spine phantom before each day of testing and step phantom before each week of testing. Analysis of each scan was performed according to Hologic landmarks. There was a period of time during the study where the DEXA machine could not be used due to software issues. As such, DEXA scans were not performed on all patients at all visits. The number of patients who had DEXA's performed is indicated in each individual chapter. Regardless, as the period of time the DEXA was not in use was at random and patient recruitment is staggered over the 5 years, the patients who did undergo DEXA's scans are likely to be representative of the whole cohort.

2.1.21 Blood biochemistry

Blood biochemistry was assessed from a sample obtained by venipuncture after an overnight fast. Serum and plasma vacutainers (BD vacutainers, Franklin Lakes, NJ, USA) were used to collect 10 mL venous blood samples following an overnight fast. Ethylenediaminetetraacetic acid vacutainers (BD vacutainers, Franklin Lakes, NJ, USA) were used to collect blood samples for oxidative stress measures. Haemoglobin, phosphate, creatinine, corrected calcium, albumin, urea, lipids, bicarbonate, glucose, and insulin were measured using standard techniques in a National Association of Testing Authorities accredited pathology laboratory at the Princess Alexandra Hospital. Insulin resistance was computed using the homeostatic model assessment of insulin resistance method (HOMA-IR).(152) The formula used is:

$$\text{HOMA-IR} = \frac{\text{Glucose (mmol/L)} \times \text{insulin}}{22.5}$$

Fasting blood samples were performed prior to any other testing. Beta-blocker medication was withheld the morning of testing.

2.1.22 Oxidative stress

Samples were stored on ice before being centrifuged at 2500 rpm for 10 min. Plasma was stored at -80°C with butylated hydroxytoluene (10 µL of 100 mM to each 1.5 mL eppendorf tube) to prevent artefactual oxidation.

Total F2-isoprostanes

Samples were analysed in duplicate using a method developed in our laboratory.(153) Total F2-isoprostanes were extracted from plasma after saponification with methanolic NaOH. Samples were spiked with 8-iso-PGF2α-d4 (Cayman Chemicals, Ann Arbor, MI, USA) as an internal standard and incubated at 42°C for 60 minutes. Samples were then acidified to pH 3 with hydrochloric acid, and hexane added and samples mixed for 10 minutes before centrifugation. The supernatant was removed and the remaining solution extracted with ethyl acetate and dried under nitrogen. Samples were reconstituted with acetonitrile, transferred into vials with silanized glass inserts and dried. Derivatization with pentafluorobenzylbromide and diisopropylethylamine and incubation at room temperature for 30 minutes followed. Samples were then dried under nitrogen before pyridine, Bis(trimethylsilyl)trifluoroacetamide 99% and Trimethylchlorosilane 1% were added and incubated at 45°C for 20 minutes. Finally, hexane was added samples were mixed, then 1µl was injected for analysis using gas chromatography tandem mass spectrometry (Version 6.9.2, Varian, Australia) in negative chemical ionization mode.

Patients were grouped by normal (≤250 pg/ml) or elevated (>250 pg/ml) oxidative stress according to plasma F2-isoprostanes levels. This was based on using a value 1.5 standard deviations (SD) from mean values obtained from a previous study on apparently healthy 18-30 year old males and females from our laboratory.(154)

GPX

Plasma glutathione peroxidase (GPX) activity was measured via the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to $NADP^+$ by modifying previous methods (155, 156) for analysis on a Cobas Mira automated spectrophotometer (Roche Diagnostics, Switzerland). The main reagent contained a KH_2PO_4 buffer, EDTA, glutathione reductase, glutathione and NADPH in $NaHCO_3$. The main reagent and sample were monitored at 340 nm for 200 s.

TAC

Plasma total antioxidant capacity (TAC) was measured spectrophotometrically (Cobas Mira, Roche Diagnostics, Australia) using an adapted method (157). Plasma was incubated with met-myoglobin and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS). After incubation, hydrogen peroxide was added and the sample incubated again. Absorbance was measured to determine total antioxidant capacity.

Protein carbonyls

Protein carbonyls were analysed using an adapted version of the methodology from Levine et al.(158) Duplicate plasma samples were incubated with 2,4 Dinitrophenylhydrazine in 2.5M hydrochloric acid (HCl) while plasma blanks were incubated in HCl only. All samples were then precipitated with trichloroacetic acid (TCA) on ice and centrifuged for 10 minutes. Supernatants were discarded and the pellets resuspended in TCA and again centrifuged as above. Supernatants were removed and the pellets resuspended in ethanol:ethylacetate solution. After centrifugation as above the pellets were washed twice more with the ethanol:ethylacetate solution, resuspended in a guanidine hydrochloride solution, and absorbance read at 370 nm with correction at 650nm (Fluostar Optima, BMG Labtech, Offenburg, Germany). Protein carbonyl concentration was normalised to plasma protein content measured using a Pierce BCA protein assay kit (Thermo Scientific Australia).

2.1.23 Inflammation

Inflammation markers IL-6, TNF-α and INF-ɣ were measured by an electrochemiluminescence technique using Human Pro-inflammatory 4-plex Ultra-sensitive Kit with the Sector Imager 6000 from Meso Scale Discovery (Gaithersburg, USA). The assays were performed according to the manufacturer's instructions. CRP was measured in a National Association of Testing Authorities accredited pathology laboratory at the Princess Alexandra Hospital.

2.1.24 Creatinine and cystatin-C measurements

Creatinine was measured by the Jaffe method on a Beckman DxC800 general chemistry analyser (Beckman Coulter Diagnostics, Brea, CA, USA). A particle-enhanced immunoturbidimetric assay (Cystatin C Tina-quant Roche/Hitachi- Roche Diagnostics GmbH. Mannheim Germany) on a COBAS Mira clinical autoanalyser (Roche Diagnostica Switzerland), was used to perform serum cystatin-C measurements according to the manufacturer's instructions. Control and calibration materials were provided by Roche Diagnostics. A 2-reagent assay system was used, in where reagent 1 is a buffer and reagent 2 is a suspension of latex particles coated with rabbit anti-cystatin-C specific polyclonal antibodies. The sample is mixed with reagent 1 in a cuvette and incubated for 3 minutes, after which reagent 2 is added. After 7.5 minutes from the start of the cohesion reaction, of wavelength 550 nm, the absorbance difference was measured. All cystatin-C measures were performed in duplicate and the average was taken. If there was a difference of greater than 10% between 2 measures, a 3rd measure was taken and if the 3 values were similar, the average was taken, or if an outlier of the 3 was detected it was deleted.

2.1.25 Biochemistry coefficients of variation

The coefficients of variation (CV) for oxidative stress measures were determined by the average inter-assay variability in all samples performed in duplicate. The oxidative stress CV's were: F2-isoprostanes- 7.6%, GPX- 2.4%, TAC- 1.9% and protein carbonyls- 7.9%. All cystatin-C samples were performed in duplicate, and the average inter-assay CV was 3.8%. The inflammation measures were performed in duplicate, with CV's of less than 20% considered acceptable, as per the manufacturer's recommendation. The average inter-assay CV's for inflammation measures were: INF-α- 7.6%, TNF-γ- 4.5% and IL-6- 4.4%.

2.1.26 eGFR measurements

Four measures of renal function were assessed by eGFR; MDRD study equation (2006),(14) CKD-EPI creatinine equation (2009),(11) CKD-EPI cystatin-C equation (2012)(12) and CKD-EPI creatinine-cystatin-c equation (2012)(12). Table 2.1outlines the calculations used for each formula. All eGFR's are made relative to a surface area of 1.73m^2. The eGFR used for inclusion in the LM3 study is by the MDRD calculation.

Table 2.2 eGFR calculations

Name	Sex	Cr	Cys	Equation
MDRD (2007)(10)				$175xcr^{-1.151}xage^{-0.203}$(x1.212, if black),(x0.742,if female)
CKD-EPIcr (2009)(11)	F	≤0.7		$144x(cr/0.7)^{-0.329}x0.993^{age}$(x1.159,if black)
		>0.7		$144x(cr/0.7)^{-1.209}x0.993^{age}$(x1.159,if black)
	M	≤0.9		$141x(cr/0.9)^{-0.411}x0.993^{age}$(x1.159,if black)
		>0.9		$141x(cr/0.9)^{-1.209}x0.993^{age}$(x1.159,if black)
CKD-EPIcys (2012)(12)	F		≤0.8	$133x(cys/0.8^{)-0.499}x0.996^{age}x0.932$
			>0.8	$133x(cys/0.8^{)-1.328}x0.996^{age}x0.932$
	M		≤0.8	$133x(cys/0.8^{)-0.499}x0.996^{age}$
			>0.8	$133x(cys/0.8^{)-1.328}x0.996^{age}$
CKD-EPIcr-cys (2012)(12)				130x(cr/0.7)-0.248x(cys/0.8)-0.375x0.995age(x1.08,if black)

MDRD= modification of diet in renal disease; CKD-EPI=CKD epidemiology collaboration; cr=creatinine; cyst=cystatin-C; F=female; M=male

2.1.27 Vascular structure and function

Applanation tonomotrey was used to measure central PWV from the carotid and femoral arteries. The measurement was performed alongside a recording of an ECG R wave using a 3-lead configuration (SphygmoCor 8.1, AtCor Medical, Sydney, Australia). The pulse wave distance was determined from the sternal notch to femoral pulse distance minus the sternal notch to carotid pulse distance. Using the distance and time between ventricular contractions at each pulse, PWV was estimated. PWV measures were performed in duplicate and the average value was taken. The CV of PWV from all visits was 6.9%.

AIx was determined using a commercial device (SphygmoCor 7.1; AtCor Medical, Sydney, Australia) through PWA by applanation tonometry of the radial artery. AIx was determined by the ratio of augmented pressure to central pulse pressure. Radial artery waveform was calibrated against the mean of duplicate brachial blood pressure measurements. PWA measurements were accepted if the operator index was ≥80 and the indices in the Quality

Control section were suggested by SphygmoCor to be inside the limits (pulse height variation, diastolic variation and shape deviation). All PWA measures were performed in duplicate and the average value was taken. The CV of AIx from all visits was 10.2%.

2.1.28 Heart rate variability

Participants completed a supine 5 minute resting ECG recording before the maximal exercise stress test. Three Ag/AgCl electrodes were used in a modified lead II Einthoven configuration connected to an analysis program (SpyhmoCor 8.1, AtCor Medical, Sydney, Australia). The R-R interval tachogram was analyzed in Kubios (2.1, Kuopio, 2012) by which ectopic beats were removed using linear interpolation of the previous and subsequent beats. HRV was assessed by time and frequency domain parameters. Standard deviation of the N-N interval (SDNN) was used as the global time domain measure of HRV. The root mean square of the standard deviation (RMSSD) of the R-R intervals was also calculated. For the frequency domain, HRV was divided by spectral power analysis into high frequency (0.15-0.40 Hz) (HF), low frequency (0.04-0.15 Hz) (LF), and very low frequency (0.0033–0.04 Hz) (VLF) powers. Total power (TP) was also calculated as a sum of all frequency powers.

2.1.29 Haemodynamics

Resting blood pressure was taken at the brachial artery after 5 minutes of quiet rest using an automatic machine (BPM-300, VSM MedTech, Vancouver, Canada). The average of three measurements taken at 1 minute intervals were used for analysis. Peak exercise blood pressure was taken from the last stage completed in the maximal treadmill test using a mercury sphygmomanometer. Central blood pressure was determined using a commercial device (SphygmoCor 7.1; AtCor Medical, Sydney, Australia) through PWA by applanation tonometry of the radial artery. PWA measurements were taken in a quiet room after the DEXA scan, HRV and PWV had been performed, thus the patient had been lying supine for approximately 20-30 minutes. Patients abstained from any caffeine or stimulants on the morning of testing.

Twenty-four hour average blood pressure measurements were recorded using ambulatory blood pressure monitoring (A&D Medical TM-2430, California, USA). Blood pressure recordings were taken every 30 minutes from 6:00am-10:00pm (day) and every hour from

10:00pm to 6:00am (night). The data was downloaded after each 24 hour period and averages were calculated by the A&D software for the total 24 hours, day time and night time.

2.2 Study 2

As study 2 is only reported in one chapter, any additional methods not already described, are reported in Chapter 8 only.

Chapter 3.

Oxidative stress contributes to muscle atrophy in CKD patients

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This chapter has been published in *Redox Report,* November 2014. This chapter contains an abridged methods section of the published paper to avoid replication of the methods described in Chapter 2.

3.1 Abstract

Background: Patients with CKD have impaired muscle metabolism, resulting in muscle atrophy. Oxidative stress has previously been identified as a significant contributor to muscle atrophy in other populations, but the contribution in CKD is unknown. The aim of this study was to investigate the association between oxidative stress, grip strength and lean mass in patients with CKD.

Methods: This is a cross-sectional study of 152 participants with stage 3 or 4 CKD (eGFR 25-60 ml/min/1.73m^2). Outcome measures include grip strength (dynamometry), lean mass (DEXA), oxidative stress (plasma total F2-isoprostanes, protein carbonyls), inflammation (interleuking-6 [IL-6], tumour necrosis factor- α [TNF-α] and interferon- γ [IFN-γ]), cardiorespiratory fitness (VO_2peak) and standard clinical measures.

Results: Thirty four (22.4%) CKD patients had elevated oxidative stress levels (plasma F2-isoprostanes >250 pg/ml), with 82% of patients below age-predicted grip strength normative values. There was a significant negative association between plasma F2-isoprostanes and grip strength (r=-0.251) and lean mass (r=-0.243). There were no associations with inflammation markers. Multiple linear regression identified plasma F2-isoprostanes as a significant predictor of grip strength independent of other predictors; sex, diabetes status, body mass index (BMI), body fat percent and phosphate (adjusted R^2=69.5, $p<0.001$).

Conclusions: Plasma F2-isoprostanes were independently associated with reduced strength in CKD patients.

3.2 Introduction

Patients with CKD develop muscle wasting, which significantly influences muscular strength.(106) Skeletal muscle atrophy is associated with a 3 fold increase in mortality over a 4-6 year period in dialysis patients.(108) The aetiology of muscle atrophy in CKD is multi-factorial and associated with metabolic acidosis, excess angiotensin II and inflammation, however to our knowledge the contribution of oxidative stress in pre-dialysis patients has not been studied.(106)

Oxidative stress occurs when there is a disruption of redox signalling and control pathways.(159) A well-documented target of oxidative injury is lipid peroxidation of arichidonic acid, which produces F2-isoprostanes.(79) As such, plasma F2-isoprostanes are the gold standard for quantifying oxidative stress.(160) Other common assays or biomarkers of oxidative stress in CKD patients include advanced oxidation of protein products, protein carbonyls, γ-Glutamyl transpeptidase and malondialdehyde.(79). Protein carbonyls quantify reactive species damaged proteins, however this assay is not a specific measure of oxidative stress as it also measures glycated proteins and bound aldehyde.(79) Reduced total antioxidant capacity (TAC) and glutathione peroxidase (GPX) may also indicate a disturbance of the redox signalling pathways. Elevated oxidative stress levels, measured by plasma free F2-isoprostanes, protein carbonyls and protein reduced thiol content, have been reported in moderate-severe CKD.(161) Oxidative stress is suggested to be associated with inflammation, endothelial dysfunction and malnutrition in the uraemic population, thereby synergistically contributing to atherogenecity and risk of a cardiovascular event occurring.(162) Despite the multi-factorial nature of oxidative stress in renal patients, it has been suggested that the retention of oxidised solutes is likely a major contributor to the disease process.(80) Only one small study has investigated the effects of oxidative stress and muscle atrophy in renal patients. Crow et al. (2007) identified that haemodialysis patients (n=10) had significantly reduced diameter size of type I and II muscle fibres when compared to sex-matched controls.(163) However, the authors found no clear association between an oxidative stress marker, serum malonaldehyde, and muscle fibre diameter. Whether an increase in oxidative stress with CKD influences muscle atrophy and strength is yet to be examined.

Grip strength is well recognized as an indicator of overall upper body strength(164) and provides risk estimates similar to those of quadriceps strength.(165) It has been suggested

that muscle strength is a greater predictor of mortality than muscle mass.(165) Moreover, it has been reported that hand grip strength is a reliable measure of lean body mass in both men and women with chronic renal failure.(166) Due to the strong validity and accessibility of grip strength measures in the community, this study focused on grip strength as a primary measure of strength.

The aim of this study was to investigate the association between oxidative stress, grip strength and lean mass in patients with CKD. It was hypothesised that CKD patients would have impaired muscle function, evidenced by grip strength lower than age-predicted normative values. Furthermore, it was hypothesised that oxidative stress would be negatively associated with lean mass and grip strength.

3.3 Methods

The data from this study is a cross-sectional baseline analysis of LM3 on 152 subjects with stage 3-4 CKD. Patients were grouped by normal (≤250 pg/ml) or elevated (>250 pg/ml) oxidative stress based on plasma F2-isoprostanes. Age predicted grip strength values were calculated by a predictive equation for males and females reported by Desrosiers et al. (1994). Appendicular lean mass percentage was calculated from the average of the 4 limbs. DEXA was performed on a representative sub-set of patients due to limited machine availability (n=75). As the machine availability was random and patient recruitment was staggered, this sub-group of patients is still likely to be representative of the whole cohort.

Statistics

Mean±standard deviation (SD) was used to describe baseline characteristics, with percentages used to describe frequencies for categorical variables. Median and interquartile range (IQR) was used to describe not normally distributed and log transformed variables. A one-way analysis of variance (ANOVA) with a Bonferroni post-hoc analysis was used to determine between group differences of grip strength tertiles. Univariate associations between variables and grip strength were evaluated using Pearsons correlation of coefficient. Not normally distributed variables were transformed using the natural logarithm. Spearmans Rho was used for not normally distributed variables that were not able to be transformed.

Significant univariate associations were included in a multivariate model to identify independent correlates, using the enter method. Regression diagnostics were assessed for identification of collinearity and variance inflation factor issues. Statistical analysis was performed using IBM SPSS statistics 21 (New York, 2012). Statistical significance was assumed at $p<0.05$.

3.4 Results

Patient Characteristics

One hundred and fifty two patients were recruited and eligible for study participation between March 2008 and February 2013. Table 3.1 shows the demographic and clinical data for this cohort. The mean age of patients was 60 years, the majority were obese and had low fitness. Nineteen patients were classified as stage 4 CKD and 141 patients were classified as stage 3 CKD. Sixty-eight (43.9%) patients had diabetes. There was no significant difference in F2-isoprostanes between patients with stage 3 and 4 CKD (p=0.91) and patients with and without diabetes (p=0.75). Thirty four patients (22.4%) had elevated F2-isoprostanes (≥250 pg/ml). There was no consequent increase in anti-oxidant status in patients with elevated F2-isoprostanes (GPX= normal F2-isoprostanes group 24.8±2.9 U/L vs. elevated F2-isoprostanes group 19.6±0.4, p=0.6; TAC= normal F2-isoprostanes group 1.6±0.1 mmol/L vs. elevated F2-isoprostanes group 1.8±0.1, p=0.2). There was also no significant correlation between F2-isoprostanes, protein carbonyls, GPX and TAC (Table 3.2). There was however a moderate correlation between GPX and TAC (r=-0.245, p=0.003).

Table 3.1 Patient characteristics

Variable	
Age (years)	59.7±10.0
Female sex (n,%)	65(42.8)
Diabetes (n,%)	68(44.7)
eGFR ($ml \cdot min \cdot 1.73m^2$)	40.2±9.0
Body mass index (kg/m^2)	33.7[8.1]
Fat (%)[#]	36.7±7.7

Appendicular lean mass (%)[#]	60±9.2
Medications	
B-blockers (n,%)	47(30.3)
Angiotensin converting enzymes inhibitor (n,%)	69(44.5)
Statins (n,%)	83(53.5)
Blood Biochemistry	
F2-isoprostanes (pg/ml)	193.0[108.5]
Protein carbonyls (nM/mg)	0.52[0.14]
Glutathione peroxidase (U/L)	24.2±4.3
Total antioxidant capacity (mmol/L)	1.7±0.4
Albumin (g/L)	3.8[5.0]
Homeostatic model assessment- insulin resistance* (%)	6.5[15.8]
HaemoglobinA1c*	7.2[1.7]
Haemoglobin (g/dL)	13.2±1.5
Phosphate (mmol/L)	1.1±0.2
Bicarbonate (mmol/L)	26.0±6.5
Inflammation	
Interferon-ɣ (pg/ml)	1.0[0.7]
Interleukin-6 (pg/ml)	2.2±2.1
Tumour necrosis factor-α (pg/ml)	6.8±4.0
C-reactive protein (mg/L)	5.1[4.8]
Exercise Parameters	
VO_2peak ($ml \cdot kg \cdot min^{-1}$)	23.3[7.2]
Physical activity levels (hours/week)	2.3 [3.3]
Grip strength- best of either hand (kg)	30.7±11.3
Haemodynamics	
Rest systolic blood pressure (mm/Hg)	137.4±20.9
Rest diastolic blood pressure (mm/Hg)	81.8±12.4

#Due to limited machine availability DEXA was performed on n=75 participants. Mean±standard deviation given for normally distributed variables, median [interquartile range] given for not normally distributed variables, number (%) given for categorical variables. eGFR=estimated glomerular filtration rate. *in patients with diabetes only.

Table 3.2 Muscle atrophy variables and univariate relationship with F2-isoprostanes

Variable	r value	p value
Sex	0.21	**0.01**
Diabetes status	-0.04	0.7
Age	-0.22	**0.01**
Glutathione peroxidase	0.02	0.8
Total anti-oxidant capacity	-0.05	0.6
Protein carbonyls	0.06	0.5
Albumin	-0.20	**0.02**
Homeostatic model assessment- insulin resistance	0.19	**0.03**
Interferon-ɣ	0.13	0.2
Interleukin-6	0.09	0.3
Tumour necrosis factor-α	0.05	0.6
C-reactive protein	0.07	0.4
Haemoglobin	-0.20	**0.01**
Grip strength	-0.25	**<0.01**
Appendicular lean mass %	-0.24	**0.04**
Body mass index	0.27	**<0.01**

F2-Isoprostanes and Muscle Atrophy Markers

The association between F2-isoprostanes and other muscle atrophy markers are represented in Table 3.2. There was a negative association between F2-isoprostanes and grip strength (Figure 3.1), and F2-isoprostanes and appendicular lean mass percentage (Figure 3.2). There was no significant relationship between F2-isoprostanes and grip strength in patients with normal F2-isoprostanes (r=-0.161, p=0.10). There were however, significant associations between F2-isoprostanes and sex, albumin, homeostatic model assessment- insulin resistance (HOMA-IR), haemoglobin and BMI. There were no associations (p>0.05) with inflammation markers INF-ɣ, TNF-α, IL-6 and C-reactive protein (CRP). The power of detecting a significant association between grip strength and F2-isoprostanes in 152 participants is 99.3%. The correlation between grip strength and F2-isoprostanes in patients with BMI <30 kg/m^2 (n=41) was statistically significant (r=-0.353, p=0.03). Despite the significant difference in F2-isoprostanes between males and females (males=177±89.1 pg/ml vs. females=214.4±99.1, p<0.01), the correlation between grip strength and F2-isoprostanes in males was only approaching significance (r=-0.211, p=0.07). Likewise, the correlation between grip strength and F2-isoprostanes in females only was not significantly correlated (r=-0.039, p=0.7).

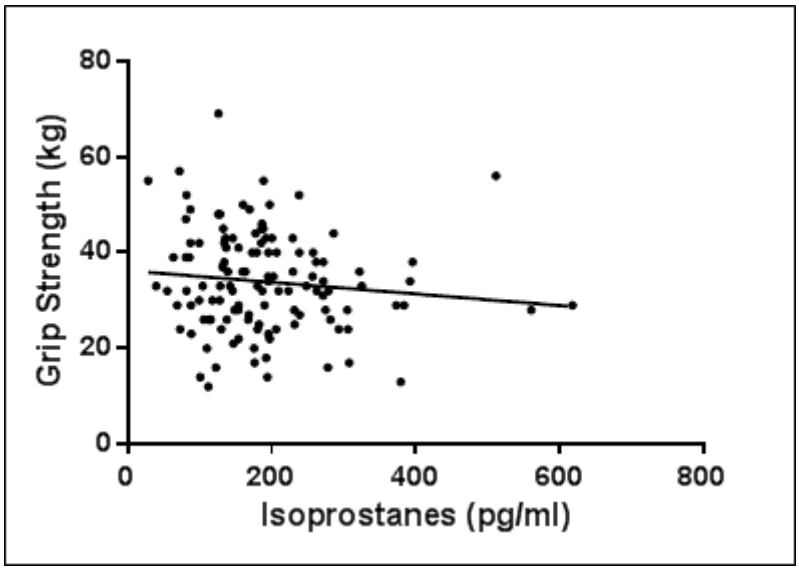

Figure 3.1 Association between F2-isoprostanes and grip strength. r=-0.251, p<0.01

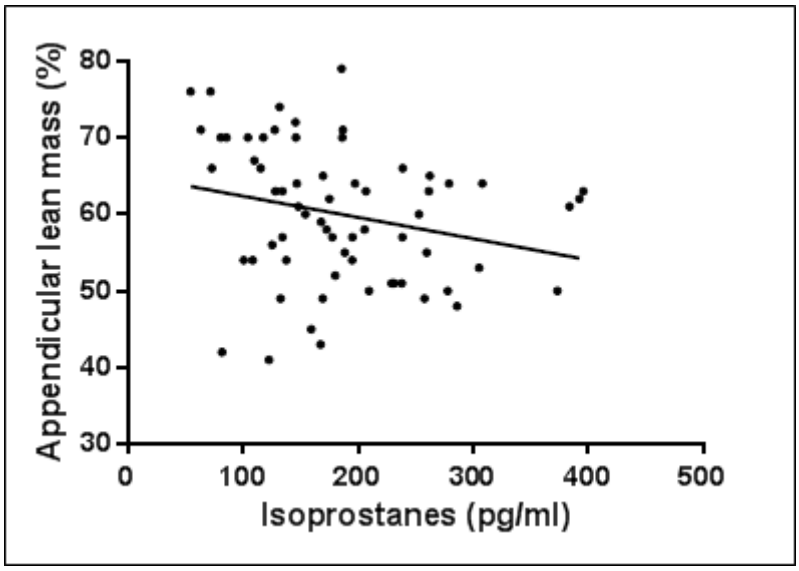

Figure 3.2 Association between F2-isoprostanes and appendicular lean mass percentage. r=-0.243, p=0.04

Predictors of Grip Strength

Grip strength was separated into relative tertiles of low (≤25 kg, n=50), moderate (26-35 kg, n=43) and high strength (≥36 kg, n=44) (Figure 3.3). There was a significant group difference between grip strength tertiles ($p<0.01$), with post-hoc analyses identifying the low strength group to have significantly higher levels of plasma F2-isoprostanes than the high strength group ($p<0.01$). The difference between low strength and moderate strength was approaching

significance (p=0.06). It was identified that 82% of all patients had grip strength values below their age-predicted grip strength (p<0.001). Factors associated with grip strength are shown in Table 3.3. There was a strong correlation between grip strength and appendicular lean mass. Multiple linear regression found F2-isoprostanes to be a predictor of grip strength (ß=-0.219, p=0.03) independent of other predictors; sex, diabetes status, BMI, body fat percentage and phosphate in a model also including age, IFN-ɣ, appendicular lean mass, haemoglobin and VO_2peak (adjusted R^2=69.5, p<0.001).

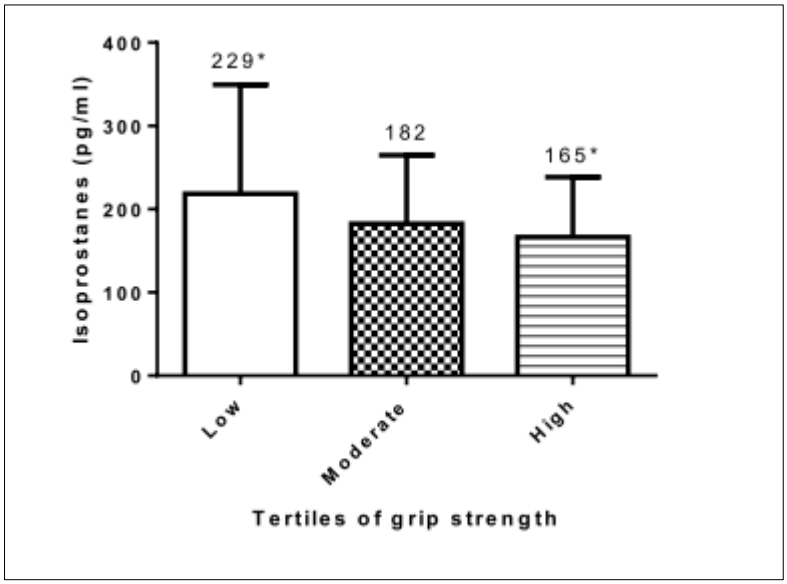

Figure 3.3 F2-isoprostanes by tertiles of grip strength. Data is reported as mean and SEM

Table 3.3 Factors associated with grip strength

Variable	Univariate (r or r_s)	p value	Multivariate (β)	p value
Age	-0.22	**0.01**	-0.19	0.12
Sex_s	-0.73	**<0.001**	-0.59	**<0.001**
Diabetes $Status_s$	-0.31	**<0.001**	-0.22	**<0.01**
eGFR	0.06	0.48		
Body mass index (Ln)	-0.23	**0.01**	0.37	**<0.01**
Total antioxidant capacity	0.01	0.89		
F2-Isoprostanes (Ln)	-0.25	**<0.01**	-0.22	**0.03**
Protein $carbonyls_s$	0.04	0.64		
Glutathione peroxidase	0.01	0.88		
Interferon-γ_s	-0.22	**0.02**	0.05	0.61
Tumor necrosis factor-α	-0.10	0.26		
Interleukin-6	-0.09	0.32		
Phosphate	-0.41	**<0.001**	-0.18	**0.05**
Haemoglobin	0.48	**<0.001**	-0.04	0.71
$Bicarbonate_s$	0.01	0.94		
C-reactive $protein_s$	-0.06	0.48		
$Albumin_s$	0.13	0.14		
Homeostatic assessment	-0.16	0.07		
VO_2peak (Ln)	0.45	**<0.001**	0.05	0.67
Physical activity $levels_s$	0.10	0.28		
Appendicular lean mass (%)	0.86	**<0.001**	-0.06	0.78
Fat (%)	-0.58	**<0.001**	-0.59	**0.013**

Adjusted R^2=0.695, p<0.001

3.5 Discussion

This is the first study to investigate the role of oxidative stress in reduced lean mass and grip strength in CKD patients. Our main findings were 1) 82% of CKD patients had grip strength lower than age-predicted normative values, 2) 22.4% of CKD patients had elevated F2-isoprostanes, 3) oxidative stress was negatively associated with variables associated with muscle atrophy, in particular, appendicular lean mass, grip strength, sex, age, albumin, HOMA-IR, haemoglobin and BMI and 4) plasma F2-isoprostanes were an independent predictor of reduced grip strength.

CKD results in significantly reduced muscle mass and strength.(105) Indeed, it was found that 82% of the CKD patients had grip strength lower than age-predicted normative values. It is likely that reduced strength is occurring at least partly as a consequence of muscle atrophy, evident by the strong positive correlation between grip strength and lean mass. Our findings are in support of the literature which identifies reduced strength in CKD patients, as a study on haemodialysis patients also found reduced strength to occur as a consequence of reduced muscle mass.(76) This is clinically relevant as reduced functional strength is closely associated with survival and low muscle mass is a potentially modifiable factor.(167) The almost linear relationship that occurs between grip strength and lean mass, supports the use of grip strength as an inexpensive and easily used test in the field.

F2-isoprostanes were elevated in a significant proportion of patients, however there was no subsequent increase in anti-oxidant status. In addition, there was no association between GPX, TAC and F2-isoprostanes. These findings are supported by Karamouzis et al. (2008) who found that as the stage of kidney disease increased, so too did the level of plasma F2-isoprostanes.(168) This study also found that TAC did not change with advancing CKD stages. The disconnect between F2-isoprostanes and GPX and TAC in our findings is suggesting that an increase in oxidants is occurring without a compensatory increase in antioxidants.(168) This imbalance may be an important finding in elucidating the pathogenesis of oxidative injury in CKD patients. This is consistent with findings from Dalla Libera et al. (2009) who found an increase in muscle protein carbonylation and a blunted expression of stress proteins involved in the anti-oxidant defence in patients with disuse muscle dystrophy. As suggested by the authors of this study, future investigations are needed to identify the mechanisms responsible for the switching off of these anti-oxidant defences, and whether challenging this mechanism can ameliorate muscle atrophy.(169)

Plasma F2-isoprostane levels were identified to be negatively associated with both lean mass and grip strength. This finding suggests a relationship between oxidative stress and resultant loss of strength through reduced muscle mass, which has not previously been identified in CKD patients. It has previously been reported in haemodialysis patients that inflammatory markers are associated with muscle mass.(170) Indeed, inflammatory cytokines have been reported to induce myofilament degradation through caspase-3 activation.(171) However, in the current study we were not able to demonstrate an independent association between inflammatory cytokines and grip strength or lean mass. It is possible that in less severe CKD

patients (ie. stage 3-4) oxidative stress may have a more significant contribution to muscle wasting than inflammation.

The significant association of F2-isoprostanes and grip strength in patients with BMI <30 kg/m^2 (n=41) suggests that F2-isoprostanes is not just a marker of muscle function in obese patients. However, future studies should identify if this relationship still exists in CKD patients with a BMI of <25 kg/m^2. Sex and F2-isoprostanes were strongly correlated in all patients and yet when separated into males and females the correlation between grip strength and F2-isoprostanes was no longer significant. This may indicate that a larger sample size is needed to detect significant correlations due to the high variability in F2-isoprostanes. Prior studies have reported inconsistent findings on sex differences and oxidative stress levels.(172-175) We have shown that female CKD patients have higher levels of F2-isoprostanes than males. Therefore future large scale studies should identify whether the correlations between oxidative stress, lean mass and strength is dependent on sex and whether changes in muscle parameters after an intervention differs between males and females.

Oxidative stress was associated with other variables related to muscle atrophy; hypoalbuminemia, increased insulin resistance, anaemia and obesity. These factors have previously been proposed as variables associated with muscle atrophy.(176) It has been reported that increased ROS bind specific muscle proteins and increases the degradation by the Ubiquitin-Proteasome System.(111) This increase in degradation results in muscle atrophy.(110) In genetic muscle diseases oxidative stress is implicated as the initiator and driver of muscle damage.(177)

An obvious approach to the problem of reduced strength in CKD is exercise training. This potentially has a dual effect of not only strengthening muscle, but also correcting the imbalance between oxidative stress and protective anti-oxidant capacity. As exercise increases oxygen uptake, there is an acute increase in ROS production through the electron transport system.(178) With repeated exercise, anti-oxidant enzymes that combat this exercise-induced oxidative stress are upregulated to provide more protection against ROS.(179) Indeed, an investigation in exercise induced malondialdehyde in rats also found a compensatory increase in glutathione peroxidase and superoxide dismutase.(180) Therefore, although exercise can cause acute increases in ROS, long term exercise can prove to be a beneficial treatment in improving oxidative stress.(181) The current evidence on the protective effects of anti-oxidant therapies in improving kidney function remains

equivocal.(182) However, using anti-oxidant therapy to attenuate ROS and increase muscle mass in the renal population has not previously been studied.(79)

Limitations

The analysis from this study employs a cross-sectional design and therefore causality between oxidative stress, reduced grip strength and lean mass cannot be determined. Despite the well-recognized validity of grip strength as an indication of overall upper body strength,(164) additional measures of strength, such as 1RM testing, may have reinforced the findings. It should be noted that whilst DEXA provides an estimate of lean body mass, skeletal muscle histology or magnetic resonance imaging to assess fibre and muscle size would have provided a better measure of muscle atrophy. Despite this limitation, the associations found with lean mass are encouraging, as DEXA has been reported to *underestimate* loss of thigh muscle mass in comparison to MRI.(183) Further work looking at the expression of other factors involved in muscle loss, such as atrogin-1 and myostatin in muscle biopsies of patients with CKD is required to better understand the role of oxidative stress in the pathogenesis of muscle atrophy.

Another limitation of this study is the classification of normal and elevated F2-isoprostanes groups based on healthy participants previously studied in our lab.(154) Aging has a well recognized influence on oxidative stress levels.(184) Considering the older age of participants in the current study, it would have been ideal to use age matched controls to assess whether CKD patients were considered to have normal or elevated levels of F2-isoprostanes. Our laboratory has not previously reported F2-isoprostane measures in older adults. Therefore, due to variations in laboratory specific protocols and coefficients of variation, age matched comparisons could not be performed for the current study. Furthermore, the association between grip strength and oxidative stress has a low r value, despite being statistically significant. This increases the likelihood that the significant association is a spurious one and should be considered when interpreting the findings.

Conclusions

CKD patients have below average grip strength when compared to age-predicted normative values. It was identified that plasma F2-isoprostanes were independently associated with reduced strength in CKD patients. The findings from this study may assist in ascertaining

appropriately targeted treatments for muscle loss, such as resistance exercises to restore muscle strength and long-term exercise training to correct the oxidative stress imbalance.

Chapter 4.
Feasibility and physiological and clinical determinants of participating in higher intensity exercise training

This chapter is in the final stages of preparation for submission to *European Journal of Applied Physiology*. This chapter contains an abridged methods section of the submitted manuscript to avoid replication of the methods described in Chapter 2.

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4.1 Abstract

Background: Higher intensity exercise (vigorous and high intensity) is more beneficial for improving cardiorespiratory fitness than moderate intensity exercise in a range of chronic disease populations. Understanding why patients would participate in this form of training has important implications for exercise prescription. The aim of this study was to investigate the feasibility and determinants of participation in higher intensity exercise when prescribed a general home exercise program after an initial supervised period.

Methods: This is an observational sub-study of a randomised controlled trial. Forty-four participants with stage 3-4 CKD (eGFR 25-60 ml/min/1.73 m^2) were allocated to undertake a 12 month individualised aerobic and resistance training LI intervention. The goal of the intervention was for all subjects to complete 150 minutes/week of moderate intensity exercise with included higher intensity exercise if possible. Pre and post intervention measurements included a physical activity questionnaire of the preceding 6 months, blood analysis, pulse wave analysis (PWA) and a maximal treadmill test to determine cardiorespiratory fitness (VO_2peak) and exercise capacity (METs).

Results: At 12 months, the number of patients who reported performing weekly higher intensity exercise in the previous 6 months of the intervention increased from 20% to 43%, with the number of patients reporting moderate intensity exercise increasing from 16% to 25%. The number of participants reported not meeting exercise guidelines decreased from 64% to 32% (<150 minutes/week). Participants exercising at higher intensities had significantly ($p<0.05$) higher cardiorespiratory fitness (27.7±9.4 ml/kg/min), exercise capacity (9.8±4.2 METs) and haemoglobin levels (142.9±16.1 g/L) at baseline compared to those reporting moderate (23.3±4.5 ml/kg/min, 8±3.1 METs, 129.8±12.9 g/L) and not meeting guidelines (19.8±5.1 ml/kg/min, 6±2 METs, 127.3±12.5 g/L). Participants completing higher intensity exercise also had a significantly greater exercise capacity at 12 months (11.9±3.7 METs) than participants reporting moderate intensity exercise (9.2±1.7 METs) and not meeting guidelines (7.6±3.2 METs).

Conclusions: The study was successful in increasing higher intensity physical activity in a large proportion of patients with CKD. Those who reported higher intensity exercise had increased haemoglobin levels, cardiorespiratory fitness and exercise capacity at baseline, underlining the importance of these measures in optimising exercise prescription.

4.2 Introduction

Patients with CKD have a known reduction in exercise capacity,(185, 186) which is one of the strongest predictors of mortality(187) and is the likely consequence of multiple mechanisms. Despite the recognised benefits of regular moderate exercise in improving cardiovascular disease risk factors and maintaining kidney function,(75) recent studies have suggested higher intensity exercise is a more effective way of evoking the largest exercise-induced adaptations in chronic disease patients.(188) The feasibility of this type of training in CKD patients is unknown.

The Active Australia questionnaire has previously been reported to have good validity and reliability, with self-reported physical activity closely correlated with accelerometry and pedometry measures in middle-aged women (140) and in older adults.(137) This questionnaire also has acceptable levels of test re-test reliability for assessing activity status and moderate reliability for assessing total minutes of physical activity.(138, 139) Furthermore, the Active Australia questionnaire is reported to be a reliable measure of self-reported physical activity intensity, with significant correlations with accelerometry data in overweight adults for each category of exercise intensity (walking, moderate and vigorous).(139) Reeves et al. (2010) has also reported the Active Australia questionnaire to be responsive to changes in physical activity when compared to a more detailed self-report measure.(189) However, the validity and reliability of the Active Australia questionnaire has not been assessed specifically in the CKD population.

Higher intensity exercise (vigorous *and* high intensity) is defined as physical activity which raises heart rate to >70% of heart rate maximum.(63) Identifying predictors of participation in higher intensity exercise is essential for targeting individuals more likely to benefit from this type of training. This study sought to investigate the feasibility and determinants of incorporating higher intensity exercise in a 12 month LI in CKD patients. It was hypothesised posteriori that higher intensity exercise would be feasible for patients with CKD, with an increase in the number of patients reporting this type of exercise. It was also hypothesised that patients reporting higher intensity exercise would have greater exercise capacity at 12 months than patients reporting moderate intensity exercise not meeting exercise guidelines.

4.3 Methods

Forty-four patients with stage 3-4 CKD were included in this sub-study. The data from this study analyses the intervention arm from the LM3 study. Out of the 82 participants randomized to the LI group, 44 participants completed twelve months of the intervention with both baseline and 12 month Active Australia questionnaire data. For the purpose of this analysis control participants are not included in this observational sub-study. Exercise measurements were taken at baseline and 12 months. Achieved VO_2 peak was compared to predicted normative data for women, [VO_2 peak = (14.7 – (0.13 * age)*3.5],(148) and men [VO_2 peak = (18.4 – (0.16 * age)*3.5. Adverse events were assessed by a Registered Nurse at 6 months and 12 months.

Statistical analysis

Mean ± standard deviation (SD) was used to describe baseline characteristics, with percentages used to describe frequencies in categorical variables. Median and interquartile range (IQR) was used to describe not normally distributed and log transformed variables. ANOVA was used to detect significant differences between groups. LSD post hoc identified where the significant differences occurred. The Kruskal-Wallis test was used to compare not normally distributed variables which did not normalize with log transformation (resting systolic blood pressure and central systolic blood pressure) and are reported as median (IQR). Univariate associations identified baseline variables with exercise capacity (METs) at 12 months. A multi-nomial logistic regression was used to identify variables that would predict the intensity of exercise that participants would undertake during the 12 month intervention (not meeting guidelines, moderate intensity or higher intensity exercise). Due to the high number of variables that may influence exercise capacity, and the relatively small sample size, a multi-level logistic regression was employed. The three models included in the regression analyses; are demographics, blood biochemistry and exercise parameters. Higher intensity exercise was used as the reference category, in order to identify the difference in participants reporting higher intensity exercise, to both moderate and not meeting guidelines groups. In variables that were identified as significantly different between higher intensity and moderate intensity exercise, the moderate intensity group was made the reference

category in the same multi-nomial logistic regression model in order to identify the odds ratio of participating in higher intensity exercise. Significance was set at $p < 0.05$.

4.4 Results

The intervention significantly increased physical activity levels. At baseline only nine people (20%) reported completing higher intensity exercise, and this increased to 19 people (43%) after 12 months (Figure 4.1). Baseline characteristics of the study population according to their physical activity levels during the intervention are displayed in Table 4.1. There were no significant group differences in age, renal function or haemodynamics. However, participants who reported performing higher intensity exercise at 12 months had significantly greater baseline VO_2peak, exercise capacity (METs) (Figure 4.2) and phosphate than participants not meeting guidelines. Table 4.2 demonstrates the difference in physical activity levels between the groups at 12 months (exercise time, MET minutes, vigorous intensity and moderate intensity all $p<0.001$). Participants reporting higher intensity exercise also had a significantly greater exercise capacity at 12 months compared to those reporting both moderate intensity exercise and not meeting guidelines (Figure 4.2). Participants in the higher intensity ($p<0.001$) and not meeting guidelines ($p<0.01$) group also had a significant within group change in exercise capacity from baseline to 12 months and comparison of the delta values between groups was not statistically significant (Figure 4.3). Haemoglobin levels were significantly greater at baseline in the participants reporting higher intensity exercise compared to both moderate and not meeting guidelines groups ($p<0.01$) (Figure 4.4).

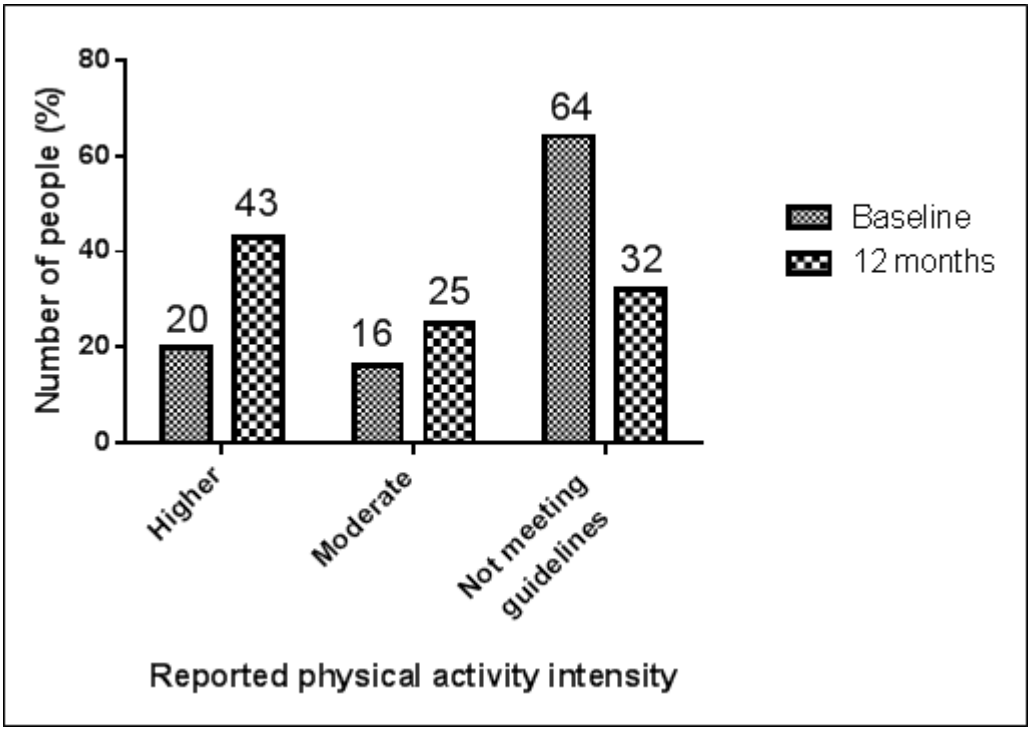


Figure 4.1 Number of participants reporting different intensities of exercise at baseline and 12 months

Table 4.1 Baseline characteristics of participants reporting different exercise intensities at 12 months

Variables	Not meeting guidelines (n=14)	Moderate (n=11)	Higher** (n=19)	p value
Age (years)	60.9±9.9	58.5±8.8	59.1±11.0	0.83
Sex (males)(%)	6(42.9)	5(45.5)	14(73.7)	0.15
eGFR (ml/min/1.73m^2)	37.6±6.6	38.2±7.4	39.4±8.5	0.79
Weight (kg)	98.5±20.8	86.5±11.5	84.6±15.5	0.06
Diabetes status	5(35.7)	4(36.4)	7(36.8)	0.98
History of CVD				
Myocardial infarction	1(7.1)	1(9.1)	2(10.5)	0.93
Previous angina	1(7.1)	1(9.1)	3(15.8)	0.63
Heart failure	1(7.1)	1(9.1)	1(5.3)	0.94
Hypertension	13(92.9)	10(90.9)	18(94.7)	0.92
Hyperlipidaemia	9(64.3)	8(72.7)	11(57.9)	0.72
Peripheral vascular disease	2(14.3)	2(18.2)	4(21.1)	0.84
Blood Biochemistry				
Haemoglobin (g/L)	127.3±12.5	129.8±12.9	**142.9±16.1***	**<0.01**
Hba1C (%)(diabetes only)	7.3±0.9	7.9±1.2	7.4±1.4	0.73
Albumin (g/L)	40.1±3.0	37.8±3.8	37.6±4.7	0.20
C-Reactive protein (mg/L)	4.0[3.3]	5.1[4.4]	2.0[2.3]	0.21
Exercise parameters				
VO_2peak (ml/kg/min)	20.7[7.4]	22.2[6.1]	**25.8[10.4]**$^{\#}$	**<0.01**
Below predicted VO_2	13/14	11/11	14/19	0.09
METs	6.0±2.6	8.0±3.1	**9.8±4.2**$^{\#}$	**0.02**
Exercise time (mins/week)	37.5[120.0]	70.0[180.0]	150.0[200.0]	0.10
Grip (kg)	30.8±13.4	36.0±12.9	38.2±12.7	0.32
Haemodynamic and vascular				
Peripheral systolic BP (mmHg)	133.0[19.0]	118.0[16.0]	124.5[25.3]	0.72
Peripheral diastolic BP (mmHg)	76.0[8.8]	76.0[8.0]	76.0[13.3]	0.50
Central systolic BP (mmHg)	118.8[9.9]	111.5[8.5]	114.8[12.9]	0.13
Central diastolic BP (mmHg)	77.8[12.5]	77.5[9.0]	76.3[9.3]	0.47
Medication				
Erythropoietin stimulating agent	0	0	0	
Iron supplementation n, (%)	3(21.4)	1(9.1)	0	0.09
β-Blocker n(%)	1(7.1)	3(27.3)	5(26.3)	0.34

Values are reported as mean±SD for normally distributed variables. Not normally distributed and log transformed variables are reported as median [IQR]. Categorical values are presented as n(%).
*= higher significantly different from both moderate and not meeting guidelines
†= higher significantly different from moderate
#= higher significantly different from not meeting guidelines
**= includes both vigorous and high intensity exercise
CVD= cardiovascular disease; eGFR = estimated glomerular filtration rate; BP = blood pressure

Table 4.2 Physical activity levels of patients reporting different exercise intensities at 12 months

Variables	Not meeting guidelines (n=14)	Moderate (n=11)	Higher** (n=19)	p value
Exercise time (mins/week)	60.0[86.3]	330.0[161.0]	280.0[250.0]	<0.001
Exercise time (MET/mins)	270.0[388.1]	1485.0[720.0]	1320.0[1275.0]	<0.001
Vigorous exercise (mins/week)	0	0	90[120]	<0.001
Moderate exercise (mins/week)	60[86.3]	330[160]	180[130]	<0.001

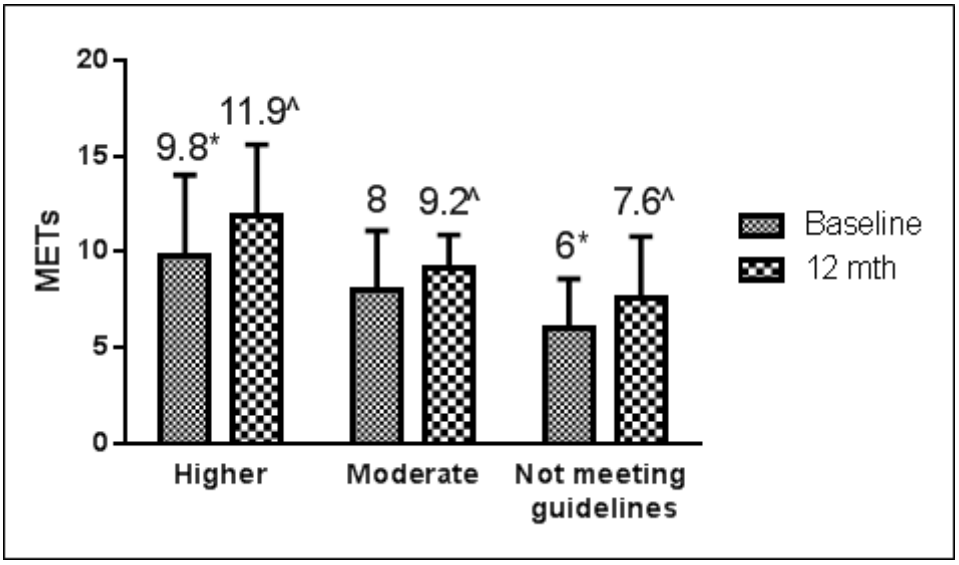


Figure 4.2 Effects of exercise intensity after 12 months of exercise training on peak exercise capacity at 12 months. Values are mean and SEM, * indicates a significant difference between baseline higher and not meeting guidelines groups (p=0.018). ^ indicates a significant difference between higher, moderate and not meeting guidelines groups at 12 months (p<0.01)

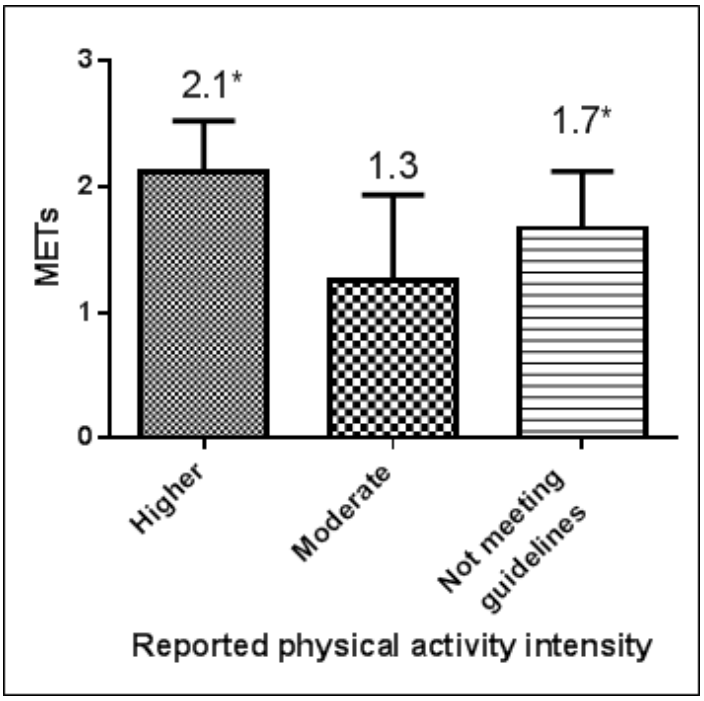


Figure 4.3 The delta scores of METs from baseline to 12 months between the groups. Values are mean and SEM, * indicates a significant within group difference between baseline and 12 months; higher ($p<0.001$) and not meeting guidelines ($p<0.01$)

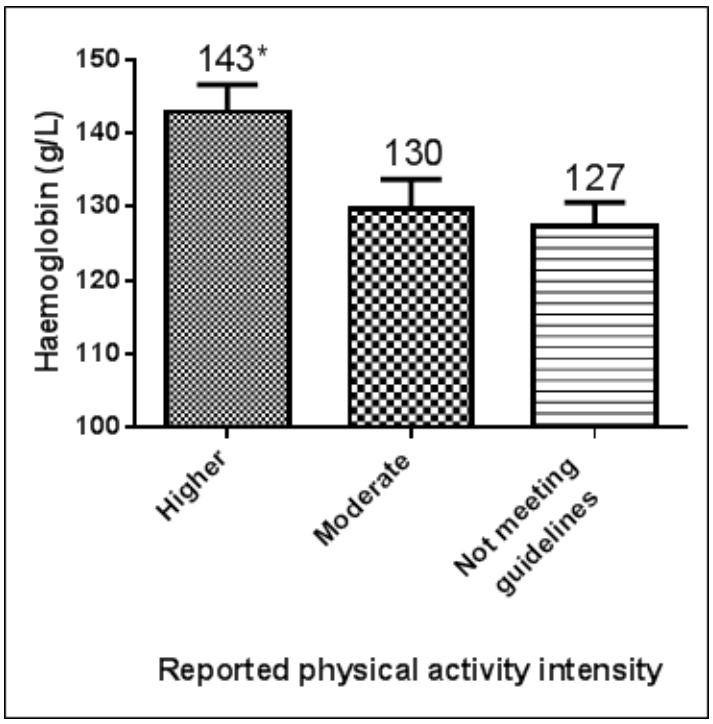


Figure 4.4 Effects of baseline haemoglobin on self-reported exercise intensity in a 12 month intervention. Participants reporting higher intensity exercise had significantly higher haemoglobin levels than participants reporting both moderate and not meeting guidelines ($p<0.01$)

Table 4.3 investigates the baseline association of exercise capacity at 12 months in order to identify whether baseline correlates are similar for change in exercise capacity and participation in higher intensity exercise. There was a strong positive association between baseline haemoglobin and exercise capacity at 12 months ($r=0.55$, $p<0.001$)(Table 4.3, Figure 4.5). A group comparison of differing haemoglobin levels in all patients, demonstrates that exercise capacity significantly increases as each level of haemoglobin increases (<115 vs. 115-130 vs. 130-150 vs. >150 g/L, $p<0.01$)(Figure 4.6). Other significant associations between baseline factors and exercise capacity at 12 months (Table 4.3) were; age, sex, diabetes status, BMI, haemoglobinA1c, central systolic blood pressure (all negative), while, VO_2peak, and grip strength were positively associated with exercise capacity.

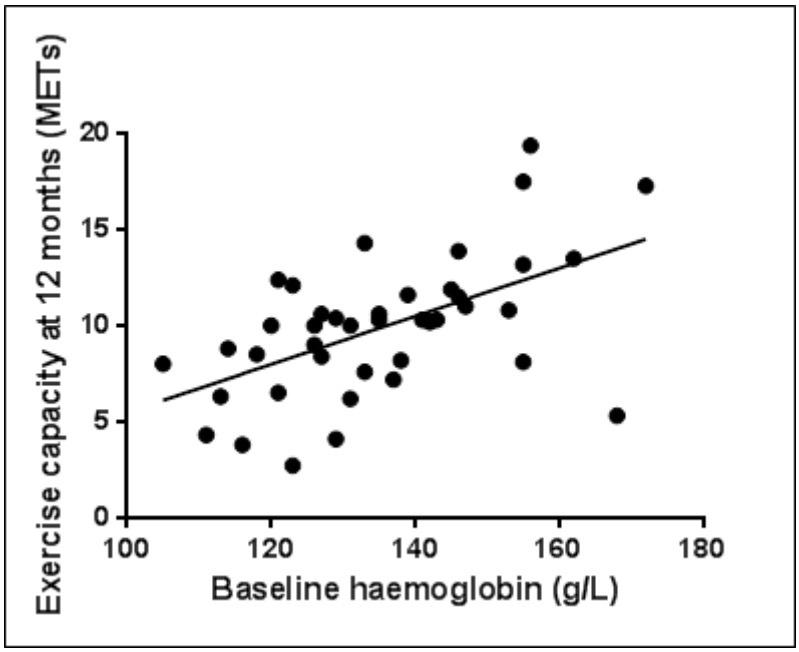


Figure 4.5 Association between baseline haemoglobin and exercise capacity at 12 months. r=0.490, p<0.01

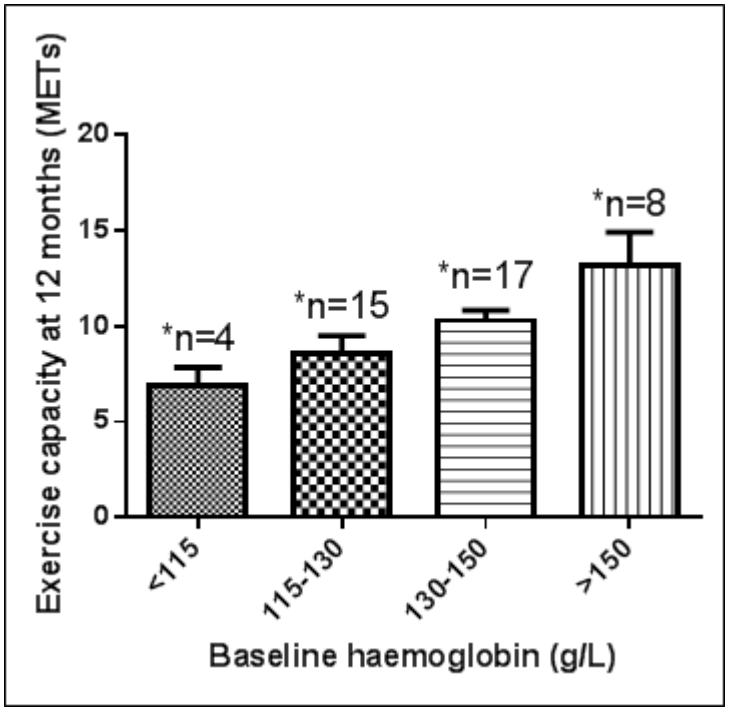


Figure 4.6 A group comparison on all patients of baseline haemoglobin on exercise capacity after 12 month intervention. Participants with haemoglobin levels >150 g/L at baseline had significantly higher exercise capacity at 12 months than each of the other three haemoglobin groups (p<0.01)

Table 4.3 The association of baseline factors with exercise capacity at 12 months

Variable	Univariate (r)	p value
Age	-0.61	**<0.001**
Sex	-0.44	**<0.01**
Diabetes	-0.49	**0.001**
Ischaemic heart	-0.11	0.52
eGFR	0.01	0.54
Weight	-0.16	0.32
Body mass index	-0.54	**<0.001**
Haemoglobin	0.55	**0.001**
Albumin	-0.15	0.36
C-reactive protein	-0.02	0.91
VO_2peak	0.77	**<0.001**
Grip strength	0.66	**<0.001**
Exercise time	0.34	**0.03**
Resting peripheral SP	-0.184	0.26
Resting peripheral DP	0.15	0.35
Central SP	-0.33	**0.04**
Central DP	-0.02	0.91

eGFR = estimated glomerular filtration rate; SP = systolic pressure; DP = diastolic pressure.
*Ischaemic heart disease=previous angina or previous myocardial infarction.

Multinomial logistic regression models were conducted to predict the exercise intensity reported by participants during the intervention. There were three models used in the regression; demographics (age, sex, BMI and diabetes status), blood biochemistry (haemoglobin, eGFR and haemoglobina1c) and exercise (VO_2peak, physical activity levels and grip strength) (Table 4.4). Only the blood biochemistry model identified those reporting higher intensity exercise to be different from those reporting moderate and not meeting guidelines (chi square = 12.846, p=0.046 with df=6). The Wald criterion (p = 0.04) demonstrated that only haemoglobin made a significant contribution to higher intensity exercise relative to moderate intensity exercise. The odds ratio indicates that when haemoglobin is higher by 1 unit (g/L) participants are 1.1 times more likely to participate in higher intensity exercise. When compared to the not meeting guidelines group, for every 1

g/L increase in haemoglobin, participants are 1.12 times more likely to participate in higher intensity exercise ($p<0.01$). There was a significant difference between the not meeting guidelines and the higher intensity group in the demographics model (chi square = 16.002, $p=0.042$ with df=8). For every 1 kg/m^2 increase in BMI, patients are 1.4 times more likely to report not meeting guidelines exercise ($p<0.01$).

Table 4.4 Multi-level multi-nomial logistic regression predicting exercise intensity

Variable (model significance)	**Not meeting guidelines**		**Moderate intensity**	
	Exp(B)	**p value**	**Exp(B)**	**p value**
Demographics (p=0.042)				
Age	1.1	0.50	1.0	0.77
Sex	0.8	0.76	0.4	0.26
BMI	1.4	**<0.01**	1.1	0.22
Diabetes status	2.0	0.52	1.4	0.73
Blood Biochemistry (p=0.046)				
eGFR	1.0	0.80	1.0	0.86
Haemoglobin	0.9	**<0.01**	0.9	**0.04**
HaemoglobinA1c	0.5	0.14	0.7	0.39
Exercise (p=0.09)				
VO_2peak	3.1	0.08	0.7	0.41
Grip	<0.01	0.95	0.9	0.34
Physical activity levels	2.7	0.10	1.3	0.26

Higher intensity is the reference category

4.5 Discussion

This is the first study to investigate the feasibility and physiological and clinical determinants of higher intensity exercise in CKD patients. Understanding the role of higher intensity training in the CKD cohort is vital, as the superiority of this type of training over moderate intensity training in many chronic disease populations has been consistently reported.(188) The main findings of this study were, 1) 43% of participants reported higher intensity exercise during the 12 month intervention and these participants had significantly greater exercise capacity at 12 months than participants reporting both moderate intensity and not meeting guidelines; 2) participants reporting higher intensity exercise had significantly higher

baseline cardiorespiratory fitness, exercise capacity and haemoglobin than participants reporting not meeting guidelines; 3) baseline haemoglobin levels was the only variable which was significantly higher in participants reporting higher intensity exercise compared to both other groups; 4) baseline haemoglobin was strongly associated with exercise capacity at 12 months and was a significant predictor of participants reporting higher intensity exercise.

Feasibility of higher intensity exercise

In the present study a large number of CKD patients reported being able to perform higher intensity exercise (43%), despite most patients being below their age predicted VO_2peak at baseline. This finding is clinically significant as recent studies have shown that performing higher intensity exercise is associated with greater health benefits when compared to moderate intensity exercise.(58) The reported benefits in these studies are increased cardiorespiratory fitness,(190-198) mitochondrial biogenesis,(192, 196) improved ejection fraction and stroke volume,(193, 197) suppression of fatty acid uptake and lipogenesis(192) and an increase in flow mediated dilatation, suggesting improved endothelial function.(191-193, 196) The CKD population have demonstrated reductions or impaired function in each of the above parameters that can potentially be improved with HIIT.(113, 124, 199) It therefore seems reasonable to suggest that implementing this type of training may be an effective option for CKD patients to improve cardiovascular health. The present study suggests that it is feasible for CKD patients to include higher intensity exercise in their training program, with 43% of participants regularly undertaking this type of training during the intervention. One way of including higher intensity training is to perform intervals- alternating short bursts of high intensity exercise with rest periods or light exercise. This type of training may be a more suitable way for chronic disease sufferers to achieve health enhancing benefits.

In this study, participants who reported completing higher intensity exercise had significantly greater exercise capacity after the intervention than those performing moderate intensity exercise and those categorised as not meeting physical activity guidelines. This is clinically significant as for every 1 MET increase there is a 12% reduced risk of mortality.(29) This finding may indicate that 60(86.3) minutes (Table 2) of moderate intensity exercise may be adequate to elicit a modest increase in exercise capacity in patients with low exercise capacity. At baseline the higher intensity group were meeting the Australian exercise

guidelines of 150 minutes/week of moderate intensity exercise (Australia's Physical Activity and Sedentary Behaviour Guidelines 2014), whereas the other two groups were reporting less physical activity time in an average week in the 6 months prior to participating in the study. This suggests that participation in higher intensity exercise may be dependent on previous physical activity levels. It is not particularly surprising that participants reporting higher intensity exercise during the intervention had higher physical activity and fitness levels at baseline than the other two groups. This finding may indicate that the perceived exertion of high intensity exercise is more attainable to someone with a higher fitness level and previous physical activity experience, even though participation in higher intensity exercise specifically at baseline was relatively low (20%). The aim of this study was to identify whether it was feasible for CKD patients to participate in higher intensity exercise. A previous study has identified that it *is* possible for patients with very low fitness levels (VO_2peak of 13±1.6 ml/kg/min) to participate in high intensity training.(193) However, the findings from the current study may suggest that it is most realistic to expect more physically active and fitter patients to participate in this type of exercise in an unsupervised home-based setting. Alternatively, if patients with lower fitness are prescribed higher intensity exercise, they may need greater support to achieve these goals.

Determinants of higher intensity exercise

Individuals who performed higher intensity exercise were more likely to have normal haemoglobin levels at baseline than those who completed both moderate and not meeting guidelines. These findings suggest additional considerations may be necessary when providing exercise prescription for chronic disease patients with low haemoglobin. Reduced haemoglobin can limit VO_2max, through the reduced oxygen-carrying capacity of the blood.(84) The attenuated oxygen carrying capacity of the blood creates a decrease in exercise ability and time to exhaustion.(200, 201) Furthermore, a lack of oxygen to the working muscles decreases physical performance by limiting muscular function.(85, 202) The kidneys synthesise and secrete most of the endogenous EPO- the hormone that regulates red blood cell production.(87) The kidney damage which occurs in CKD, can impair the synthesis and release of EPO, resulting in a chronic reduction in red blood cells.(87) As a result of this, anaemia is a common problem in CKD patients, as demonstrated in a study by McClellan et al. (2004) of 5222 males and females, in where 47.7% of patients had low

haemoglobin <12 g/dL.(88) However, it was identified that only 15.9% of the patients in the current study were considered to have low haemoglobin (<12 $g \cdot dL^{-1}$) at baseline.

Notwithstanding the physiological influence of anaemia on exercise performance, the fatigue that occurs with lower haemoglobin levels may also influence the motivation to partake in physical activity and in particular, higher intensity exercise. Some studies have indirectly assessed the influence of haemoglobin on physical performance. Leikis et al. (2006), followed 12 stage 3-4 CKD patients over two years and identified that VO_2peak declined, alongside renal function, despite maintenance of haemoglobin levels.(90) However, it should be noted the haemoglobin levels were reasonably high in this study (12.9±9 dg/L). Likewise, Kaysen et al. (2011) found haemoglobin to be inversely related with a physical function questionnaire but not with a short physical performance battery.(91) The findings from the present study suggest that haemoglobin levels are indeed a strong predictor of exercise capacity at 12 months in CKD patients. The effect of haemoglobin on exercise capacity was independent of diabetes status and kidney function. The lack of difference in eGFR and physical activity levels between participants with differing levels of haemoglobin, suggests that the haemoglobin levels are not just an indication of overall health. Rather, the fact that cardiorespiratory fitness, previous physical activity and haemoglobin were all higher in participants reporting higher intensity exercise, may suggest that haemoglobin could be used as a surrogate marker of exercise capacity.

As anaemia is associated with increased risks of morbidity and mortality,(89, 203) studies have looked at the effects of normalising haemoglobin levels. However, studies looking at normalization of haemoglobin levels in CKD and anaemia such as the CREATE,(204) TREAT,(205) NEPHRODIAB2(206) and CHOIR(207) studies, have found that correcting haemoglobin to normalized levels (130-150g/L) did not improve mortality outcome and moreover may be detrimental to cardiovascular health. The avoidance of prescribing erythropoietin stimulating agents to CKD patients is due in part to exacerbation of hypertension and subsequent increase in events of stroke and mortality.(208) Hypertension is the most common complication in CKD and can be aggravated by ESA treatment. Rather than promote the normalization of haemoglobin levels in CKD patients, this study aims to highlight the effects anaemia may have on participation of high intensity exercise. Exercise Physiologists should consider a patient's haemoglobin levels and tailor exercise training programs appropriately. As such, patients with low haemoglobin may require additional

supervision and motivation to ensure adherence to the exercise program. Alternatively, it may be more suitable to prescribe moderate intensity exercise to patients with lower haemoglobin, if that is more likely to be adhered to.

The lack of association between eGFR at baseline and METs at 12 months may suggest kidney function is not a limiting factor in improving exercise capacity.(209) Nonetheless, this lack of association may be a reflection of the narrow range of eGFR's which were included in this study. Ischaemic heart disease was also not significantly different between the three groups, nor did it correlate with exercise capacity at 12 months. Promisingly, this would suggest that existing cardiovascular disease is not a limiting factor in CKD patients participating in higher intensity exercise.

Based on the findings from this study, it seems reasonable to suggest that there are certain characteristics which would typify a person most suitable to performing higher intensity exercise.(30) Table 4.5 provides a proposed guideline of patient characteristics which may elicit the greatest improvements in exercise capacity from higher intensity exercise. This guideline is not designed to omit individuals from higher intensity exercise who do not possess these attributes, rather emphasising that they may require more supervision or guidance in performing this type of exercise. Certainly, exercise prescription requires an individualized approach and should be assessed on a case by case basis.

Table 4.5 Proposed guidelines for characteristics of patients most likely to see the greatest improvements in exercise capacity from higher intensity exercise, in order of significance

Characteristic	Ideal value
VO_2peak (ml/kg/min)	>22.1
METs	>7.3
Grip strength (kg)	>34
Age (years)	<61
BMI (k/m^2)	<32.4
Diabetes	No
Haemoglobin (g/L)	>133
Sex	Male
Exercise time (minutes/week)	>73.5

Not surprisingly, BMI was shown to be a significant contributor to participants reporting not meeting physical activity guidelines. This is an obese population and the majority of participants were reporting no physical activity at baseline. This association is likely bi-directional in that being overweight or obese provides a further barrier for participation in physical activity.(210) Although it is feasible for obese individuals to participate in higher intensity exercise,(196) the breathlessness associated with obesity(211) may limit the motivation to participate in this type of exercise. Increased breathlessness associated with being overweight may have limited the motivation to participate in higher intensity exercise. Indeed, this may be evident in the higher weight seen in the not meeting guidelines group. To reverse this cycle it is imperative ways to improve physical activity levels in CKD patients are identified. Despite an improvement in physical activity in a number of participants there were still 32% of participants not meeting physical activity guidelines after 12 months of the intervention. This may suggest that eight weeks of training followed by a home program, telephone follow-up calls and gym refresher sessions is not sufficient to maintain physical activity for 12 months in many CKD patients. It is likely that for some CKD patients, constant supervision may be the only option in successfully integrating a positive lifestyle change.(212, 213)

Limitations

The relatively small sample size is a limitation to the study. It is also an observational study of the intervention arm of a randomized control trial and therefore true predictions/causations cannot be gained. As this is the first study of its kind, it provides ground work for a randomized control trial to establish the longitudinal effects of different exercise intensities in CKD patients. Considering the main findings of the paper are based on self-reported exercise intensity undertaken throughout the intervention, the use of a recall questionnaire is a limitation to this study. The psychometric properties of the Active Australia questionnaire has been correlated with pedometer steps and the total physical activity minutes has been reported to be significantly correlated with physical function scores, demonstrating acceptable convergent validity for community-dwelling older adults.(137) However, another study by the same author, reported that some older adults were confused with questionnaire phrasing, misunderstood the scope of activities to include in answers, misunderstood the time frame of activities to report and struggled to accurately estimate the frequency and duration

of their activities.(214) Nonetheless, clarity in the Active Australia questionnaire was ensured by administering of the questionnaire by an Exercise Physiologist. However, concurrent use of an objective measure of physical activity, such as accelerometry or pedometry, to provide cross-validation of the Active Australia questionnaire at certain time points would have confirmed the reported physical activity levels.

This study focusses on the physiological and clinical determinants of participation in higher intensity exercise in patients with CKD. There are a number of other social and environmental factors which may also influence participation in higher intensity exercise. The narrow scope of this thesis to only quantitative outcome measures is a limitation to the study. Indeed, other factors such as motivation, education, socioeconomic status, mental health, peer influences, nutritional status, physical environment and co-morbidities, to name a few, may also influence participation in higher intensity exercise. A prediction model including all possible quantitative factors relating to exercise involvement (as mentioned above) would help explicate the true range of determinants related to higher intensity exercise participation. Future studies investigating the feasibility and determinants of higher intensity exercise should also include qualitative analysis of the psychosocial aspects of participation in exercise training. Qualitative exploration may also elucidate the enjoyment and motivation to undertake this type of training, to ensure long-term exercise adherence. It should also be noted that as this is a lifestyle intervention, the impact of patient interactions with the nurse practitioner, dietitian, diabetic educator and psychologist may also have provided support to participate in differing exercise intensities, rather than just support solely from the exercise physiologist.

Conclusions

The study was successful in increasing higher intensity exercise in some patients with CKD. Participants who reported completing higher intensity exercise also had the greatest exercise capacity after the 12 month intervention compared to patients reporting moderate intensity and not meeting guidelines. Participants who reported performing higher intensity exercise were more likely to have higher haemoglobin levels at baseline. These findings suggest that in CKD patients haemoglobin levels are a strong predictor of exercise capacity at one-year and have a likely influence of fatigue on motivation. This preliminary study provides interesting findings and suggestions for future high intensity training to be performed in the

CKD population. Future studies should identify whether low haemoglobin levels in CKD patients influences the change in exercise capacity with higher intensity exercise.

Chapter 5.
Agreement between cystatin-C and creatinine based eGFR estimates after a 12 month lifestyle intervention

This manuscript is in preparation for *American Journal of Kidney Diseases*. This chapter contains an abridged methods section to avoid replication of the methods described in Chapter 2.

5.1 Abstract

Background: Estimation of glomerular filtration rate (eGFR) using formulae based on serum creatinine concentrations are commonly used to assess kidney function. Physical exercise can increase creatinine turnover and lean mass; therefore this method may not be suitable for use in exercising individuals. Alternatively, cystatin-C based eGFR formulae may be a more accurate measure of kidney function in studies examining the impact of exercise in CKD patients. The aim of this study was to assess the agreement of four different creatinine and cystatin-C based estimates of GFR before and after a 12 month LI intervention. It was hypothesised that there would be poor agreement between these measures after 12 months of exercise training due to the impact of exercise on serum creatinine.

Methods: 142 participants with stage 3 or 4 CKD (eGFR 25-60 ml/min/1.73 m^2) were included. Subjects were randomised into either a control group (standard nephrological care) or a LI group (12 months of predominantly home-based aerobic and strength based exercise training). Four eGFR formulae were compared at baseline and after 12 months: 1) modification of diet in renal disease creatinine (MDRDcr), 2) CKD-epidemiology collaboration creatinine (CKD-EPIcr), 3) CKD-EPIcystatin-C (CKD-EPIcys) and 4) CKD-EPIcr-cys.

Results: The LI resulted in significant improvements in exercise capacity (1.9±1.8 METs). Lean mass ($r=0.319$, $p<0.01$) and grip strength ($r=0.391$, $p<0.001$) were associated with serum creatinine at baseline. However, there were no significant correlations between cystatin-C and the same measures. There was no change in lean mass in both control and LI groups during the 12 months. CKD-EPIcys was considerably lower than CKD-EPIcr at both baseline and 12 months (both groups baseline= -7.9±8.6 and LI at 12 months= -8.4±12.3 and Control at 12 months= -13.1±11.8 ml/min/1.73 m^2), CKD-EPIcr-cys (both groups baseline= -3.1±3.7 and LI at 12 months= -2.5±5.5 and Control at 12 months= -4.5±4.5 ml/min/1.73 m^2) and MDRDcr (both groups baseline= -8.5±8.6 and LI at 12 months= -9.2±12.2 and Control at 12 months -14.4±11.4 ml/min/1.73 m^2).

Conclusions: Cystatin-C and creatinine based eGFR both provided similar estimates of kidney function between baseline and after 12 months of participating in a predominantly home-based exercise training program. However, CKD-EPIcys was considerably lower than CKD-EPIcr and MDRDcr at both baseline and 12 months. A combination of cystatin-C and

creatinine in eGFR measurements may provide the most accurate assessment of kidney function in exercising individuals.

5.2 Introduction

The most common approach used to assess kidney function is by estimation of glomerular filtration rate based on serum creatinine concentrations, as it can be calculated routinely from standard tests and is inexpensive. Physical exercise can cause an increase in serum creatinine;(215) therefore this method may not be suitable for use in exercising individuals. Alternatively, cystatin-C based eGFR is suggested to be an improved method for eGFR measurement and may provide a more precise estimate of kidney function.(216) The agreement between cystatin-C and creatinine based eGFR before and after an LI in CKD patients is yet to be studied.

The current reference method for measuring kidney function is to quantify the clearance of an exogenous marker such as inulin to determine GFR.(8) However, this measure is rarely used clinically due to the test complexity and cost. Indirect assessment of endogenous markers to provide an estimation of GFR is commonly used for routine clinical measurements via different formulas.(9) These formulae use either a measure of creatinine or cystatin-C in serum or urine, along with patient characteristics; age, sex and race to estimate filtration rates. The four most frequently used eGFR equations are: 1) MDRDcr(10), 2) CKD-EPIcr(11), CKD-EPIcys(12) and CKD-EPIcr-cys(12).

Creatinine based eGFR measures are commonly used as a ‘first test’ and for routine clinical assessment of kidney function.(12, 18) Creatine phosphate is taken up by muscle after it is released into the circulatory system following synthesis in the liver. Creatinine is formed as the by-product of muscle creatine breakdown during muscle contraction(217) and therefore can be directly influenced by muscle mass.(218, 219) This is an important limitation for estimation of GFR as muscle mass is often reduced in the CKD population.(219) Furthermore, intense exercise may cause a breakdown of muscle leading to an increase in serum creatinine levels,(215) potentially making creatinine-based eGFR measures inaccurate in individuals participating in higher intensity exercise. Cystatin-C is a low molecular weight cystine protease inhibitor that is produced at a constant rate by all nucleated cells and is not influenced by muscle mass.(220) The use of cystatin-C in the eGFR equation is suggested to

be the gold-standard measure of eGFR due to its increased association with risk of death and progression to end-stage renal disease.(221)

Exercise training is important for patients with CKD as it has been shown to improve physical function and cardiovascular disease risk factors in a number of studies.(39, 75, 77) Therefore, it is important to identify whether there is a difference in the agreement between cystatin-C and creatinine based eGFR measures after exercise-induced adaptations occur. It is also important to assess the impact of exercise training on kidney function – such as by slowing the rate of decline or the potential for harm by volume depletion.

The aim of this study was to assess the agreement between cystatin-C and creatinine based eGFR estimates following an LI. It was hypothesised that there would be poor agreement between these measures after 12 months of exercise training due to the impact of exercise on serum creatinine.

5.3 Methods

This study included 142 subjects with stage 3-4 CKD, with 74 participants randomized to the intervention group and 68 to the control group (Figure 5.1). This analysis is looking at the first 12 months of the LM3 study in both control and intervention groups.

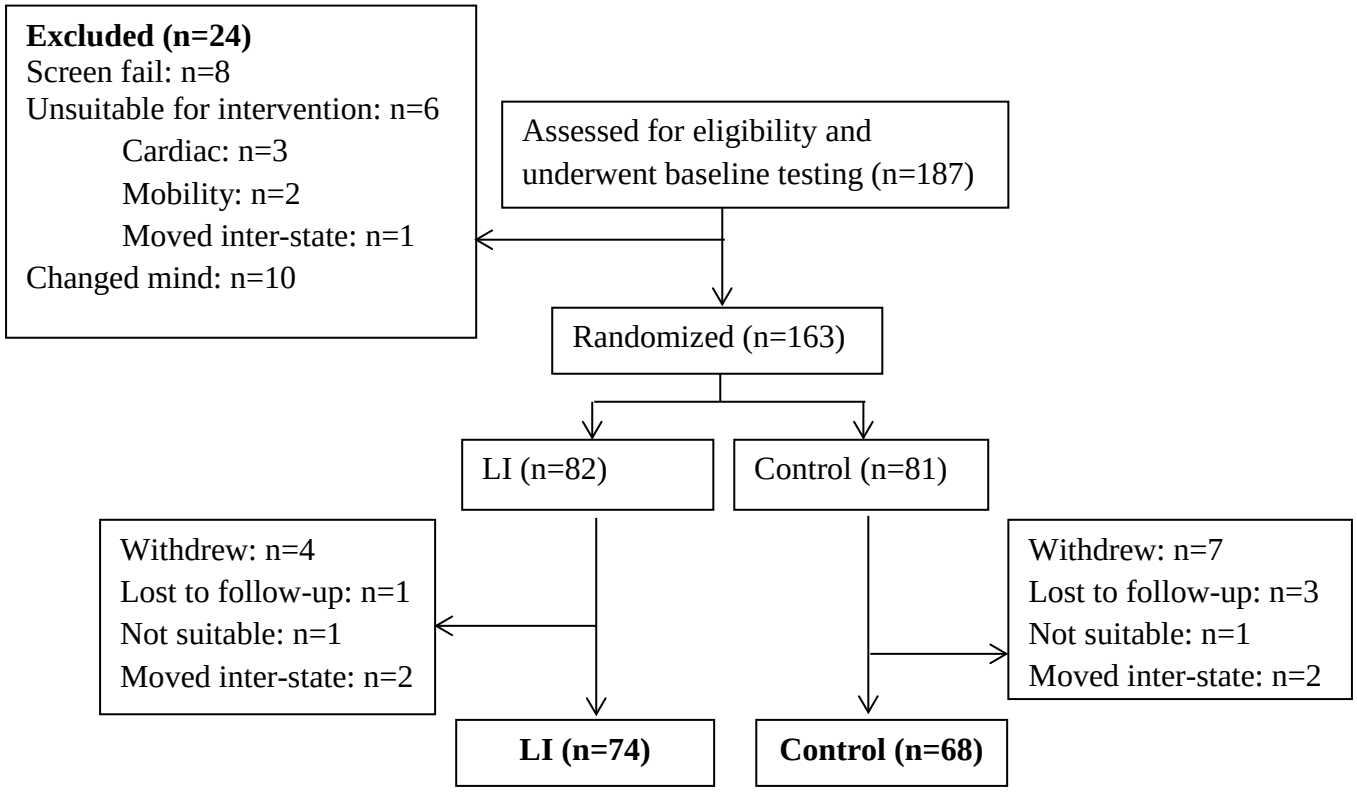


Figure 5.1 Consort diagram

Four measures of renal function were assessed by eGFR; MDRDcr$_{-175}$ study equation (2007),(10) CKD-EPIcr equation (2009),(11) CKD-EPIcys equation (2012)(12) and CKD-EPIcr-cys equation (2012)(12). All eGFR's are made relative to a body surface area of 1.73m^2. An improvement in either total MET minutes or vigorous MET minutes is determined from the change from baseline to 12 months. Due to the changes in total mass influencing the percentage of lean mass, baseline and change in appendicular lean mass are reported in absolute values. DEXA was performed on a sub-set of patients due to limited machine availability (n=73). Blood was collected after an overnight fast prior to any other tests. Participants were asked to refrain from any exercise on the morning of the test.

Statistics

Mean±standard deviation (SD) was used to describe normally distributed baseline characteristics, with percentages used to describe frequencies for categorical variables. Median [interquartile range (IQR)] was used to describe not normally distributed variables

and variables transformed by the natural logarithm. Pearson's correlation coefficient was used to assess association for normally distributed and log transformed variables. Spearman's correlation was used for associations of categorical variables, or when when there was at least one variable not normally distributed. Comparison between groups was assessed by an independent t-test of the delta from baseline to 12 months. The Mann-Whitney U test was performed on not-normally distributed variables. Differences between groups for categorical variables were analysed using Pearson's Chi Square test. Within group differences were assessed by paired t-tests for normally distributed and log transformed variables. Wilcoxon-Sign rank test was used for not normally distributed variables. Participants were considered to have improved their lean mass, grip strength, total MET minutes and vigorous intensity exercise if the delta from baseline to 12 months was greater than zero. Bland-Altman analysis was used to assess agreement between eGFR measurements using GraphPad Prism 7. All other statistical analyses were performed on IBM SPSS Statistics 22. Statistical significance was set at $p<0.05$.

5.4 Results

Patient characteristics

There were no significant differences between groups for any of the baseline characteristics or medication use (Table 5.1). Patients were on average 62 years of age and generally obese with an average body mass index (BMI) of 32 kg/m^2. Mean VO_2peak for the group was considered very poor, according to the American College of Sports Medicine (ACSM) (2010) normative values for men and women aged 60-69, at 23.6 ml/kg/min.(222) The number of participants who changed their hypertensive or diuretic medication (commenced, ceased or no change) was not significantly different between the control and LI groups (Table 5.2).

Table 5.1 Baseline characteristics

Variable	Control (n=68)	LI (n=74)	p value
Age (years)	63.5[9.4]	60.5[14.2]	0.23
Male sex, n(%)	41(60.3)	44(59.5)	0.92
African American, n(%)	0(0)	1(1.4)	0.34
Diabetes, n(%)	27(42.2)	32(43.8)	0.85
Systolic blood pressure (mmHg)	133.0[26.5]	130.0[18.0]	0.18
Diastolic blood pressure (mmHg)	80.0[12.0]	78.0[10.0]	0.56
ACE inhibitor, n(%)	34(53.1)	34(47.9)	0.54
ATRB, n(%)	30(45.5)	44(59.5)	0.10
Thiazide, n(%)	16(25)	13(18.3)	0.35
Spironolactone, n(%)	1(1.4)	3(4.9)	0.89
Loop diuretics, n(%)	14(20.6)	15(21.2)	0.24
Statin, n(%)	41(64.1)	46(64.8)	0.93

ACE=angiotensin-converting-enzyme; ATRB=angiotensin receptor blocker. Median[IQR] and n(%) is reported

Table 5.2 Change in hypertensive and diuretic medications during the 12 month study period

Medication	Control		LI		p value
	Commenced	Ceased	Commenced	Ceased	
ACE inhibitor, n(%)	+1(1.5)	-5(7.4)	+1(1.4)	-7(9.5)	0.83
ATRB, n(%)	+4(5.9)	-2(2.9)	+6(8.1)	-6(8.1)	0.30
Thiazide, n(%)	+1(1.5)	-2(2.9)	+1(1.4)	-3(4.1)	0.90
Spironolactone, n(%)	0	-2(2.9)	0	0	
Loop diuretics, n(%)	+6(8.8)	-3(4.4)	+4(5.4)	-3(4.1)	0.76

ACE=angiotensin-converting-enzyme; ATRB=angiotensin receptor blocker

Fitness measures

Table 5.3 demonstrates the baseline and within group changes in all patient characteristics in the LI and control groups. Compared to the control group, the LI group had significant ($p<0.05$) improvements in VO_2peak and METs and a close to significant ($p=0.05$) increase in

6 minute walk time. There were also treatment effects of get up and go time, moderate intensity activity, time spent walking and total activity, such that there was a significant improvement in the LI compared to the control group. There were no significant ($p>0.05$) between-group differences in appendicular lean mass, grip strength or vigorous intensity exercise. There were also no within group changes in lean mass or vigorous intensity exercise in the LI group.

Table 5.3 Change in patient characteristics, exercise parameters and eGFR measurements from baseline to 12 months

Variable	Control		LI		p value
	Baseline	Δ 12m	Baseline	Δ 12m	
Weight (kg)	94.9±22.2	**1.3±4.5***	93.9±20.3	**-1.8±6.1*#**	**<0.01**
Body mass index (kg/m^2)	33.3±6.9	**0.5±1.6***	33±6.1	**-0.6±2.2*#**	**<0.01**
VO_2peak (ml/kg/min)	23.2±5.4	**-0.9±4.2***	23.2±7.6	**1.7±3.7*#**	**<0.01**
Exercise capacity (METs)	7.5±2.6	-0.1±1.9	7.5±3.7	**1.9±1.8*#**	**<0.001**
6 min walk (m)	483.8±95.9	3.0±67.9	493.8±106.4	**29.5±63.9***	0.05
Grip strength (kg)	32.8±10.3	-1.6±6.7	35.4±11.8	**-2.2±7.2***	0.67
Get up and go (s)	4.8(1.3)	**0.4(1.1)***	5.1(2.0)	**0.0(1.1)#**	**0.02**
Appendicular lean mass (kg)	23.6±5.7	0.0±0.0	24.1±5.6	0.0±0.0	0.31
Walking/week (hours)	0.7(2.3)	0.0(1.0)	0.7(2.0)	**0.5(1.6)*#**	**0.02**
Moderate/week (hours)	0.0(0.0)	0.0(0.0)	0.0(0.0)	**0.2(1.5)*#**	**<0.01**
Vigorous/week (hours)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.3)	0.27
Total activity (hours)	1.2(3.5)	0.0(2.7)	1.0(3.0)	**1.8(3.2)*#**	**<0.01**
Total activity (METhours)	3.8(13.5)	0.0(11.0)	3.3(11.8)	**8(13.7)*#**	**<0.01**
Kidney function measures					
Creatinine (µmol/L)	143.8±33.3	4.6±26.8	150.3±33.9	6.3±29.2	0.75
Cystatin-C (mg/L)	2.1±0.5	**0.2±0.4***	2.1±0.5	0.1±0.4	0.14
MDRDcr (ml/min/1.73m^2)	42.7±9.7	0.0±8.1	41.2±9.1	-0.8±8.5	0.62
CKD-EPIcr (ml/min/1.73m^2)	41.9±10.1	-0.2±8.4	40.7±9.6	-0.8±8.9	0.67
CKD-EPIcys (ml/min/1.73m^2)	30.4±8.9	**-2.4±6.2***	31.5±10.3	-0.7±7	0.17
CKD-EPIcr-cys (ml/min/1.73m^2)	34.5±8.5	**-1.5±6.3***	34.6±9.4	-1.5±5.8	0.97
Protein:creatinine	38.0(85.5)	-0.5(23.8)	38.0(88.8)	-1.0(32.8)	0.42
Albumin:creatinine	14.0(63.5)	0.0(21.5)	14.5(69.4)	0.0(17.7)	0.47

Delta is calculated by 12 month minus baseline. Mean ± standard deviation is presented for normally distributed delta variables. Median(IQR) is presented for not normally distributed delta variables. p value presented indicates significance of delta values between groups.
*= within group statistical significance, # and values in bold in the p value column=between group statistical significance of delta values (baseline to 12 months)

Kidney function

Table 5.3 shows no significant within group changes in the LI group for any of the kidney function measures. The control group had a significant increase in cystatin-C over 12 months with subsequent decreases in CKD-EPIcys and CKD-EPIcr-cys. There were no significant between group differences for any of the changes in eGFR measures over the 12 months. Further analysis was conducted by combining control and intervention participants and then separating into two groups; those that had an increase in lean mass (n=17) and those who decreased appendicular lean mass (Table 5.4). There were still no significant changes in any eGFR measures between those who increased appendicular lean mass and those who decreased appendicular lean mass. Likewise, there was no significant change in eGFR in those who did/did not increase their total physical activity time (METhours) (n=72), and those who did/did not increase their grip strength (n=45).

Table 5.4 The difference between those who improved and declined appendicular lean mass, total physical activity time and grip strength

	Appendicular lean mass (kg)			Total physical activity time (hours)			Grip strength (kg)		
	Improvement	**Decline**	**p value**	**Improvement**	**Decline**	**p value**	**Improvement**	**Decline**	**p value**
MDRDcr	-3.6±7.7	-0.7±5.9	0.24	-1.6±8.7	-2±8.6	0.69	0.3±9.0	1.3±7.7	0.36
CKD-EPIcr	-3.8±7.9	-0.6±5.9	0.21	-1.5±9.0	1.9±9.6	0.79	0.2±9.5	-1.4±7.9	0.35
CKD-EPIcys	-1.1±7.4	-2.5±4.7	0.53	-1.7±7.3	-1±8.6	0.79	-1.4±6.2	-1.9±6.9	0.68
CKD-EPIcr-cys	-2.7±4.9	-2.1±4.8	0.77	-0.4±7.2	-2±5.6	0.37	-1.1±6.1	-2.4±5.3	0.27

Association between creatinine and lean mass and strength

Table 5.5 shows correlations between kidney function estimates with fitness and body composition measures in all patients at baseline. Creatinine was significantly associated with lean mass (r=0.32, p<0.01) and grip strength (r=0.39, p<0.001). However, Cystatin-C was not correlated with lean mass or grip strength. There were also no significant associations between the change in creatinine or cystatin-C and the change in fitness and body composition measures (Table 5.6).

Table 5.5 Associations between kidney function estimates with fitness and body composition measures in all patients with creatinine and cystatin-C based eGFR measures at baseline (correlation coefficient presented as r)

	Baseline (r value)					
	Creatinine	Cystatin-C	EPIcr	EPIcys	EPIcr-cys	MDRD
VO_2peak (L/min)	0.11	-0.04	**0.20***	0.16	**0.20***	0.18
VO_2peak/lean (L/min/kg)	-0.15	-0.10	0.17	0.12	0.15	0.11
Appendicular lean mass (kg)	**0.32***	0.12	-0.06	0.004	-0.01	-0.05
Grip strength (kg)	**0.39***	0.02	0.01	0.11	0.08	0.03

*= significantly correlated (p<0.05)

Table 5.6 Associations between delta kidney function estimates with delta fitness and body composition measures from baseline to 12 months

	Delta creatinine (r value)	**Delta cystatin-C (r value)**
Delta VO_2peak (ml/kg/min)	0.01	-0.17
Delta exercise capacity (METs)	0.02	-0.12
Delta appendicular lean mass (kg)	<-0.01	0.08
Delta grip strength (kg)	-0.02	-0.10

Agreement of eGFR measures

Table 5.7 shows the agreement between CKD-EPIcys with the other three estimates at baseline in all patients. The Bland-Altman analysis identified CKD-EPIcys and CKD-EPIcr-cys to be considerably lower than CKD-EPIcr and MDRDcr for estimating GFR. Figure 5.2 indicates CKD-EPIcys to have lower average values than CKD-EPIcr at baseline in all patients (eGFR 7.9 below CKD-EPIcr). This bias was consistent at 12 months in both LI (eGFR 8.4 below CKD-EPIcr) and control groups (eGFR 13.1 below CKD-EPIcr). Only CKD-EPIcys and CKD-EPIcr are reported in Figure 5.2 (baseline), Figure 5.3 (12 months LI patients) and Figure 5.4 (12 months control group) in order to demonstrate the difference between cystatin-C and creatinine using similar equations (ie. CKD-EPI equation).

Table 5.7 Bland-Altman agreement of CKD-EPI cys with the other three estimates at baseline in all patients

	EPIcr	**EPIcr-cys**	**MDRDcr**
EPIcys	-7.9±8.6	-3.1±3.7	-8.5±8.6
EPIcr		7.3±12.7	0.5±1.6
EPIcr-cys			-7.9±12.1

Data is reported as bias ± SD of bias.

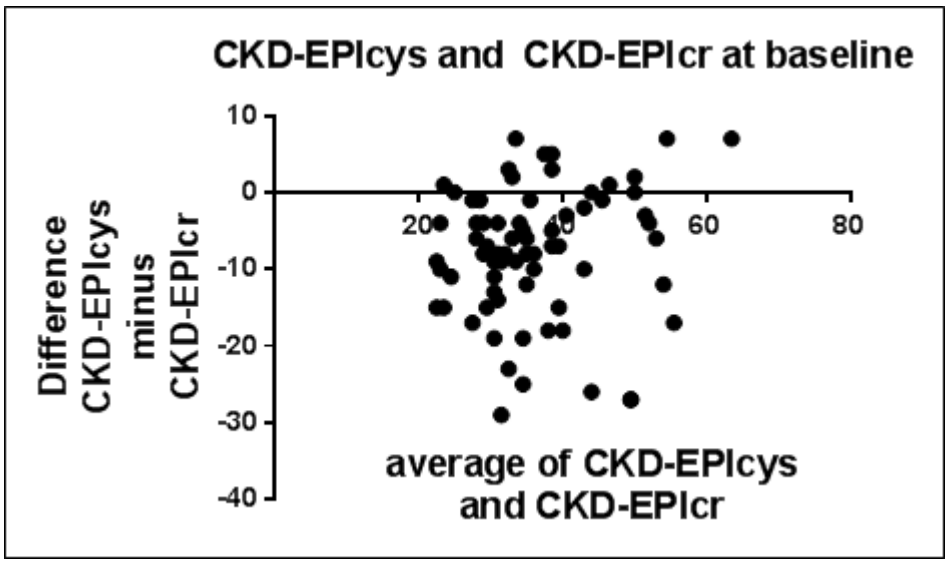


Figure 5.2 Bland-Altman plots at baseline for CKD-EPIcr compared to CKD-EPIcys. CKD-EPIcys minus CKD-EPIcr is divided by the mean of CKD-EPIcys+CKD-EPIcr. CKD-EPIcys is shown to be 7.9±8.6 ml/min/1.73m^2 less than CKD-EPIcr at baseline

Table 5.8 Bland-Altman agreement of CKD-EPIcys with the other three estimates at 12 months in LI patients

	EPIcr	EPIcr-cys	MDRDcr
EPIcys	-8.4±12.3	-2.5±5.5	-9.2±12.2
EPIcr		5.9±6.9	-0.5±2.2
EPIcr-cys			-6.7±6.8

Data is reported as bias ± SD of bias.

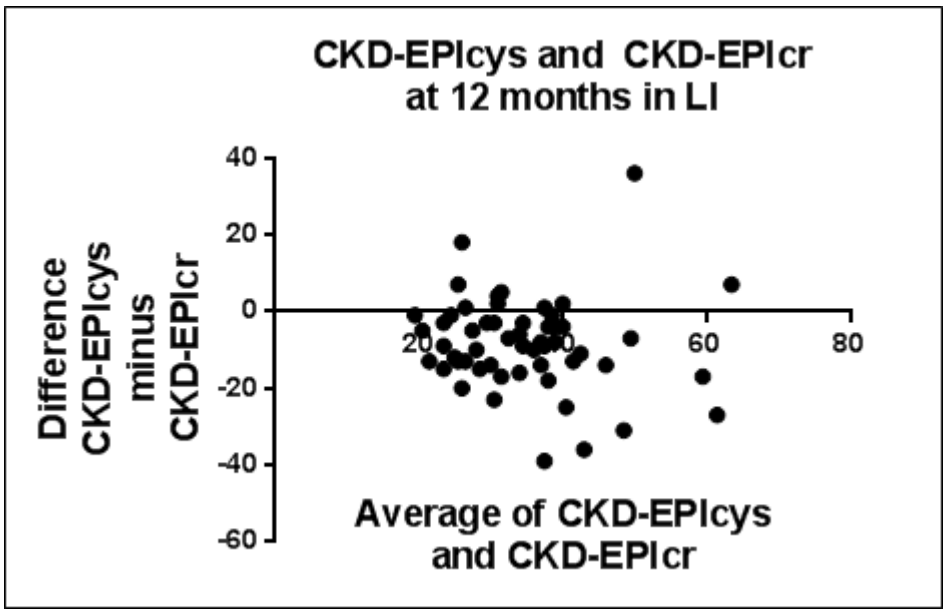


Figure 5.3 Bland-Altman plots at 12 months in LI patients for CKD-EPIcr compared to CKD-EPIcys. CKD-EPIcys minus CKD-EPIcr is divided by the mean of CKD-EPIcys+CKD-EPIcr. CKD-EPIcys is shown to be 8.4±12.3 ml/min/1.73m^2 less than CKD-EPIcr

Table 5.9 Bland-Altman agreement of CKD-EPIcys with the other three estimates at 12 months in control patients

	EPIcr	EPIcr-cys	MDRDcr
EPIcys	-13.1±11.8	-4.5±4.5	-14.1±11.4
EPIcr		8.6±7.4	-1.0±1.5
EPIcr-cys			-9.6±7.0

Data is reported as bias ± SD of bias.

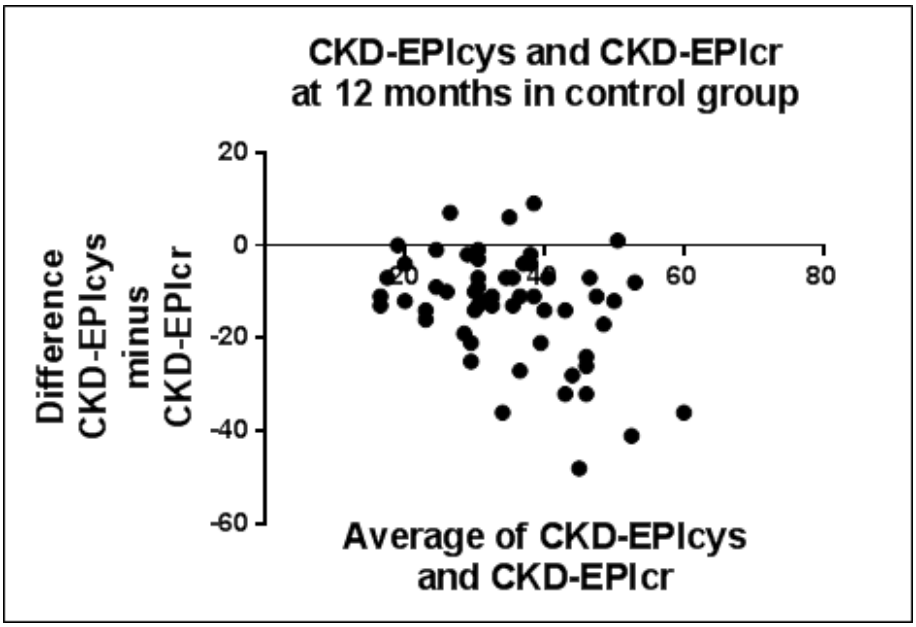


Figure 5.4 Bland-Altman plots at 12 months in the control group for CKD-EPIcr compared to CKD-EPIcys. CKD-EPIcys minus CKD-EPIcr is divided by the mean of CKD-EPIcys+CKD-EPIcr. CKD-EPIcys is shown to be 13.1±11.8 ml/min/1.73m^2 less than CKD-EPIcr

5.5 Discussion

This is the first study to investigate the agreement between cystatin-C and creatinine based eGFR measures before and after a 12 month LI. The findings from this study indicate that 1) cystatin-C based eGFR estimates are considerably lower than creatinine eGFR estimates; 2) the agreement between cystatin-C and creatinine eGFR was consistent at baseline and after the LI; and 3) lean mass and grip strength were correlated with creatinine and *not* cystatin-C at baseline.

The Bland-Altman analysis showed a considerably lower eGFR measured by CKD-EPIcys than by CKD-EPIcr and MDRDcr. Not surprisingly, the agreement between CKD-EPIcr and MDRDcr was similar. Furthermore, as expected the difference between CKD-EPIcr-cys and the other measures was midway between CKD-EPIcr and MDRDcr, and CKD-EPIcys. An overestimation of eGFR using the MDRDcr equation has previously been reported by Lamb et al. (2003).(223) Although, in this study comparisons are made to creatinine clearance rather than CKD-EPI equations or cystatin-C based measures. CKD-EPIcr is thought to produce less biased estimates of eGFR at higher levels of kidney function, however is less accurate when GFR falls below 60 ml/min/1.73m^2.(136) On the other hand, cystatin-C is suggested to be a more sensitive marker of GFR than creatinine.(224) This is supported in our study, evident by the lower CKD-EPIcys and CKD-EPIcr-cys identified by the Bland-Altman plots when compared to CKD-EPIcr and MDRDcr. The lower lean mass and strength in the study population (69) may be contributing to the higher CKD-EPIcr and MDRDcr measures compared to CKD-EPIcys. Despite the lower CKD-EPIcys at baseline, it is important to note the agreement between the cystatin-C and creatinine based eGFR measures do not significantly differ after 12 months of exercise training. As CKD-EPIcr and MDRDcr are used as routine measurement in standard clinical care, the consistency of these findings support the use of creatinine based eGFR measures in exercising individuals.

The findings from this study identified that lean mass and grip strength were correlated with creatinine and *not* cystatin-C at baseline. This finding is in agreement with evidence that lean mass is significantly related to serum and urinary creatinine but not with cystatin-C, even after adjustment for physical activity levels.(219) From the baseline findings of creatinine correlating with lean mass and grip strength, it would appear that muscle mass and strength would be influencing creatinine based eGFR estimates (CKD-EPIcr and MDRDcr). An intervention resulting in significant increases in strength and mass such as with a specific hypertrophy resistance training program, may potentially provoke a decline in eGFR using creatinine based equations. Indeed, the lack of change in lean mass in the current study limits the ability to make conclusions regarding the effects of exercise induced gains in lean mass on eGFR measures. As such, clinical decisions based on creatinine based eGFR's of patients undertaking hypertrophy training should be considered with caution. Although the small sample size limits the ability to detect a significant change in eGFR,(136) the findings from this study suggest that resistance exercise as commonly prescribed in a health-enhancing

exercise program was not enough to elicit changes in eGFR. It was not expected that this intervention would not provide any significant changes in grip strength or lean mass. However, in this generalizable CKD cohort the lack of increase in grip strength and lean mass is important and warrants further investigation. Future studies should investigate whether specific hypertrophy interventions influences creatinine based eGFR measures.

The equations used to estimate GFR have advanced in precision and accuracy. In 1999, the MDRDcr equation was suggested to be more accurate than the accepted Cockcroft-Gault and creatinine clearance methods.(13) More recently it was proposed that CKD-EPIcr provided a more precise estimate of GFR and categorization for risk of mortality compared to the MDRDcr study equation.(16) CKD-EPIcr was developed using the same variables as MDRDcr but with different coefficients, which created a moderate improvement in overall accuracy.(17) It has been suggested that standardized serum creatinine assays be used as a first test for assessing eGFR in adults due to its cost-effectiveness and that Cystatin-C be used as a more precise confirmatory test if a below normal creatinine eGFR is detected.(18)

Banfi et al. (2006) identified elevated serum creatinine concentration levels in athletes compared to sedentary controls, likely due to a higher muscle mass in the athletes.(225) It is suggested that the observed eGFR reductions are limited to periods when the athlete is unaccustomed to the training load. This is not unlike a CKD patient commencing an exercise program after an extended sedentary period. However, it is promising to note there was no reduction in eGFR in the LI group of the current study, despite a large increase in physical activity levels. This is supported by a number of studies who have shown maintenance of kidney function with exercise training.(36, 75)

Participation in higher intensity exercise may be an important consideration in determining appropriate kidney function estimates. Indeed, Poortmans et al. (1996) showed that post-exercise proteinuria was directly related to the intensity of exercise rather than its duration. Creatinine is derived almost entirely in the muscle as a by-product of creatine synthesis.(226) Subject to the intensity of the exercise, muscle breakdown that occurs during exercise may be evident in serum and urine and as such may transiently underestimate kidney function. Physical activity undertaken in the 24 hour period preceding testing was not recorded in the current study. If the exercise was of high enough intensity it may potentially have resulted in transient increases in serum creatinine. Nonetheless, it was shown by Lippi et al. (2008) that

there was no difference in half-marathon runner's serum creatinine levels 24 hours post moderate-high intensity exercise. There was, however, a statistically significant difference shown immediately post-exercise and a clinically significant difference shown at 3 hours and 6 hours. Nevertheless, in the current study testing was completed in the morning after an overnight fast, and participants were asked to refrain from any exercise the morning of the test. Moreover, it has been reported that measuring renal function a day after moderate-high intensity exercise does not influence kidney function values.(227) However, it is presumed that the rate of creatinine clearance post exercise in half-marathon runners would be higher than the creatinine clearance of CKD patients. Therefore, the rate of creatinine clearance in the study by Lippi et al. (2008) should be interpreted with caution when extrapolated to the CKD population. There are no studies to date which investigate the clearance rate of creatinine after an acute exercise session in CKD patients. This is an area which warrants further research, to ensure 'spot' measures of creatinine based eGFR are not being influenced by prior exercise training. Future studies should evaluate creatinine based eGFR clearance at regular time intervals following exercise sessions of differing intensities.

Cystatin-C also has some reported limitations in its use as a kidney function measure, due to its associations with cardiovascular disease risk factors. After modelling to adjust for measured GFR, Rule et al. (2013) found residual associations of CKD-EPIcys with CKD risk factors, including hypertension, BMI, and C-reactive protein (CRP).(20) This confounding association makes it difficult to establish whether cystatin-C is a true measure of renal function or rather a reflection of CKD risk factors.(220) Due to the limitations of both creatinine and cystatin-C, it has been suggested that using the combination of both measures in the CKD-EPI equation may provide the most accurate assessment of kidney function.(18-20)

Limitations

A significant limitation which needs to be addressed is the large sample size needed to see a change in eGFR. As suggested by Lamb et al (2014), 1000 participants are needed to detect differences in accuracy of measurement between MDRDcr and CKD-EPIcys.(136) The authors of this pilot study found in 1000 subjects, the simulations showed an 87% power at the 5% significance level to detect a difference of 5%. The current study did not have the resources to provide longitudinal exercise training on this scale. For this reason, agreement,

rather than change, was the focus of this analysis. Future large scale exercise interventions comparing creatinine and cystatin-C estimates of GFR measures are needed to explicitly address the hypothesis from this study. Also, as mentioned above, not measuring physical activity levels the day prior to blood collection is a limitation of the study, as recent physical activity may have resulted in transient increases in serum creatinine.

Another limitation of the study is the use of a 'spot' measurement of creatinine and cystatin-C samples. In the initial phases of the program it is likely that patients were more unaccustomed to the exercise training load than closer to the 12 month testing visit. Therefore, the relative exercise intensity (and perhaps an increase in training volume) in the initial intervention period may have resulted in different creatinine and cystatin-C measures than what was observed at 12 months.

Conclusions

The findings from this study found no difference in the agreement between creatinine and cystatin-C estimates of GFR after a 12 month LI. As previously reported, a combination of creatinine and cystatin-C based estimates of GFR will undoubtedly provide the most accurate estimate of GFR, particularly in light of the considerably lower eGFR found with CKD-EPIcys than CKD-EPIcr and MDRDcr. However, if only creatinine based measures are accessible to the treating Physician, the findings from this study suggest it is reasonable to expect creatinine based measures to provide consistent results after prescription of a health-enhancing exercise program. Future exercise training studies comparing cystatin-C and creatinine based eGFR measures in specific hypertrophy and high intensity training programs are warranted to confirm the current findings.

Chapter 6.
Effects of a 36 month lifestyle intervention on exercise capacity and cardiovascular disease risk factors

This chapter contains an abridged methods section to avoid replication of the methods described in Chapter 2.

6.1 Abstract

Background: Exercise training has the potential to improve health outcomes in CKD patients, by improving fitness and cardiovascular disease risk factors; however the feasibility and effectiveness of a long-term lifestyle intervention (LI) that includes predominantly home-based exercise training has not been investigated in this population. The aim of this study was to assess the feasibility and efficacy of a three-year LI on cardiorespiratory fitness, exercise capacity, arterial stiffness and autonomic function.

Methods: Ninety-four patients with stage 3-4 CKD (25-60 ml/min/1.73m^2) were randomized to either a control group (standard nephrological care) or a LI group. The LI comprised aerobic and strength based exercise training, nurse practitioner care, dietary advice and diabetic education (if applicable) in addition to usual nephrological care. Outcome measures assessed every year included exercise capacity (METs), cardiorespiratory fitness (VO_2peak), arterial stiffness (pulse wave velocity [PWV] and augmentation index [AIx]) and autonomic function (heart rate variability [HRV]). Functional capacity (six minute walk time), physical activity levels, grip strength, body composition and standard clinical measures were performed every 6 months.

Results: After 36 months there was a significant increase in physical activity levels in the LI group (LI= +112.5±224.5 mins/week), while the control group decreased slightly (= -12.7±171.1 mins/week, $p<0.01$). The number of patients in the LI group meeting physical activity guidelines (≥150 minutes/week) doubled (baseline = 31.3% to 36 months = 62.2%) compared to a decrease in the control group (baseline = 34.9% to 36 months = 30.6%). Exercise capacity significantly improved in the LI group (LI= +2.2±2.7, control= -0.2±2.2 METs, group x time $p=0.01$) and PWV decreased (LI= -0.1±2.5, control= +0.6±3.1 m/s, group x time $p=0.03$). Furthermore, the LI ameliorated the significant decline in cardiorespiratory fitness seen in the control group (VO_2peak control= -4.2±4.7 ml/kg/min, $p=0.01$ and LI= -1.2±8.5 ml/kg/min, $p=0.92$).A multiple linear regression model including group allocation, age, baseline grip strength and baseline METs significantly predicted the change in exercise capacity from baseline to 36 months ($R^2=0.47$, $p<0.01$). Within group analyses found that the LI significantly improved HRV (total power; LI= +558.0±757.1, $p=0.01$ and control=+150.0±368.3 m/s^2 $p=0.73$).

Conclusions: A 3 year LI doubled the number of patients with moderate CKD meeting physical activity guidelines, as well as significantly improving exercise capacity and arterial stiffness compared to the control group. This study indicates that a LI was feasible and effective in CKD patients over 3 years.

6.2 Introduction

The accelerated cardiovascular disease which is common in patients with CKD means that these individuals are more likely to die of cardiovascular disease than progress to renal failure.(228) The high prevalence of traditional risk factors in CKD patients does not completely explain the increased cardiovascular disease risk and high mortality associated with this disease.(229) Non-traditional risk factors, such as reduced exercise capacity may account for some of the augmented risk. Indeed, reduced exercise capacity is a stronger predictor of survival than many traditional risk factors in end stage kidney disease patients.(187) Patients with CKD have significantly impaired cardiorespiratory fitness and exercise capacity compared to normative values.(209) It has been suggested that muscle wasting, which is common in the CKD population,(106) may be a significant contributor to reduced exercise capacity.(230) Physical inactivity, which contributes to a lower exercise capacity and cardiorespiratory fitness, is a potentially modifiable cardiovascular disease risk factor. Patients with CKD are typically sedentary,(209) thus exercise training seems to be a currently underutilized therapy.

Exercise training should include a combination of aerobic and resistance training,(30) in order to target the reduced exercise capacity *and* muscle atrophy which is prevalent in this population.(69, 209) Despite this, only three non-randomized studies and one randomized study have used a combined (resistance and aerobic) exercise training approach in CKD patients.(51-55) As such, current exercise guidelines for CKD patients are based on limited evidence.(30) However, the improvements in outcomes measures in these studies include increased exercise capacity,(51-53) cardiorespiratory fitness,(54, 55) muscular strength(51) and functional ability(52, 53). Supervised exercise training is suggested to have a cardio-protective effect and improve cardiovascular disease risk factors such as arterial compliance, autonomic control and cardiac function,(231) however this approach is time and resource intensive. Considering approximately 16% of the population aged over 25 are reported to

have at least one marker of kidney damage,(1) evaluating the effectiveness of economical home-based, non-supervised exercise training approaches are warranted.

Telephone interventions have the potential to cost-effectively reach a broad population.(232) Indeed, a six month telephone based intervention by Wilcox et al. (2008) was shown to have similar improvements in physical activity as a 20-week group-based lifestyle behaviour change program in sedentary adults over 50 years of age.(233) Our group has also conducted a predominantly telephone-based intervention of combination aerobic and resistance exercise training in patients with type two diabetes mellitus.(212) In this study participants underwent four weeks of supervised gym based training followed by 48 weeks of telephone follow-up phone calls. This approach resulted in significant improvements in cardiorespiratory fitness and body composition. Our group has previously assessed the benefits of a 12 month predominantly home-based combination training program with telephone intervention in the same CKD cohort as reported in this study.(234) The exercise training was identified to improve cardiorespiratory fitness, body composition and diastolic function. However, the aim of the current study was to determine whether these enhancements can be maintained for a longer period of time. The feasibility of a longer-term home-based combination aerobic and resistance training has not been reported in the CKD population.

Therefore, the aim of this study was to assess the feasibility and efficacy of a LI on cardiorespiratory fitness, exercise capacity, arterial stiffness and autonomic function over a 36 month period. It was hypothesised that the LI would elicit significant improvements in cardiorespiratory fitness, exercise capacity, arterial stiffness and autonomic function compared to a control group.

6.3 Methods

Ninety-four patients with stage 3-4 CKD were included in this analysis. The data from this randomized control trial (RCT) examines the intervention and control arms over the 36 month period of the LM3 study. The LM3 study was terminated due to a lack of funding before 30 participants could complete the testing at 36 months. These patients were excluded from the current analysis. Details of patients included and excluded in this study are outlined in Figure 6.1. A comprehensive explanation of the methods and inclusion and exclusion criteria are reported in Chapter 2. Tests were performed annually (maximal exercise stress

test, DEXA, arterial stiffness and autonomic function) and six monthly (anthropometry, blood biochemistry, functional tests, blood pressure measures and physical activity questionnaire). The term exercise capacity refers to METs (derived from the treadmill, based on the exercise time) and cardiorespiratory fitness refers to VO_2peak.(235) Due to limited availability of the DEXA machine, only a sub-set of patients had follow-up scans (LI=15, control=8). Also, due to limited availability of ambulatory blood pressure monitors, only a sub-set of patients had follow-up 24 hour blood pressure monitoring (LI=20, control=17).

Statistical analysis

Mean±standard deviation (SD) was used to describe normally distributed baseline characteristics, with percentages used to describe frequencies for categorical variables. Median[IQR] was used to describe not normally distributed variables and variables transformed by the natural logarithm. Normality was assessed using the Shapiro-Wilk test and visual interpretation of a histogram. Comparison between groups for baseline variables was assessed by an independent t-test on normally distributed and log transformed variables. The Mann-Whitney U test was performed on not-normally distributed variables. Differences between groups for categorical variables were analysed using Pearson's Chi Square test.

Within group differences were assessed by paired t-tests for normally distributed and log transformed variables. Wilcoxon-Sign rank test was used for not normally distributed variables. A GEE was used to assess the main effect of group allocation on each variable, including the 4 or 7 visits over the 36 month period as within subject effects. Robust estimator was used as the covariance matrix and exchangeable was selected for the working correlation structure. A repeated measures ANOVA was used to assess the changes over each visit in the fitness measures (physical activity levels, VO_2peak, METs and grip strength). Friedman's ANOVA was used to assess the change in physical activity time every 6 months due to the not-normal distribution of this variable. Sphericity assumed or Greenhouse-Geisser was used as appropriate to detect group x time interactions. LSD post-hoc analyses were used to compare main effects of time in each group. A multiple linear regression model was used to identify baseline predictors of change in estimated METs from baseline to 36 months using the enter method. Variance inflation factor was used to assess collinearity between predictor variables. A per-protocol analysis was performed on patients in the LI group who reported

achieving physical activity guidelines at the 36 month visit (≥150 minutes in an average week in the preceeding 6 months). Paired t-tests were used to investigate significant differences between baseline and 36 months in patients meeting physical activity guidelines for the variables VO_2peak, METs, grip strength and eGFR. All statistical analyses were performed on IBM SPSS Statistics 22. Figures were constructed on GraphPad Prism 7. With many repeated variables in a large sample size, a small amount of missing data for variables were inevitable. The GEE uses an all available pairs approach, therefore participants with missing values for certain visits are still included in the analysis. However, the repeated measures ANOVA deletes cases with any missing data. The sample size for each repeated measures ANOVA performed is included in the results text. Statistical significance was set at $p \leq 0.05$.

6.4 Results

Patient characteristics

One hundred and eighty-seven participants with stage 3-4 CKD were recruited for this study (Figure 6.1). After 163 participants were randomized, 94 patients completed testing at 36 months. There were 15 patients from each group who were not included in the final analysis as they did not complete the entire 36 month period before the study was ceased due to funding issues (Figure 6.1). In order to assess the completion percentage of participants who were given the opportunity to finish the study, these 15 participants from each group were excluded from the total of randomized participants (ie. LI n=67 and control n=66). Therefore, it was identified that 71.6% (n=48) of patients completed the LI and 69.7% (n=46) completed the control phase.

There were no significant differences between age, sex or diabetes status between the control and LI groups (Table 6.1). The control group had a higher rate of previous myocardial infarction, however there were no differences between groups for other cardiovascular co-morbidities. There were no significant differences between groups for medication use. Table 6.2 shows diabetes to be the most common known cause of renal disease in both groups.

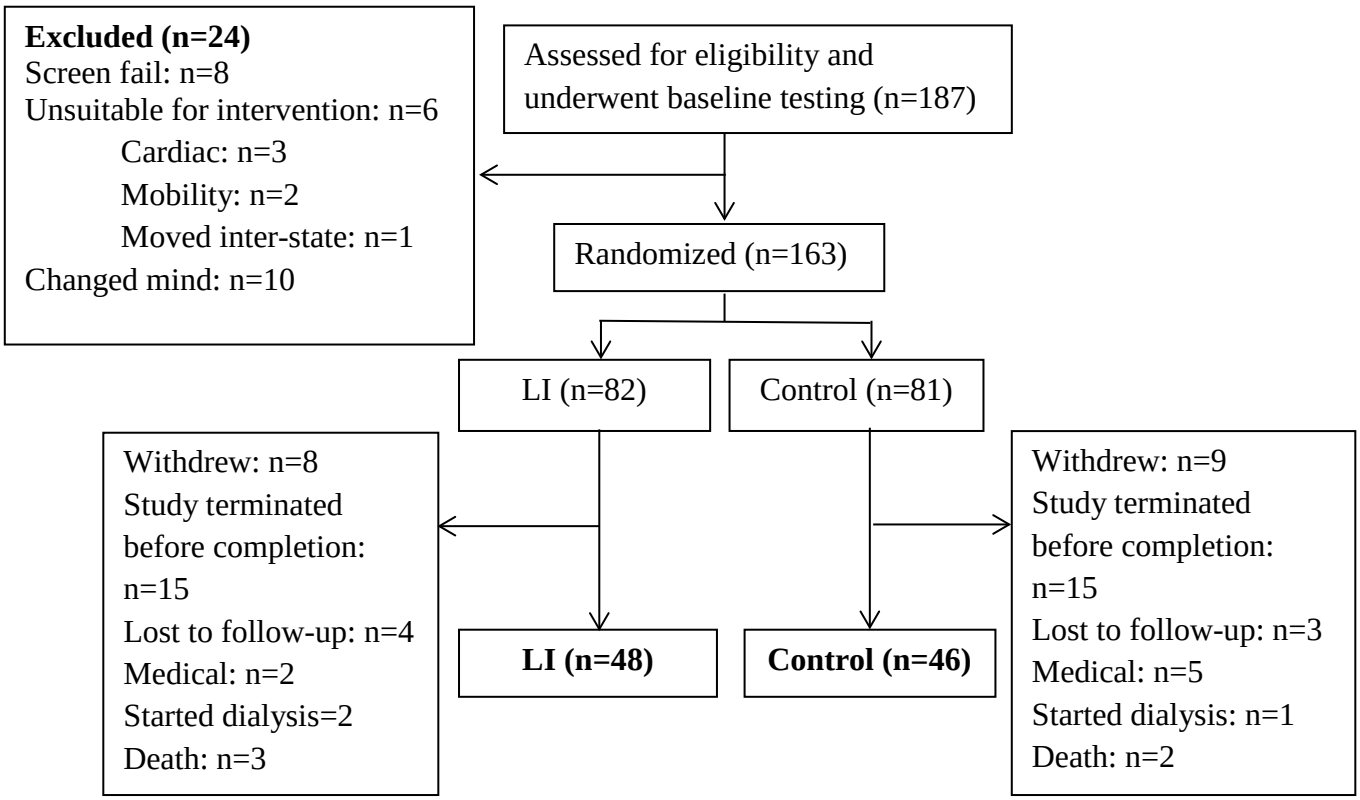


Figure 6.1 Consort diagram

Table 6.1 Patient characteristics

Variable	Control (n=46)	Lifestyle Intervention (n=48)	p value
Age (years)	61.4±8.5	59.3±8.9	0.24
Sex (male)	26.0(56.5)	29.0(60.4)	0.70
Patient history			
Diabetes	19(41.3)	19(39.6)	0.87
Hyperlipidaemia	31(68.9)	34(70.8)	0.84
Myocardial infarction	10(21.7)	3(6.4)	**0.03**
Heart failure	1(2.2)	3(6.4)	0.33
Peripheral vascular disease	5(10.9)	10(21.3)	0.17
Hypertension	44(95.7)	45(93.8)	0.68
Stent	6(13)	5(10.6)	0.72
CABG	4(8.7)	2(4.3)	0.38
Previous angina	12(27.9)	9(20.9)	0.45
Medications			
ACEi	25(53.2)	24(52.2)	0.92
ARB	22(47.8)	28(58.3)	0.31
Beta-blocker	14(29.8)	18(39.1)	0.34
Calcium channel blocker	26(56.5)	20(41.7)	0.15
Thiazide	8(17)	12(26.1)	0.29
Statin	33(70.2)	28(60.9)	0.34
Insulin	9(19.1)	11(23.9)	0.58

Values are mean±SD for normally distributed variables and n(%) for categorical variables. Value in bold is statistically significant between groups ($p<0.05$).
LI=lifestyle intervention; CABG=coronary artery bypass graft; ACEi=ace inhibitor; ARB=angiotensin receptor blocker

Table 6.2 Cause of renal disease

Cause	Control	Lifestyle Intervention
T2DM insulin	9(19.6)	3(6.3)
T2DM non-insulin	2(4.3)	4(8.3)
Glomerulonephritis	2(4.3)	5(10.4)
Polycystic kidney disease	3(6.5)	2(4.2)
IgA nephropathy	2(4.3)	1(2.1)
Renal vascular disease	1(2.2)	2(4.2)
FSGS	1(2.2)	1(2.1)
Reflux nephropathy	1(2.2)	1(2.1)
Analgesic nephropathy	0	1(2.1)
Calculi	0	1(2.1)
T1DM	0	1(2.1)
Other	17(37)	16(33.3)
Unknown	8(17.4)	10(20.8)

T2DM= Type 2 diabetes mellitus; IgA= Immunoglobulin FSGS= Focal segmental glomerulosclerosis; T1DM= Type 1 diabetes mellitus

Physical activity levels

There was a significant main effect of group over time, with an increase in the LI group for time spent walking per week, (delta LI=79.6±180.8, control= -27.9±139.2 minutes), total physical activity time per week (delta LI= 112.5±224.5, control= -12.7±171.1 minutes)(Figure 6.2, n=26 in ANOVA) and total MET minutes per week (delta LI= 548.0±1335.2, control= -202.0±1096.3 MET minutes, all group x time $p<0.01$) (Table 6.3). It was identified that 31.3% of participants in the LI group were meeting physical activity guidelines (≥150 minutes/week) at baseline and this increased to 62.2% at 36 months. On the other hand, 34.9% of participants in the control group were meeting physical activity guidelines at baseline and this decreased at 36 months to 30.6% (group x time $p<0.01$). Table 6.3 shows that 37.8% of participants in the LI group reported going from not meeting physical activity guidelines to meeting guidelines. On the other hand, only 11.8% participants in the control group reported not meeting guidelines at baseline and meeting physical activity guidelines at 36 months.

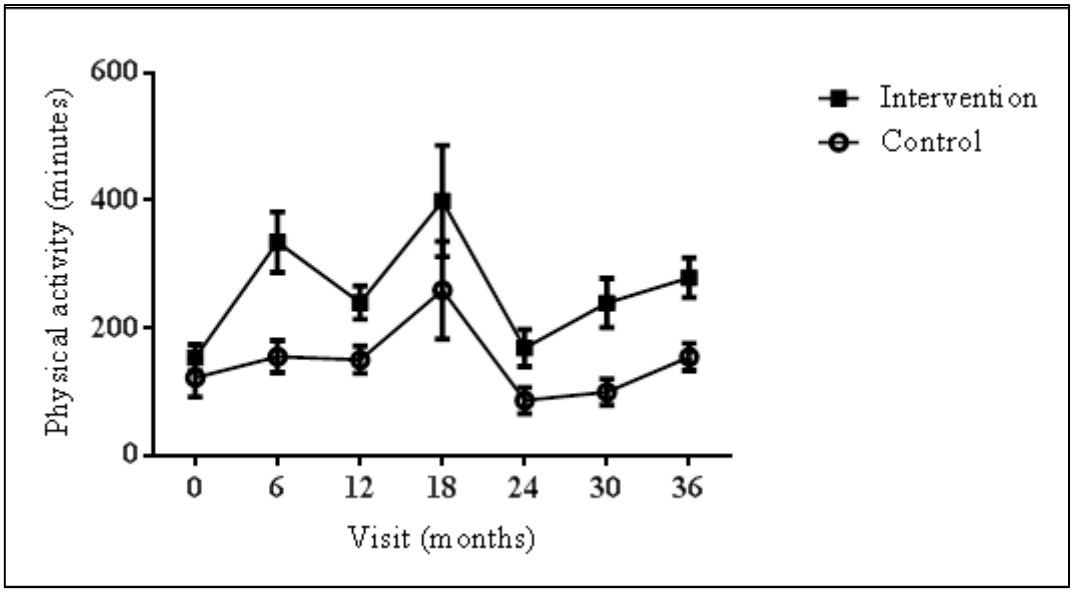


Figure 6.2 Total physical activity time measured every 6 months for 36 months

Table 6.3 Change in meeting physical activity guidelines after 36 months

Change in meeting physical activity guidelines	Control	Lifestyle Intervention
Now meeting guidelines (n,%)	4(11.8)	14(37.8)
No longer meeting guidelines (n,%)	24(70.6)	20(54.1)
No change (n,%)	6(17.6)	3(8.1)

Fitness measures

There was a main effect of group over time, with the LI group improving exercise capacity compared to the control group (LI= +2.2±2.7, control= -0.2±2.2 METs, group x time p=0.01) (Table 6.3). A repeated measures ANOVA (n=57) also demonstrated a significant increase in METs at both 12 months, 24 months and 36 months compared to baseline in the LI group (time effect $p<0.001$) with no change in the control group (time effect p=0.82) (Figure 6.3).

There was no main effect of group over time for cardiorespiratory fitness, measured by VO_2peak (p=0.28). However, a repeated measures ANOVA (n=47) identified a significant improvement in VO_2peak between baseline and visit 3 (12 months) in the LI group (time effect p<0.01)(Figure 6.4). In contrast, a significant decline in VO_2peak from baseline to visit 5 (24 months) and baseline to visit 7 (36 months) was identified in the control group (time effect p<0.001). However, Figure 6.4 indicates that after the initial increase in VO_2peak at 12 months in the LI group, the decline in cardiorespiratory fitness appears to occur at the same rate for both groups.

There was no significant main effect of group over time for grip strength (Table 6.3). However, a repeated measures ANOVA (n=37) showed the control group to have a significant decline in grip strength (time effect p<0.01) at visit 5 (24 months), 6 (30 months) and 7 (36 months) compared to baseline that was not seen in the LI group (time effect p=0.20)(Figure 6.5). However, the results in Figure 6.5 indicate that after the initial increase in grip strength at 12 months, the decline rate is similar between groups. There was a main effect of group over time approaching significance for the get up and go test (group x time p=0.07). A within group analysis showed the control group to significantly increase their get up and go time (+0.34[1.1] s, paired t-test p<0.01) with minor non-significant increases seen in the LI group (+0.05[1.3] s, paired t-test p=0.77).

Table 6.4 Effects of a lifestyle intervention on fitness measures in patients with CKD

Variable	**Control**		**Lifestyle Intervention**		**Group x time**
Fitness measures	**Baseline**	**36mth**	**Baseline**	**36mth**	**p value**
VO_2peak (L/min)$_{(Ln)}$	2.1[0.9]	**1.8[0.7]***	2.0[0.7]	1.8[0.6]	0.92
VO_2peak (ml/kg/min)$_{(Ln)}$	23.5[7.0]	**18.9[6.6]***	21.0[6.7]	22.2[7.6]	0.33
Exercise capacity (METs)$_{(Ln)}$	7.3[5.2]	7.0[5.1]	6.3[3.4]	**9.3[3.4]***	**0.01**
Respiratory quotient	1.03±0.1	1.05±0.2	1.05±0.1	1.07±0.1	0.06
Six minute walk (m)	492.8±94.7	482.3±98.6	477.7±108.2	493.8±115.7	0.16
Grip strength (kg)	31.3±11.3	**28.7±10.0***	33.0±12.7	30.6±10.1	0.36
Get up and go (s)$_{(Ln)}$	5.0[1.7]	**5.4[2.1]***	5.1[1.8]	5.4[2.1]	0.08
Walk time (minutes)	50.0[127.5]	47.5[127.5]	60.0[135.0]	**140.0[180.3]***	**<0.01**
Vigorous time (minutes)	0.0[0.0]	0.0[7.5]	0.0[0.0]	0.0[0.0]	0.11
Moderate time (minutes)	0.0[0.0]	0.0[0.0]	0.0[0.0]	0.0[71.3]	0.06
Total time (minutes)	80.0[187.5]	**70.0[187.5]***	70.0[155.0]	**210.0[295.0]***	**<0.01**
Total MET minutes	641.5[1314.0]	431.7[1314.0]	438.0[1172.0]	**1155.0[1677.2]***	**<0.01**
Meeting guidelines	15(34.9)	**11(30.6)***	15(31.30)	23(62.2)	**<0.01**

Generalized estimating equations identifying the main effect of group over time on fitness measures performed every year (maximal exercise stress test) and every 6 months (functional measures and Active Australia questionnaire). Bold group x time p values indicate significance ($p<0.05$) using the generalized estimating equation. *values in bold indicate within group statistical difference from baseline to 36 months (paired t-test<0.05). Normally distributed variables are reported as mean±standard deviation. Not normally distributed variables and log-transformed variables are reported as median[IQR]. (Ln)= transformed with the natural logarithm for statistical tests. METs=metabolic equivalent task

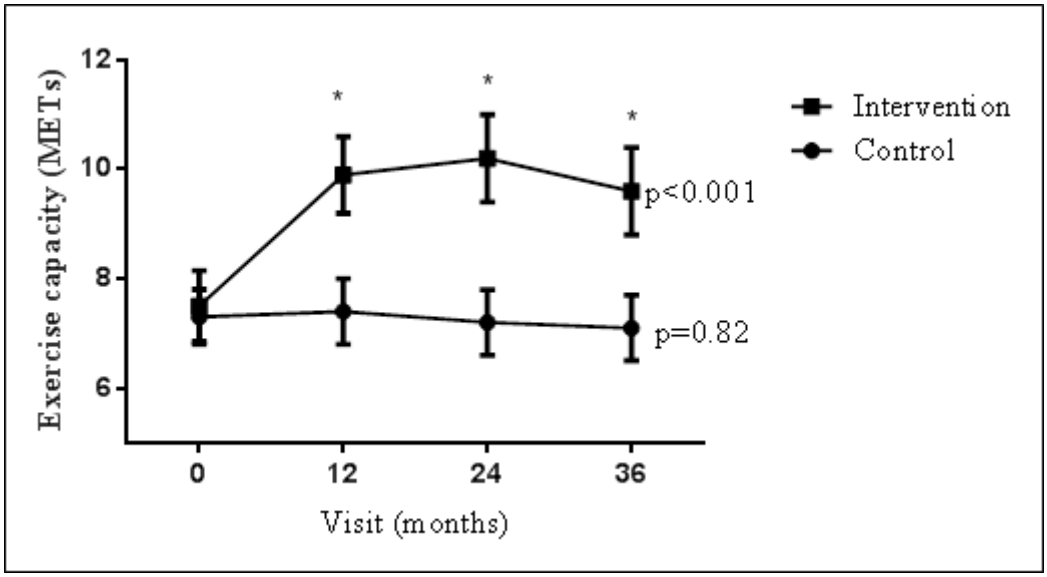


Figure 6.3 Exercise capacity measured every 12 months for 36 months

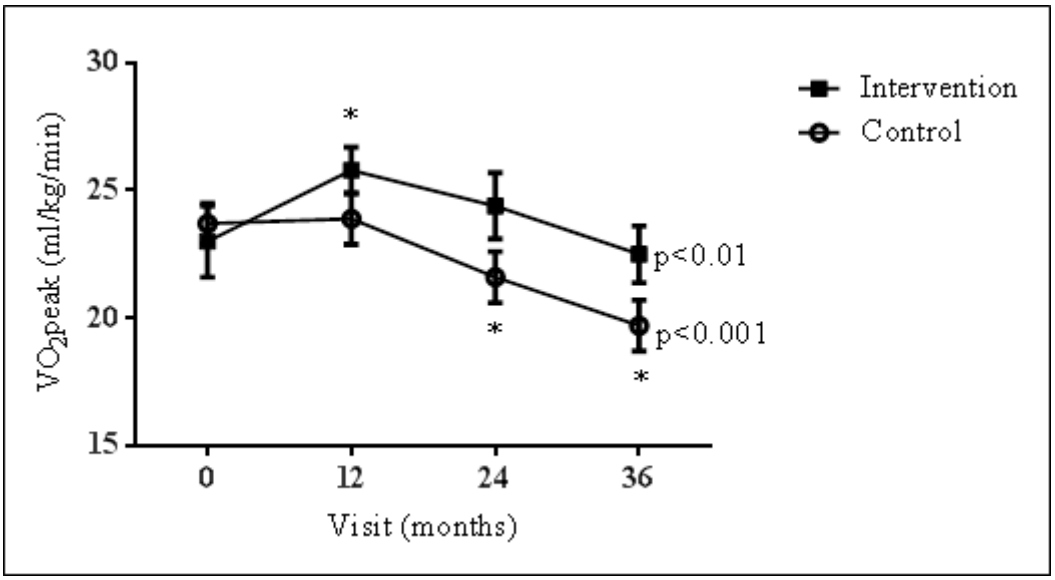


Figure 6.4 Cardiorespiratory fitness measured every 12 months for 36 months

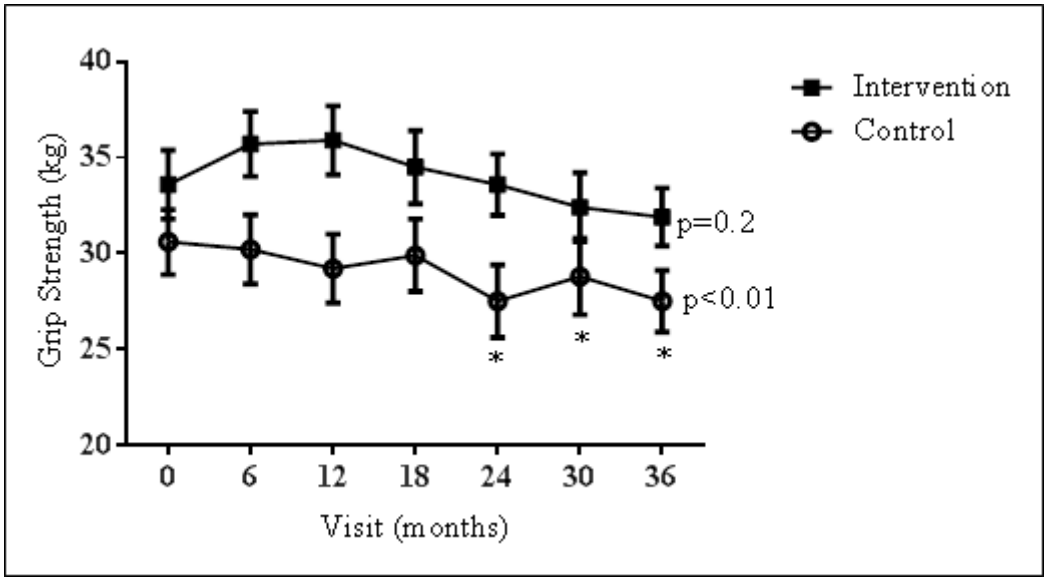


Figure 6.5 Grip strength measured every 6 months for 36 months

Body composition measures

The intervention had no effect on anthropometric and body composition measures; weight, BMI, waist:hip, fat mass or lean mass (Table 6.4). Moreover, there were similar non-significant reductions in fat mass percentage in both control (-1.4±6 %, paired t-test p=0.52) and LI groups (-1±5.1 %, paired t-test p=0.46).

Table 6.5 Effects of a lifestyle intervention on body composition and anthropometry measures

Variable	**Control**		**Lifestyle Intervention**		**Group x time**
Body composition	**Baseline**	**36mth**	**Baseline**	**36mth**	**p value**
Weight $(kg)_{(Ln)}$	91.5[31.8]	93.6[30.8]	90.5[25.1]	89.4[23.9]	0.40
Body mass index $(k/m^2)_{(Ln)}$	31.1[9.1]	31.6[7.5]	32.6[7.8]	31.5[9.8]	0.32
Waist $(cm)_{(Ln)}$	103.0[30.0]	105.0[19.0]	103.0[38.4]	101.5[25.8]	0.31
Hip $(cm)_{(Ln)}$	104.0[16.0]	107.0[15.0]	109.0[70.0]	109.5[25.0]	0.52
Waist:hip	0.9±0.1	0.9±0.1	1.0±0.1	1.0±0.1	0.38
DEXA[#]					
Lean mass (kg)	47.3±10.1	48.9±7.4	54.6±10.0	54.5±10.2	0.44
Lean mass (%)	58.4±7.3	60.0±5.0	61.8±8.1	62.9±9.4	0.77
Fat mass $(kg)_{(Ln)}$	31.5[30.2]	29.1[9.8]	29.4[15.6]	27.1[20.9]	0.36
Fat mass (%)	38.7±5.1	37.3±5.1	35.3±8.6	34.2±9.9	0.77
Total mass $(kg)_{(Ln)}$	79.6[29.9]	81[20.9]	89.8[20.9]	87.4[24.5]	0.44

Generalized estimating equations identifying the main effect of group over time on body composition measures performed every year for the DEXA measures and every six months for the other anthropometry measures. Normally distributed variables are reported as mean±standard deviation. Not normally distributed variables and log-transformed variables are reported as median[IQR]. (Ln)= transformed with the natural logarithm for statistical tests. DEXA= dual-energy x-ray absorptiometry. [#]Due to limited machine availability DEXA was performed in a sub-set of patients (n=23)

Blood biochemistry

The intervention had no main effect of group over time on creatinine, eGFR, blood lipids, C-reactive protein (CRP) or haemoglobin (Table 6.5). The LI group showed a significant decline in eGFR (-3.9±9.0 ml/min/1.73m^2, paired t-test p=0.03), although the change in creatinine was only mildly and not significantly increased (21.9±56.2 μmol/L, paired t-test p=0.31). The LI also had significant decreases in total cholesterol (TC) (-0.5±0.7 mmol/L, paired t-test p<0.001) and low-density lipoproteins (LDL) (-0.4±0.6 mmol/L, paired t-test p<0.01).

Haemodynamics, vascular function and heart rate variability

There was no significant main effect of group over time on any haemodynamic variables. However, within-group analyses showed the control group to significantly decrease resting heart rate (-10.5±12 bpm, paired t-test p<0.01), peak heart rate (-11.2±17.2 bpm, paired t-test p<0.01) and peak systolic blood pressure (-26.5±37.8 mm/Hg, paired t-test p<0.01). There was a significant main effect of group over time on PWV (p=0.03). Although there were no within-group differences for this measure, the LI group had a decrease in PWV (-0.1±2.5 m/s, paired t-test p=0.67) and the control group had an increase in PWV (0.6±3.1 m/s, paired t-test p=0.12)(Table 6.2). There was a significant improvement in total power (TP) in the LI group +558.0±757.1 m/s^{2,} paired t-test p=0.01), however there was no main effect using a GEE (group x time p=0.12). Other variables of HRV- standard deviation of the N-N interval (SDNN), root mean square of the standard deviation (RMSSD), very low frequency (VLF), low frequency (LF) and high frequency (HF) were unchanged over the 3 years in both groups (Table 6.6).

Table 6.6 Effects of a lifestyle intervention on blood biochemistry

Variable	**Control**		**Intervention**		**Group x time**
Blood biochemistry	**Baseline**	**36 mth**	**Baseline**	**36 mth**	**p value**
eGFR (ml/min/1.73m^2)	39.8±8.6	38.6±14.5	38.7±7.8	**34.8±11.6***	0.25
Creatinine (µmol/L)$_{(Ln)}$	145.0[54.3]	140.5[64.3]	157.5[47.0]	160.5[76.8]	0.31
Fasting glucose (mmol/L)$_{(Ln)}^{\#}$	7.1[4.0]	7.0[5.7]	6.9[5.0]	6.2[2.9]	0.76
Total cholesterol (mmol/L)$_{(Ln)}$	4.3[1.4]	4.4[1.4]	4.6[0.9]	**4.0[1.3]***	0.22
Triglycerides (mmol/L)$_{(Ln)}$	1.6[1.5]	1.4[1.0]	1.6[1.3]	1.4[0.9]	0.08
HDL (mmol/L)$_{(Ln)}$	1.2[0.5]	1.2[0.5]	1.2[0.8]	1.2[0.5]	0.28
LDL (mmol/L)$_{(Ln)}$	2.5[1.4]	2.4[1.4]	2.5[0.7]	**2.0[0.9]***	0.52
VLDL (mmol/L)$_{(Ln)}$	0.7[0.6]	0.6[0.3]	0.7[0.5]	0.6[0.4]	0.26
C-reactive protein (mg/L)$_{(Ln)}$	4.0[4.9]	3.2[5.1]	3.2[5.8]	2.3[3.6]	0.77
Haemoglobin (g/L)	132.3±14.6	130.2±15.5	135.2±16.0	131.7±13.9	0.97
Haematocrit (%)	40.4±4.0	39.9±4.6	42±4.0	41.0±3.7	0.99

Generalized estimating equations identifying the main effect of group over time on blood biochemistry measures performed every 6 months. Normally distributed variables are reported as mean±standard deviation. Not normally distributed variables and log-transformed variables are reported as median[IQR]. (Ln)= transformed with the natural logarithm for statistical tests. *values in bold indicate within group statistical difference from baseline to 36 months (paired t-test $p<0.05$). $^{\#}$= in patients with diabetes only (n=38). eGFR=estimated glomerular filtration rate; HDL=high-density lipoprotein; LDL=low-density lipoprotein; VLDL=very low-density lipoprotein

Table 6.7 Effects of a lifestyle intervention on haemodynamics and vascular function

Variable	**Control**		**Intervention**		**Group x time**
Haemodynamics	**Baseline**	**36 mth**	**Baseline**	**36 mth**	**p value**
Resting heart rate (bpm)	82.0±10.8	**71.5±13.5***	83.5±14.4	79.9±13.5	0.23
Peak heart rate (bpm)	147.2±18.1	**136.1±23.5***	147.8±30.2	144.6±25.9	0.16
Resting systolic BP $(mm/Hg)_{(Ln)}$	142.7[35.0]	136.7[28.0]	134.0[24.3]	129.0[22.6]	0.99
Resting diastolic BP $(mm/Hg)_{(Ln)}$	80.0[14.7]	77.7[12.4]	83.3[14.3]	80.0[12.4]	0.08
Peak systolic BP (mm/Hg)	181.6±40.2	**155.1±21.2***	167.3±46.1	164.3±24.4	0.73
Peak diastolic BP (mm/Hg)	85.5±11.4	83.4±10.9	81.9±13.4	81.9±11.2	0.70
24hr systolic BP $(mm/Hg)_{(Ln)}^{\#}$	136.0[13.0]	137.0[26.0]	128.0[26.3]	129.5[16.8]	0.05
24hr diastolic BP $(mm/Hg)_{(Ln)}^{\#}$	75.0[12.0]	75.0[16.5]	77.0[14.8]	73.5[11.8]	0.73
Central systolic BP $(mm/Hg)_{(Ln)}$	116.5[18.0]	115.5[25.3]	112.0[23.0]	114.5[14.0]	0.52
Central diastolic BP $(mm/Hg)_{(Ln)}$	71.0[18.0]	70.0[23.0]	76.5[10.0]	72.0[12.0]	0.77
Vascular function					
Pulse wave velocity $(m/s)_{(Ln)}$	9.8[3.2]	9.9[3.8]	9.1[2.9]	8.6[3.9]	**0.03**
AIx (%)	24.9±8.7	24.7±10.8	26.6±8.6	26.7±10.8	0.22
Heart rate variability					
SDNN $(m/s)_{(Ln)}$	20.7[13.7]	21.4[9.3]	29.9[19.0]	24.6[15.6]	0.89
RMSSD $(m/s)_{(Ln)}$	10.9[13.4]	14.2[11.7]	20.0[13.4]	13.1[17]	0.76
Total power $(m/s^2)_{(Ln)}$	313.0[45.5]	335.0[377.8]	**288.0[38.5]**$^{\wedge}$	**576.1[964.6]***	0.14
Very low frequency $(m/s^2)_{(Ln)}$	115.1[272]	123.6[261.6]	284.3[441.0]	298.7[371.9]	0.46
Low frequency $(m/s^2)_{(Ln)}$	52.2[119.0]	88.2[106.7]	185.0[306]	92.6[245.5]	0.54
High frequency $(m/s^2)_{(Ln)}$	31.4[98]	55.3[97.2]	142.2[146.0]	85.8[235.5]	0.98

Generalized estimating equations identifying the main effect of group over time on haemodynamic and vascular measures performed every year. Normally distributed variables are reported as mean±standard deviation. Not normally distributed variables and log-transformed variables are reported as median[IQR]. (Ln)= transformed with the natural logarithm for statistical tests. Bold group x time p values indicate significance ($p<0.05$) using the generalized estimating equation.*bold values indicate significant within group difference ($p<0.05$). ^bold values indicate significant between group difference at baseline ($p<0.05$). BP=blood pressure; SP=systolic pressure; DP=diastolic pressure; AIx=augmentation index; SDNN=standard deviation of N-N interval; RMSSD=root mean square of the standard deviation. #Due to limited machine availability 24 hour blood pressure monitoring was performed in a sub-set of patients (n=37)

Per-protocol analysis

Given that 14 (29.2%) LI participants at 36 months reported not meeting physical activity guidelines, analyses were conducted in those who *were* meeting guidelines. A within group analysis of variables from baseline to 36 months was performed in LI patients who reported achieving ≥150 minutes in an average week at 36 months (n=34)(Table 6.7). The changes in cardiorespiratory fitness (VO_2peak) and exercise capacity (METs) over the 3 years were similar to the changes found with all participants (Table 6.3). It was established that even in the patients meeting physical activity guidelines, VO_2peak remained unchanged (paired t-test p=0.40) over the intervention, whereas METs significantly increased (paired t-test p=0.01). Grip strength was also shown to decline over the 36 months in patients meeting exercise guidelines (paired t-test p=0.01). It was identified that in LI patients who were meeting physical activity guidelines there was no change in eGFR measures (paired t-test p=0.99), which is in contrast to the previous finding which detected a difference in all patients in the LI group. There was a significant improvement in the cardiovascular disease risk factors total cholesterol and low-density lipoproteins (both paired t-test p=0.03) and total power (paired t-test p=0.01).

Table 6.8 Within group comparison of patients in the lifestyle intervention group who achieved physical activity guidelines at 36 months

Variable	Baseline	36 months	p value
VO_2peak (ml/kg/min)$_{(Ln)}$	22.6[9.8]	22.0[11.2]	0.40
Exercise capacity (METs)$_{(Ln)}$	8.0[4.8]	9.7[4.5]	**0.01***
Grip strength (kg)	32.0±11.7	28.6±9.3	**0.01***
eGFR (ml/min/1.73m^2)	40.3±6.7	40.3±12.8	0.99
Total cholesterol (mmol/L)$_{(Ln)}$	4.7[1.1]	4.1[1.6]	**0.03***
Triglycerides (mmol/L)$_{(Ln)}$	1.1[1.0]	1.1[1.1]	0.63
High-density lipoprotein (mmol/L)$_{(Ln)}$	1.4[0.6]	1.3[0.5]	0.10
Low-density lipoprotein (mmol/L)$_{(Ln)}$	2.5[0.7]	2.1[1.1]	**0.03***
Very low-density lipoprotein (mmol/L)$_{(Ln)}$	0.5[0.5]	0.5[0.5]	0.93
Body fat (%)	32.8±9.5	33.8±9.1	0.57
Resting average systolic BP (mm/Hg)$_{(Ln)}$	136.3[23]	132.5[24.3]	0.71
Resting average diastolic BP (mm/Hg)$_{(Ln)}$	81.7[17.3]	79.9[9.8]	0.56
Pulse wave velocity (m/s)$_{(Ln)}$	8.4[3.1]	8.1[3.5]	0.40
AIx (%)	24.5±8.9	27.0±11.6	0.37
Total power (m/s^2)$_{(Ln)}$	287.5[40.0]	577.4[1477.5]	**0.01***
SDNN (m/s^2)$_{(Ln)}$	27.9[16.2]	26.8[17.4]	0.54
RMSSD (m/s^2)$_{(Ln)}$	19.6[15.4]	14.0[25.3]	0.53
High frequency (m/s^2)$_{(Ln)}$	113.6[174]	120.2[324.5]	0.31
Low frequency (m/s^2)$_{(Ln)}$	112.1[315]	89.7[292.0]	0.37
Very low frequency (m/s^2)$_{(Ln)}$	246.9[412.0]	333.3[533.4]	0.40

Physical activity guidelines are ≥150 minutes/week of exercise. Normally distributed variables are reported as mean±standard deviation. Not normally distributed variables and log-transformed variables are reported as median[IQR]. (Ln)= transformed with the natural logarithm for statistical tests. *bold values indicate significant within group difference ($p<0.05$). BP=blood pressure; SDNN=standard deviation of N-N interval; RMSSD=root mean square of the standard deviation.

Predictors of changes in exercise capacity

A multiple linear regression assessed the relationship between baseline variables related to fitness and the change in METS from baseline to 36 months. It was identified that sex, diabetes status, eGFR, BMI, physical activity levels, VO_2peak and PWV were not significantly associated with change in exercise capacity (Table 6.8). On the other hand, randomization to the LI group and grip strength were positively associated with change in METs and age and baseline METs were negatively associated with change in METs. The model including these four variables explained 47% of the variability in the change in METs (R^2=0.47, p<0.01) and had a variance inflation factor <3.

Table 6.9 Baseline predictors of change in METs over 36 months

Variable	**Standardized co-efficients β**	**p value**
Group	-0.37	**<0.01**
Sex	-0.25	0.16
Age	-0.38	**<0.01**
Diabetes status	0.11	0.38
eGFR	0.09	0.46
Body mass index $_{(Ln)}$	-0.27	0.07
Grip strength	0.46	**<0.01**
Total MET minutes	0.04	0.74
Exercise capacity (METs)$_{(Ln)}$	-0.37	**0.01**
VO_2peak $_{(Ln)}$	0.05	0.75
Pulse wave velocity $_{(Ln)}$	-0.06	0.63

R^2=0.47, p=<0.01

6.5 Discussion

This is the first long-term RCT to investigate the effects of a LI on fitness and cardiovascular disease risk factors in patients with moderate CKD. The main findings from this study are that; 1) the LI doubled the number of patients meeting physical activity guidelines, 2) the LI significantly improved exercise capacity, 3) the LI significantly increased VO2peak at 12 months followed by a similar rate of decline as the control group, 4) there was a significant decrease in arterial stiffness in the LI group compared to the control group, 5) the LI group

significantly improved autonomic function and blood lipids, and 6) group allocation, age, baseline grip strength and METs independently predicted the change in exercise capacity from baseline to 36 months.

Physical activity

It was identified that 30.6% of participants in the LI group were meeting physical activity guidelines of ≥150 minutes/week(5) in the 6 months preceding participation in the study, and this increased to 62.2% on completion of the study at 36 months. This finding is expected to be clinically important as it has been identified that physical inactivity is the 3rd biggest predictor of cardiovascular mortality in CKD patients, only behind left ventricular hypertrophy and smoking.(236) It is estimated that physical inactivity is responsible for 15 deaths per 1000 person-years.(236) Therefore, employing strategies which improve physical activity levels are important in improving outcomes for CKD patients.

Exercise capacity and cardiorespiratory fitness

The LI was successful in improving exercise capacity, as measured by METs. Furthermore, the multiple linear regression model identified group allocation to be the most significant predictor of change in exercise capacity. This finding indicates that randomization to the LI improved exercise capacity independent of other significant associates- age, baseline exercise capacity and baseline grip strength. However, only small, non-significant increases in cardiorespiratory fitness, as measured by VO_2peak, were seen in the LI group. The greatest increase seen in VO_2peak at 12 months may be due to the high supervision in the initial 8 week period and the more frequent gym refreshers sessions and telephone follow-up calls for the first year thereafter. Potentially, motivation declined when participants were expected to have greater autonomy in completing their home program. There have been a number of studies investigating the influence of researcher contact on adherence to exercise training programs and health outcomes. Indeed, studies have identified poor adherence, higher drop-out rates and subsequently a decline in health related outcomes after participants have been prescribed a home exercise program following a period of supervised exercise.(237-240) However, like the current study, Menses et al. (2011) found that despite a decline in adherence to home-based walking training after a supervised period in patients with

claudication, initial claudication distance and total walking distance was higher than baseline values.(237) A study by Bock et al. (1997), established that exercise maintenance at follow-up from a home-based period subsequent to supervised training was associated with motivational readiness. Further, a study by Loprinzi et al. (2012) reported that breast cancer survivors had greater adherence to unsupervised exercise training if they had higher self-efficacy and behavioural processes at the transition from supervised exercise.(241) The regular follow-up telephone calls and gym refresher sessions were designed to maintain the support and encouragement that was needed for adherence to the intervention at home. Perhaps, mandatory supervised sessions at more regular intervals would have encouraged greater adherence for the entire 36 month intervention. Furthermore, ensuring patient self-efficacy and motivational readiness during the supervised period may have assisted in greater outcomes in the home-based period. However, it must be considered that this study was designed as a feasible and cost-effective intervention and more frequent supervised sessions may not be practical in standard rehabilitation. Future studies should conduct an economic analysis comparing the cost of more frequent supervised sessions against the potential improvements in health outcomes. This would help determine the most applicable exercise prescription that can be realistically incorporated into standard nephrological care.

Despite the lack of a significant *increase* in VO_2peak in the LI group in the current study, the LI seemed effective in ameliorating the significant reduction in cardiorespiratory fitness from baseline to 36 months which was seen in the control group. Figure 6.4 clearly demonstrates that after the significant increase in VO_2peak at 12 months in the LI group, the decline rate is similar to that of the control group. Potentially the initial 8 week period of more intensive exercise training may have been sufficient to ameliorate the overall decline in fitness from baseline at 36 months. The significant reduction in arterial stiffness at 36 months may indicate that this more intensive initial period of exercise may have a legacy effect on cardiovascular risk factors. Indeed, whether a legacy effect does exist after a short period of intense exercise training followed by a maintenance phase of moderate intensity exercise warrants further study. This finding has important implications for minimum dose of exercise training for cardiovascular response. This decline in fitness in the control group was also identified in the study by Headley et al. (2012), who found a similar decrease in VO_2peak in a usual care control group after 48 weeks of combination training.(55) As every 1 ml/kg/min increase in VO_2peak is reported to result in a 15% decrease in risk of death in patients with

coronary heart disease, preventing this decline has a likely effect on health outcomes in CKD patients.(78)

Cardiorespiratory fitness non-responders

The significant increase seen in METs, without a subsequent increase in VO_2peak, indicates a disconnect between exercise capacity and cardiorespiratory fitness. The LI group had a significant increase in METs at 12 months, which was maintained until 36 months. On the other hand, VO_2peak increased significantly at 12 months, yet declined to baseline levels by the end of the study. This finding may indicate that an improvement in exercise efficiency is occurring, rather than a physiological adaptation. Other aspects that can influence exercise capacity are the environment, subject motivation and familiarity with maximal testing.(242) The findings from the per-protocol analysis of LI patients meeting physical activity guidelines also suggests there is an improvement in exercise capacity occurring without a subsequent increase in cardiorespiratory fitness. It has been suggested that non-responders to changes in VO_2peak are not necessarily non-responders in other measurements of training response.(243) Scharhag-Rosenberger et al. (2012) found that after middle-aged, untrained participants underwent a year of endurance training, some individuals displayed no changes in VO_2max yet had significant changes in anaerobic threshold and exercise heart rate.(244)

There may be other mechanisms occurring through the pathogenesis of CKD which are dampening the physiological adaptations to exercise training. Indeed, baseline autonomic activity has been reported to influence exercise training response.(245-247) A study by Huatala et al. (2003) has found baseline HRV in sedentary adults explains 27% of the variation in VO_2max response after an exercise training program.(248) Our group has identified HRV to be lower in CKD patients than age matched healthy controls and found HRV in CKD patients to be associated with VO_2peak (Appendix 11.7). Therefore, the low HRV in this population may be contributing to the lack of response in VO_2peak over a long-term training program. There are no longitudinal studies looking at whether chronic disease patients who are non-responders to cardiorespiratory fitness, yet improve other training responses such as exercise capacity, can achieve the same health enhancing benefits.(243)

The results of the linear regression, which identified baseline predictors of change in METs, may highlight patients who are not as responsive to a LI. Indeed, the linear regression

identified that alongside group allocation, younger age and higher baseline exercise capacity and grip strength predicted the greatest change in exercise capacity over 3 years. Although this lack of responsiveness in older patients with low exercise capacity and strength may be attributed to by physiological limitations, psychosocial factors may also be influencing the effectiveness of the LI. It seems reasonable to hypothesise that patients with lower exercise capacity and strength have the greatest opportunity for improvement, however this did not appear to be the case in the current study. Conversely, older patients with lower exercise capacity and strength may potentially need greater supervision and support from an exercise physiologist in order to receive the optimal outcomes from an exercise intervention. Indeed, patients with these characteristics may need greater support in reaching a higher level of exercise intensity and/or greater volume of exercise training. Furthermore, if a reduction in strength is a contributing factor in non-responsiveness to change in exercise capacity, a greater focus on resistance training may be necessary. Moreover, as the effort required to increase exercise capacity may be greater for older patients with lower exercise capacity and strength and greater fatigue, working closely with a behavioural psychologist may provide the support necessary to improve factors such as motivation and perceived exertion, in order to increase exercise duration and frequency.

Arterial stiffness

It was identified that the LI significantly improved (decreased) patient's PWV, with no changes seen in the control group. Mustata et al. (2010) also identified an improvement in arterial stiffness, measured by AIx, after exercise training in patients with stage 3-5 CKD. Rather than provide an indirect assessment of the stiffness of the aorto-iliac pathway such as with PWV, AIx applies a generalised transfer function to estimate the central pressure waveform and provide an indice of systemic arterial stiffness.(249) On the other hand, the study by Kosmadakis et al. (2012) showed no change in PWV or AIx after a six month walking program.(42) It has been suggested that regular aerobic exercise training increases arterial distensibility through improved endothelial function (250) and a reduction in sympathetic tone.(247) The magnitude of endothelial cell stretch with shear stress (such as which occurs with an increase in pulse pressure during exercise training) determines Akt and eNOS phosphorylation.(251) The change in mechano-biochemical signalling with exercise training improves vasorelaxation and subsequent compliance of the arterial wall.(252)

Patients in all stages of CKD have a known impairment of arterial function compared to healthy controls.(120) Dysregulation of the calcium-phosphorous balance, anaemia, inflammation and oxidative stress may all contribute to the disease process of vascular calcification and endothelial dysfunction.(100, 231) This impairment is significant, as arterial stiffness is associated with abdominal aortic, thoracic aorta and coronary artery calcification in haemodialysis patients (253) and is an independent risk factor for all-cause and cardiovascular disease mortality in patients with CKD.(254) The increase in cardiovascular disease risk with greater arterial stiffness is likely due in part to the effects of increased arterial pressure on the left ventricle,(255) causing left ventricular hypertrophy and subsequent systolic and diastolic dysfunction.(256) Indeed, it has previously been reported by our group that 61% of CKD patients have diastolic dysfunction and 8% have systolic dysfunction.(234) Consequently, decreasing arterial stiffness with exercise training has the potential to improve cardiac function in a large proportion of the CKD population. The association of arterial stiffness and cardiorespiratory fitness may also be bi-directional. It seems likely that by decreasing arterial stiffness there is a reduction in afterload, which increases cardiac output.(257) Therefore, decreasing arterial stiffness in these patients may also contribute to improvements in exercise capacity.

Autonomic function

Carotid artery stiffness is associated with impaired autonomic function. Sympatho-vagal balance is affected by greater activation of the arterial baroreceptors, as a consequence of increased arterial stiffness.(258) Autonomic dysfunction is also related to an increased risk of mortality in haemodialysis patients and therefore seems an important therapeutic target in reducing the rate of cardiovascular events in CKD patients.(259) The findings from this study indicate a significant improvement in the global HRV frequency parameter, TP, in the LI group which was not seen in the control group. Exercise training has been shown to improve global HRV time parameters, SDNN and RMSSD, in patients with haemodialysis.(117, 260) On the other hand, a study by Camillo et al. (2011) identified changes in SDNN and RMSSD after high intensity and not low intensity training in patients with chronic obstructive pulmonary disease.(261) This may suggest that higher intensity exercise is necessary to elicit changes in HRV time parameters, although, TP wasn't assessed in this study. The increase in shear stress on the arterial baroreceptors which occurs with high intensity exercise

encourages vagal outflow as reflected by HF power.(262) It is not clear why improvements in TP occurred without improvements in global time, or other frequency parameters. To elucidate these findings, future studies in CKD patients should investigate the effects of exercise training on 24-hour HRV, which may provide more substantiated results.

Muscle strength

Grip strength was found to be a significant predictor of change in exercise capacity from baseline to 36 months in the multiple linear regression model. This finding may indicate that people with greater muscle mass and strength at baseline are more likely to see the greatest improvements with exercise training. Furthermore, the decline in grip strength seen in the LI group may indicate that the thera-band and body weight exercises used for resistance training in this study may not have been sufficient to ameliorate the decline in grip strength. Indeed, it was reported in the Active Australia questionnaire that the increase in physical activity was predominantly through walking. This may indicate that the prescribed home resistance exercises were not being adequately adhered to. Further, it has been identified by Watson et al. (2013) that depletion of intramuscular amino acids occurred after a 6 month moderate intensity walking program in CKD patients undergoing standard bicarbonate supplementation.(44) In particular, there was a diminution in the branched chain amino acid L-Leu, which has anabolic effects on skeletal muscle. Conversely, no significant depletions of any intramuscular amino acids occurred in the control group who did not exercise. Therefore, it seems that the reduction of intramuscular amino acids with aerobic moderate intensity training may in fact be limiting the adaptations to changes in lean mass. In light of the findings from the current study and Watson et al., to effectively increase lean mass it seems imperative to place a significant emphasis on resistance training when prescribing an exercise program to patients with CKD.

Kidney function

The LI group appeared to have a significant decline in eGFR from baseline to 36 months. However, in the per protocol analysis there was no difference in eGFR in only those LI patients who reported achieving physical activity guidelines. This finding indicates that exercising training is not in fact contributing to the decline in eGFR. Rather, it seems the

reduced eGFR seen in the LI group may be attributed to a decline in kidney function in patients *not* considered physically active. It is difficult to ascertain the mechanisms as to the decline in eGFR in patients not meeting the physical activity guidelines, or alternatively the maintenance of eGFR in patients meeting physical activity guidelines. Potentially, the decline in eGFR in patients not meeting physical activity may be indicative of patients who have a greater disease state and are therefore the more 'unwell' patients who are less likely to participate in physical activity. On the other hand, the physical activity may have a protective benefit against a decline in kidney function and the longer time period in the 36 month intervention may demonstrate the natural decline in eGFR (263) in patients not meeting physical activity guidelines. The mixed results of exercise training on kidney function in the literature make it difficult to ascertain the exact causes of the current findings. Indeed, most studies have reported either no change, (36, 40, 51, 55) or an improvement in kidney function measures with exercise training.(37, 39, 45) On the other hand, Boyce et al. (1997) did find a decrease in eGFR as measured by creatinine clearance after 4 months of exercise training, which was maintained in the two months de-training period once exercise had ceased.(38) A patient with a renal trajectory of >3 ml/min/1.73m^2 in one year is considered to need closer renal follow-up as they are at a greater risk of morbidity and mortality.(264) Therefore, a decline of 3.9 ml/min/1.73m^2 over 3 years in the LI patients not meeting physical activity guidelines is considerably less than what is considered a high risk decline in kidney function. As such, it seems reasonable to suggest that the current findings indicate that physical activity *does* provide a protective benefit to kidney function. However, the large sample size needed to adequately assess changes in eGFR (~n=1000)(136) indicates any changes in kidney function in smaller sample sizes should be interpreted with caution.

Exercise training only formed part of the nurse-led multi-disciplinary LI. Indeed, nurse practitioner care, dietary advice, and diabetic education and psychology (if needed) were also likely to contribute to the outcomes of patients in the intervention group. As such it is difficult to ascertain the individual influences of each multi-disciplinary professional involved in this study. However, it seems reasonable to suggest that changes in physical activity and fitness measures, which were the primary outcomes in this chapter, are attributable to the exercise training specifically. Although this chapter focusses on the influence of exercise training specifically, multi-disciplinary care is still considered best practice for holistic treatment of patients with CKD.(265)

Limited statistical power (68.4%) because of the modest sample size in the present study (n = 94) may have played a role in limiting the significance of the independent t-test of the primary outcome, change in VO_2peak between groups. A post hoc power analysis revealed that on the basis of the mean, a difference in population size of 2.9 ml/kg/min and a mean standard deviation between groups of 6.6, a sample size of approximately 164 participants would be needed to obtain statistical power ($p<0.05$) at the recommended .80 level.(266)

Limitations

There are a number of limitations to the current study. The Active Australia questionnaire is based on patient's recall of their average physical activity levels per week in the previous 6 months. The use of recall for the measure of physical activity may have limited the accuracy of the reporting. However, although wearing an accelerometer may have provided more accurate data on physical activity for a single week, the average physical activity recall over the 6 month period was important due to the high rate of sickness and injury in many patients. Potentially, a 7 day analysis may not have provided a true representation of a standard week. The psychometric properties of the Active Australia questionnaire have been correlated with pedometer steps and physical function scores in community-dwelling older adults.(137) However, a cross-check validation at certain time points with steps recorded by pedometry, accelerometry or exercise diary may have confirmed the validity of the Active Australia questionnaire in the CKD population. Nonetheless, clarity in the Active Australia questionnaire was ensured by administering of the questionnaire by an Exercise Physiologist. Furthermore, specific activities reported from the Active Australia questionnaire were not recorded. Anecdotally, the activities reported in the Active Australia questionnaire were mostly intentional exercise sessions, with the most physical activity time attributed to moderate intensity walking with some theraband exercises. Collecting data on the exact activities performed throughout the intervention may have provided useful information on what activities CKD patients undertake when prescribed an aerobic and resistance home-based exercise program. However, the aim of this study was to assess the feasibility of this type of LI in a real-world setting. The increased burden placed on participants to collect detailed self-report diaries for a 3-year intervention would likely result in a higher drop-out rate.

There are a number of factors which may have influenced physical activity and exercise behaviours in this multi-disciplinary LI. As the study was designed as a feasible intervention that may be applied in the future in standard nephrological care, the delivery of the intervention was based around a standardized structure, yet was still individualized to best suit the patient. All multi-disciplinary aspects of the LI differed in timing of contact/visits and prescription according to individual needs. As such, this makes it difficult to provide specific information on the delivery and consistency of the LI. Furthermore, those patients who engaged more readily with the research team may have developed relationships which further encouraged their active participation. Due to the long duration of the study and the nature of this population, there were inevitably times when due to ill health patients were unable to participate in exercise training. Likewise, there were instances when participants refused to engage in follow-up telephone calls, gym refresher sessions or nurse practitioner and dietitian appointments. As this study aimed to replicate real-world scenarios and outcomes, these patients were still included in the final analysis. Therefore, the adherence of the LI differed for many people at varying times throughout the 36 month period and as such the efficacy of exercise training in this population needs to be considered in regards to the study outcomes. It is likely that had this been a shorter intervention with a strict compliance protocol, the efficacy of the exercise training on outcomes would have been greater.

We can only assume that the greatest change in VO_2peak seen in the LI group at 12 months is due to the initial supervised 8 week period and more concentrated telephone follow-up calls and gym refresher sessions in the first year of the study. Conducting another testing session at 8 weeks would have elucidated whether the greatest changes in cardiorespiratory fitness at 12 months are indeed attributable to the initial 8 weeks of supervised training. Nonetheless, the goal of this study was to assess the effects of longer duration training on fitness.

Potentially, a bias may exist from the type of patients who are willing to participate in a LI. Indeed, despite a cardiorespiratory fitness level below age-predicted normative values,(209) participants in this study may have been at a stage of change which is more likely to embrace a behaviour modification. Indeed, the control group appeared to slightly increase their level of physical activity in the first 18 months of the study.

Conclusions

This study found a 36 month LI doubled the number of CKD patients meeting physical activity guidelines. In addition, the intervention resulted in a significant increase in exercise capacity over the 36 months and a significant increase in cardiorespiratory fitness at 12 months followed by a decline rate similar to the control group. Furthermore, there was a significant improvement in arterial stiffness and autonomic function in the LI compared to the control group.

SECTION 2- High Intensity Interval Training

Chapter 7.
Systematic review and meta-analysis: High-intensity interval training in cardiometabolic diseases

This chapter has been published in the *British Journal of Sports Medicine*, October 2013

7.1 Abstract

Background: Cardiorespiratory fitness is a strong determinant of morbidity and mortality. In athletes and the general population it is established that HIIT is superior to MICT in improving cardiorespiratory fitness. This is a systematic review and meta-analysis to quantify the efficacy and safety of HIIT compared to MICT in individuals with chronic cardiometabolic lifestyle diseases.

Methods: Included studies were required to have a population sample of chronic disease, where poor lifestyle is considered a main contributor to the disease. Procedural quality of the studies was assessed by use of a modified Physiotherapy Evidence Base Database (PEDro) scale. A meta-analysis compared the mean difference (MD) of pre vs. post-intervention cardiorespiratory fitness (VO_2peak) between HIIT and MICT.

Results: Ten studies with 273 patients were included in the meta-analysis. Participants had coronary artery disease, heart failure, hypertension, metabolic syndrome and obesity. There was a significantly higher increase in VO_2peak after HIIT compared to MICT (MD 3·03 ml/kg/min, 95% confidence interval [CI] 2·00 to 4·07), equivalent to 9·1%.

Conclusions: HIIT significantly increases cardiorespiratory fitness by almost double that of MICT in patients with lifestyle-induced chronic diseases.

7.2 Introduction

Lifestyle-induced chronic diseases significantly alter the quality of life of sufferers. In many cases the disease itself can be potentially avoided or successfully managed with appropriate lifestyle modifications.(267) Diseases such as type II diabetes, atherosclerotic cardiovascular disease and the metabolic syndrome are closely related and often stem from the same preventable risk factors. Therapy for most chronic diseases involves exercise training to slow or reverse disease progression. Despite the known benefits of regular moderate intensity exercise in regulating risk factors in chronic disease, the majority of patients are still physically inactive.(222)

Rehabilitation for cardiac patients prior to the 1950's comprised abstention from all forms of physical activity. This was thought to diminish the cardiac load and assist in the reparative

process of the healing myocardial scar.(268) Levine and Lown (1952) appear to be the first to document challenging the idea of complete immobilization by introducing the then controversial ‘armchair treatment’ where patients were encouraged to sit in an armchair as much as possible during hospitalization post myocardial infarction.(269) When the weight of evidence suggesting the benefits of light to moderate activity in patients with chronic disease became irrefutable, exercise guidelines were created for this population.(270) Interval training appears to be first studied in cardiac rehabilitation in 1972, when patients were asked to cycle at high workloads for 60 seconds with a 30 second rest between intervals. Using the intervals, the patients were able to exercise for at least twice as long as what they were able to do when cycling continuously.(271) In 1977, a study on healthy participants examined the effects of a 10 week program which included a combination of interval and moderate continuous training. Interestingly, the authors found a linear increase in VO_2max over the 10 weeks, contrary to the studies hypothesis that the VO_2 would plateau over time. (272) In 1979 it was suggested that high intensity exercise was required to provoke the necessary training adaptations needed to improve exercise capacity in patients with recent myocardial infarctions.(273) One of the first studies to investigate intense exercise in patients with cardiovascular disease, found that if exercise is intense and prolonged enough, than it can instigate a reduction in myocardial ischaemia.(274) These findings were revolutionary, as they were established before widespread percutaneous coronary interventions were performed. From there, differing study protocols in the 1980’s and 1990’s in cardiac patients led to conflicting findings that appears to have decreased the interest in this approach.(275-277) Forty years after the first reported use of interval training in cardiac patients, the interest in high intensity interval training (HIIT) in higher risk patients has now led to a number of studies in this area.

HIIT involves alternating short bursts of high intensity exercise with recovery periods or light exercise. Studies in athletes and the general population have shown that increasing the intensity of exercise amplifies the training stimulus and associated adaptations, such as augmenting maximal oxygen uptake, anaerobic threshold, stroke volume, and performance.(57, 278) A commonly cited barrier to physical activity is lack of time. Including HIIT in a training program implies that greater health enhancing benefits could be gained in less time; making HIIT a more time efficient and attractive option. Moreover, short bursts of activity may address another common limiting factor, lack of motivation, as it may be a more enticing option than the prospect of continuously exercising for an extended period

of time. Short work periods at a higher intensity also results in a reduction in the ventilatory response and resultant dyspnoea which, in many chronic disease patients, are limiting factors to continuous exercise.(61)

VO_2max is a strong predictor of mortality.(279) Indeed, a 1-MET increase is associated with a 10-25% improvement in survival.(280) Furthermore, it is established that cardiorespiratory fitness is more cardioprotective than overall physical activity levels.(281) This provides further evidence regarding the benefits of higher intensity exercise compared to lower intensity general physical activity. It seems likely that improving VO_2max will improve the prognosis of chronic disease patients.

The aim of this systematic review is to compare and quantify the effects of HIIT against moderate intensity continuous training (MICT) in improving VO_2max in patients with lifestyle-induced chronic diseases. Furthermore, safety of HIIT will be discussed along with the feasibility of this approach with suggestions to standardise terminology and protocol recommendations.

7.3 Methods

Electronic searching of the Medline, PubMed, Embase and Cinahl databases were conducted from the earliest available date to April 2013, limited to the English language. The Medical Subject Heading (MeSH) database was employed to establish all related articles on HIIT and chronic disease. MeSH terms used were “cardiovascular diseases” OR “metabolic diseases” OR “chronic diseases” and their related terms. Text words used in conjunction with the MeSH terms were “high intensity training/exercise”, OR “interval training/exercise”, OR “intermittent training/exercise”, OR “low volume training/exercise” OR “aerobic interval training/exercise”. Reference lists of retrieved articles were also searched for other appropriate studies.

Inclusion criteria

Only full text, randomised control trials were considered for inclusion. The studies were required to have a cardiometabolic chronic disease population, where poor lifestyle is considered a main contributor. The main characteristics of such diseases, such as coronary artery disease, heart failure, diabetes, hypertension, obesity and metabolic syndrome are

recognisable by the common presence of cardiovascular disease risk factors. Other systematic reviews on the effects of HIIT have been conducted with specific conditions; pulmonary disease(61) and coronary artery disease.(282) Inclusion criteria were: exercise protocols using cardiorespiratory exercise training; a comparator group that completed MICT (matched to HIIT); an accepted measure of intensity (i.e. heart rate or rating of perceived exertion); intervention of four weeks; appropriate intensities for both groups (e.g. 60-75% PHR for MICT, 85-95% PHR for the high intensity intervals, or 80-100% peak work rate (PWR)); and a direct measure of VO_2peak/max. Due to the difficulty of chronic disease patients in reaching a plateau in VO_2max, VO_2peak was reported in nine of the studies.(191, 193-198, 283, 284) Studies including other activities (e.g. resistance training) on top of the prescribed aerobic intervention were also included, provided the same volume and modality of resistance exercises were given to both MICT and HIIT groups.

Data collection and analysis

After full text analysis of the retrieved articles, ten articles were identified to have met the inclusion criteria for the purpose of meta-analysis (Figure 7.1). These trials involved 273 patients consisting of men and women. This included 137 in HIIT groups and 136 in the MICT groups. The corresponding authors of two eligible studies were contacted for VO_2 data, but no information was provided.(285, 286)

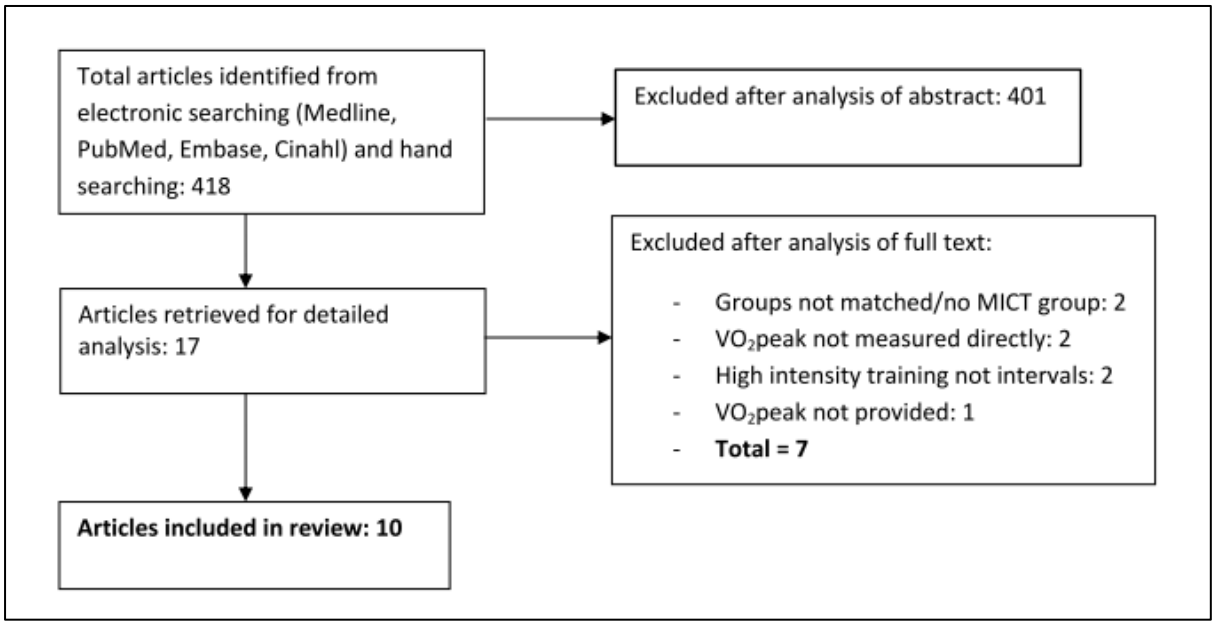


Figure 7.1 Systematic flow of article included from initial search to inclusion. The systematic review process identified ten articles for inclusion in the meta-analysis

Procedural quality of the studies was assessed by use of the modified PEDro scale. One point was awarded for each of the 10 criteria. Review Manager statistical software 5·0 (Nordic Cochrane Centre, Copenhagen, Denmark) was used to determine the mean difference and 95% CI. A forest plot is used to portray the treatment effects of each study.

7.4 Results

Seven of the ten studies included in this review studied cardiovascular disease (Table 7.1); Rognmo et al. (2004),(194) and Moholdt et al. (2005)(283) investigated coronary artery disease and Wisloff et al. (2007),(193) Roditis et al. (2007),(284) Freyssin et al. (2012),(198) Fu et al. (2011)(197) and Iellamo et al (2012)(195) recruited heart failure patients. Molmen-Hansen et al. (2012)(191) studied participants with hypertension, Schjerve et al. (2008)(196) looked at participants with obesity and Tjonna (2008) investigated the metabolic syndrome.(192) Six of the 10 studies were conducted at the same institute, the Norwegian University of Science and Technology.(191-194, 196, 283)

Table 7.1 Summary of studies comparing the effects of HIIT vs. MICT

Study	Population	Intervention	Interval Parameters	Program	Outcomes of HIIT compared to MICT
Rognmo et al., (2004)(194)	21 stable coronary artery disease patients HIIT (n=8), MICT (n=9)	Uphill treadmill walking HIIT- 4 x 4 min intervals (85-95% PHR), 3 min recovery (65-75% PHR), 33 mins MICT- 65-75% PHR, 41 mins	Ratio:4/3 MI:80% Amplitude: 25%	Supervised 3x/week 10 weeks	⇑ VO_2peak
Roditis et al., (2007)(284)	21 stable CHF patients HIIT (n=11), MICT (n=10)	Electromagnetically braked cycle ergometer HIIT – 30 sec intervals (120% pWR), 30 sec recovery (0% pWR), 40 mins MICT – 60% pWR, 40 mins	Ratio:1/1 MI:60% Amplitude: 72%	Supervised 3x/week 12 weeks	⇔ VO_2peak

Wisloff et al., (2007)(193)	27 stable postinfarction heart failure patients HIIT (n=9), MICT (n=9), C (n=9)	Uphill treadmill walking HIIT- 4 x 4 min intervals (90-95% PHR), 3 min recovery (50-70%), 38 mins MICT- 70-75% PHR, 47 mins C- standard PA advice	Ratio: 4/3 MI: 76% Amplitude: 43%	2x/week supervised, 1x/week unsupervised for 12 weeks HIIT and MICT groups	⇑ VO_2peak ⇑ LVEF ⇑ LV remodelling ⇑ EF ⇑ mitochondrial function ⇑ QOL
Schjerve et al., (2008)(196)	40 obese patients, BMI >30 kg/m^2 HIIT (n=14), MICT (n=13), strength training (n=13)	Treadmill walking or running HIIT- 4 x 4 min intervals (85-95% PHR), 3 min recovery (50-60% PHR), 38 mins MICT- 60-70% PHR, 47 mins Strength- abdominal, back leg strength programme	Ratio: 4/3 MI: 80% Amplitude: 25%	2x/week supervised 1x/week unsupervised 12 weeks all groups	⇑ VO_2peak ⇑ peak O_2 pulse ⇑ PGC-1a ⇑ sarcoplasmic reticulum Ca^{2+} uptake ⇑ FMD ⇔ artery diameter, shear rate ⇓ LDL and body weight in MICT

Tjonna et al., (2008)(192)	32 patients with metabolic syndrome HIIT (n=12), MICT (n=10), C (n=10)	Uphill treadmill walking/running HIIT- 4 x 4 min intervals (90% PHR), 3 min recovery (70%), 40 mins MICT- 70% PHR, 47 mins C- followed advice from family physician	Ratio:4/3 MI:80% Amplitude: 25%	Supervised 3x/week 16 weeks	⇑ VO_2max ⇑ PGC-1a ⇑ sarcoplasmic reticulum Ca2+ uptake ⇑ EF ⇑ insulin action ⇑ lipogenesis
Moholdt et al., (2009)(283)	59 CABG HIIT (n=23), MICT (n=25)	Treadmill walking HIIT- 4 x 4 min intervals (90% PHR), 3 min recovery (70%), 37 min MICT- 70% PHR, 46 min	Ratio:4/3 MI:80% Amplitude: 25%	Supervised 5x/week 4 weeks. Unsupervised until 6mth time point	After 4 weeks: ⇔ VO_2peak After 6 months: ⇑ VO_2 peak ⇑ HR recovery after 6 months. ⇔ QOL

Fu et al. (2011)(197)	45 CHF HIIT (n=15), MICT (n=15), control (n=15)	Cycle ergometer HIIT- 5 x 3 min intervals (80% VO_2peak), 3 min recovery (40% VO_2peak), 33 minMICT- 60% VO_2peak, 36 min	Ratio: 1 MI: 60% Amplitude: 67%	Supervised 3x/week 12 weeks.	⇑ VO_2peak ⇑ CO ⇑ TPR ⇑ LVEF ⇓ BNP, MPO, IL-6 ⇑ QOL
Freyssin et al., (2012)(198)	26 stable CHF patients HIIT (n=12), MICT (n=14)	Uphill treadmill walking and cycle ergometer HIIT – 3 x (12 x 30 sec intervals, 60 sec complete rest), 80% pWR, 5 mins rest between sets, 54 mins MICT – 45 mins, intensity at 1st ventilator threshold	Ratio:1/2 MI: Amplitude:	Supervised 6x/week 8 weeks	⇑ VO_2peak ⇑ exercise test duration ⇑ oxygen pulse ⇑ VO2 at 1st ventilator threshold
Iellamo et al., (2012)(195)	16 post-infarction HF HIIT (n=8), MICT (n=8)	Uphill treadmill walking HIIT – 4 x 4 min intervals (75-80% HRR), 3 min recovery (45-50% HRR), 20-34MICT – 45-60% HRR, 30-45 min, 30-45	Ratio: 4/3 MI: 62.5% Amplitude: 48%	Supervised 2x/week progressing to 5x/week 12 weeks	⇔ VO_2peak ⇔ CO, SV, LVEF ⇔ lipids, HOMA-IR

Molmen-Hansen et al., (2012)(191)	88 patients with essential hypertension stage 1-2, SBP 140-170 mmHg and DBP 90-109 mmHg HIIT (n=25), MICT (n=23), C (n=25)	Treadmill walking HIIT – 4 x 4 min intervals (90-95% PHR), 3 min recovery (60-70%), 38 mins MICT – 70% PHR, 47 mins C – standard recommendations for hypertension, including light-moderate exercise without supervision	Ratio: 4/3 MI: 75.25% Amplitude: 40.5%	Supervised 3x/week, 12 weeks	⇑ VO_2peak ⇑ heart rate recovery ⇑ endothelial function ⇑ EF, SV ⇓ 24 hour SBP ⇔ LV mass, TPR ⇑ QOL

Ratio = relationship between duration of interval and recovery. MI = Mean intensity between high intensity interval and recovery. Amplitude = difference between interval and recovery intensities, divided by the mean intensity. 1-RM = 1 repetition maximum for plantar flexion, SaO2 = oxygen saturation, ABI = ankle brachial index, FMD = flow mediated dilatation, LV = left ventricular, EF = ejection fraction, QOL = quality of life, SV = stroke volume, TPR = total peripheral resistance * No within group differences, BNP = brain natriuretic peptide, MPO = myeloperoxidase, IL-6 = interleukin-6

The quality assessment of the studies was determined by 2 reviewers (Table 7.2). Where there was a discrepancy, the average was taken. The studies achieved a mean PEDro score of 7·35/10. There was no blinding of all assessors in any of the studies reviewed. A between groups statistical comparison was provided by all studies with measures of variability given for VO_2peak.

Table 7.2 Methodology quality of the HIIT studies conducted in chronic disease patients. + = criteria is achieved and 1 point is given. - = criteria not achieved. ± = whether or not the criteria was met was viewed differently by 2 different reviewers and therefore half a point is given

	1	2	3	4	5	6	7	8	9	10	Total
Rognmo 2004	+	+	+	+	-	+	+	+	+	-	**8**
Roditis 2007	-	+	-	+	-	+	+	+	+	-	**6**
Wisloff 2007	+	+	-	+	-	+	+	+	+	-	**7**
Schjerve 2008	+	+	-	+	+	-	+	+	+	-	**7**
Tjonna 2008	±	+	-	+	-	+	+	+	+	+	**7.5**
Moholdt 2009	+	+	+	+	-	±	±	+	+	+	**8**
Fu 2011	+	+	-	+	-	+	+	+	+	-	**7**
Freyssin 2012	+	+	-	+	-	-	+	+	+	+	**7**
Iellamo 2012	+	+	-	+	-	+	+	+	+	-	**7**
Molmen-Hansen 2012	+	+	-	+	+	+	+	+	+	+	**9**

PEDro Criteria:

1. Eligibility criteria were specified
2. Subjects were randomly allocated to groups
3. Allocation was concealed
4. The groups were similar at baseline regarding the most important prognostic indicators
5. There was blinding of all assessors who measured the primary outcome
6. Measures of at least one key outcome were obtained from more than 70% of the subjects initially allocated to groups
7. All subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analysed by “intention to treat”

8. The results of between-group statistical comparisons are reported for the primary outcome
9. The study provides both point measures and measures of variability for at least one key outcome
10. Sample size calculations were explained

Meta-Analysis

Nine studies included means and SD of VO_2peak to allow the calculation of an overall mean difference.(191-196, 198, 283, 284) One study provided means ± standard error of mean (SEM), allowing the SD to be calculated.(197) Homogeneity between the studies was displayed with I^2=9%, p=0·36. The mean difference in VO_2peak from the ten studies was 3·03 ml/kg/min (95% CI 2·00 to 4·07), significantly (p<0·001) favouring HIIT (Figure 7.2).

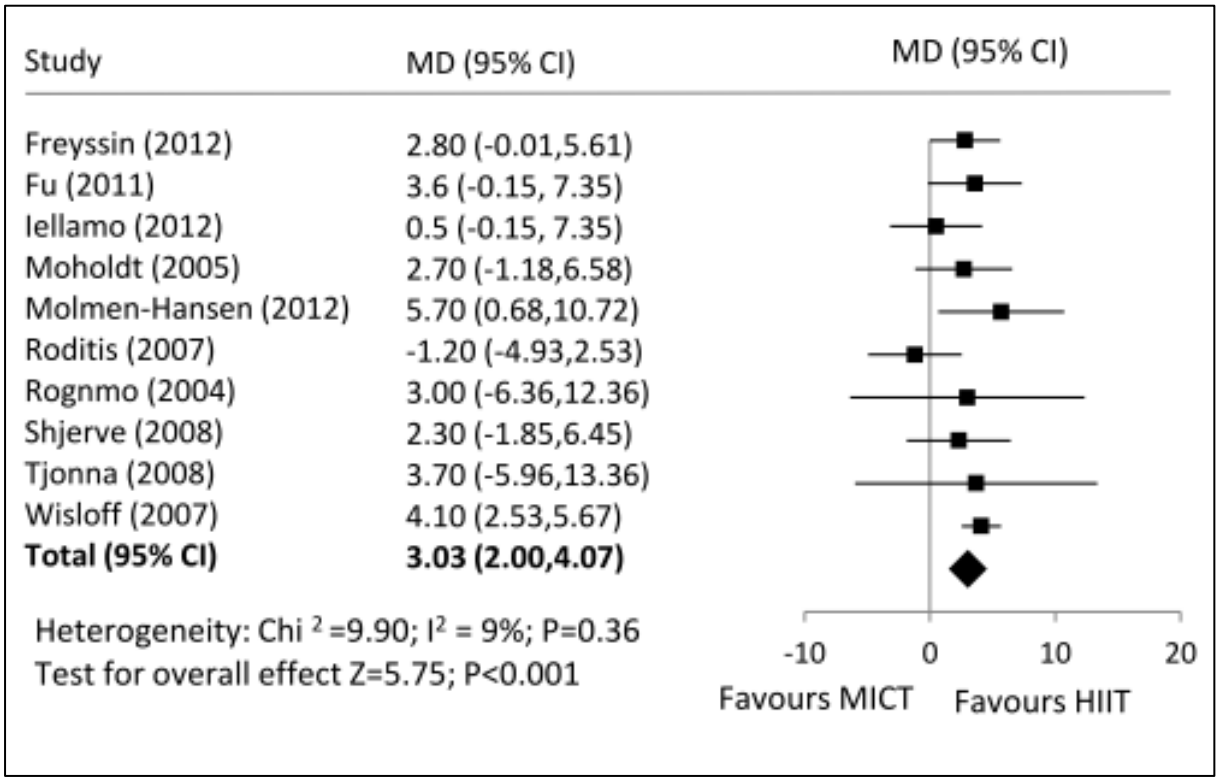


Figure 7.2 Forest plot from meta-analysis of HIIT and VO2 peak. HIIT elicits a 3.03 ml/kg/min greater mean difference than MICT

Exercise Protocols

Mode

Seven studies used uphill walking/running on a treadmill as the primary exercise modality.(191-196, 283) One study described the exercise undertaken as 'treadmill walking', therefore the differences in heart rate between intervals could have been achieved either through change in speed or change in incline.(283) In three studies, cycle ergometers were used for the duration of the program.(197, 198, 284) Freyssin et al. used strengthening, stretching and relaxing exercises in addition to the treadmill and cycle program (198).

Intensity

Intensities were set based on baseline maximal/peak testing data. Six studies used a percentage of PHR or maximal heart rate (MHR), two used a percentage of PWR or peak power and two studies used VO_2peak/heart rate reserve (VO_2R/HRR). The Norwegian studies used a heart rate of between 85 and 95% for the high intensity interval with a recovery of 50-75% MHR.(191-194, 196, 283) Two studies on the cycle ergometer were set at an intensity of 80 and 120% PWR for the high intensity interval with a passive recovery.(198, 284) The two studies using HRR/VO_2peak to determine intensity used 75-80% for the interval and 40-50% for an active recovery.(195, 197)

Total Duration

A common approach to compare HIIT and MICT sessions is to match the energy expenditure, resulting in the HIIT session being shorter. Seven of the studies differed the duration of the HIIT and MICT so that training sessions were isocaloric.(191-194, 196, 197, 283) The median duration of exercise times was 38 minutes for the HIIT group and 46 minutes for the MICT group. One study kept the duration the same between both groups but altered the mean intensity between groups.(284) Another study used a different method (training impulse method) to alter the duration of each session based on the average change in heart rate.(195)

Interval Duration

Seven of the studies included high intensity intervals of 4 minutes with 3 minutes of active recovery.(191-196, 283) One study included 5x3 minute intervals with 3 minute recoveries.(197) Two studies included shorter interval and recovery periods, with 30 seconds

used for both interval and recovery,(284) and 30 seconds used for interval and 60 seconds for recovery. (198)

Supervision

Three studies included both a supervised training component and a self-administered home program,(193, 196, 283) the remaining were purely supervised sessions. Direct supervision allows the instructor to adjust the intensity, monitor the patient and provide motivation. The length of supervised HIIT or MICT within the studies varied from four to sixteen weeks. One study was supervised for the first four weeks, thereafter subjects were advised to continue the training program at home for the following six months.(283) The studies prescribed three-six training sessions/week, with two studies including one home session/week out of the three for both HIIT and MICT groups.(193, 196) Due to constant changes in the heart rate response to exercise, supervision allows for closer monitoring to ensure rates are within the desired zones. Therefore, the presence of a supervisor is likely to improve the accuracy of the intensity.

Outcomes

Cardiorespiratory Fitness

There were improvements in VO_2peak from both HIIT and MICT in nine of the ten studies (Table 7.3).(191-198, 283) The average of pre training values was 22·5 ml/kg/min and increased to 27·9 ml/kg/min after HIIT (a 19·4% increase). MICT had baseline values of 22·6 ml/kg/min that increased to 25·2 ml/kg/min after the intervention (10·3% increase). The mean difference in the change in VO_2peak between HIIT and MICT was 3·03 ml/kg/min. A measure of exercise capacity (six-minute walk test), as well as VO_2peak, was assessed in one study. It was found that the distance walked improved in both HIIT and MICT groups.(198)

Cardiometabolic risk factors

The study by Tjonna et al. found that MICT and HIIT both reduced blood pressure; approximately 10 mmHg systolic and 6 mmHg diastolic.(192) Molmen-Hansen et al. also showed a significant reduction of 12 mmHg for systolic blood pressure in the HIIT group.(191) Other studies showed no change in systolic blood pressure after the interventions. Significant reductions in oxidized low density lipoproteins were found in HIIT

and not MICT.(192, 193) However, in one study the opposite was found.(196) Furthermore, it was established that high density lipoprotein was increased by 25% in the HIIT group.(192) These changes were supported by Wisloff et al. who also found an increase in high density lipoprotein, lower TG and improved fasting glucose in the HIIT group.(193) An improvement in TG was identified by Moholdt et al. for both HIIT and MICT groups.(283) Iellamo et al. found no significant differences in the metabolic profile (lipids, HOMA-IR), with the exception of an improvement in fasting glucose with HIIT.(195) Fu et al. found improvements in oxidative stress/inflammatory markers plasma brain natriuretic peptides, myeloperoxidase and IL-6.(197)

Two studies demonstrated no positive improvements in weight loss, BMI or body composition between the HIIT and MICT groups post intervention.(192, 195) Furthermore, one study on obese patients reported a greater decrease in body weight with MICT than with HIIT.(196) However, reduced fatty acid transport protein-1 and fatty acid synthase levels (markers of fatty acid uptake and lipogenesis) were identified, which was associated with improvements in anti-insulin receptor activation in HIIT but not MICT.(192)

The findings from Tjonna et al. showed increased circulating adiponectin and improved insulin sensitivity and β-cell function in the HIIT group.(192) No changes in adiponectin were found by Moholdt et al. at four weeks, however after the six months intervention both HIIT and MICT increased circulating levels.(283)

Skeletal Muscle

Three studies identified significant increases in PGC-1alpha, indicating improved mitochondrial biogenesis in the HIIT group.(192, 193, 196) These studies also significantly increased the maximal rate of Ca^{2+} reuptake into the sarcoplasmic reticulum by 50- 73%. Change in total myoglobin concentration and tissue oxygenation in the vastus lateralis was observed in HIIT but not MICT both acutely and after the 12 week intervention.(197)

Myocardium and Vasculature

Flow mediated dilatation was improved after both HIIT and MICT in studies that assessed endothelial function.(191-193, 196) An increased availability of nitric oxide was found with HIIT but not MICT.(192) Furthermore, a greater increase in antioxidant status found in HIIT than MICT, supports the finding of increased production and availability of nitric oxide.(193) An improvement in antioxidant status was not supported by the findings of Schjerve et

al.(196) The heart failure patients demonstrated improved left ventricular ejection fraction by 10% and a positive reversal of left ventricular remodelling.(193) An improvement in left ventricular ejection fraction was also demonstrated by Fu et al., alongside increases in cardiac output and total peripheral resistance. (197) In contrast, Iellamo et al. found no difference in cardiac output, stroke volume and left ventricular ejection fraction in their heart failure patients.(195) The hypertensive patients also had significantly improved ejection fraction, stroke volume, systolic flow velocity, end-diastolic volume, early diastolic mitral annulus tissue velocity, isovolumic relaxation rate.(191)

Adherence

A minimum attendance of 70% and 90% of training sessions was an inclusion criterion for four of the studies.(191, 194, 196, 283) Only one participant was excluded from analysis from one of these studies based on this criteria.(194) For the first four week supervised period of Moholdt et al.'s study, the HIIT group attended 82% of sessions and the MICT group attended 84%. The attendance rate during the remainder of the six month home program was not identified.(283) Both groups in six of the other studies reported attendance rates above 85%.(192-195, 197, 198) One study did not mention adherence to the protocol for either groups.(284)

Enjoyment and Quality of Life

HIIT was reported to be more enjoyable than MICT.(192) Three studies demonstrated that HIIT improved quality of life more than MICT, (191, 193, 197) whereas another showed similar increases between the two groups (283). Anxiety and depression also had similar improvements with both the HIIT and MICT.(198)

Adverse Events

Reporting of adverse events are important in determining the safety of applying this type of training to high risk populations. No studies described their adverse event monitoring protocol. However, four reported no adverse events due to the exercise training (193, 194, 198, 283) and six did not mention adverse events.(191, 192, 195-197, 284)

Table 7.3 Adaptations occurring significantly more with HIIT compared to MICT

↑ VO_2peak
↓ Systolic and diastolic blood pressure
↑ High density lipoproteins
↓ Triglycerides and fasting glucose
↓ Oxidative stress and inflammation
↓ FATP-1 and FAS
↑ Adiponectin, insulin sensitivity and β-cell function
↑ PGC-1alpha
↑ Maximal rate of Ca^{2+} reuptake
↑ Availability of nitric oxide
↑ Cardiac function
↑ Enjoyment of exercise
↑ Quality of life

FATP-1=fatty acid transport protein 1; FAS=fatty acid synthase

7.5 Discussion

The main finding from this meta-analysis is that HIIT is superior to MICT in improving cardiorespiratory fitness in patients with lifestyle-induced chronic diseases. Indeed, the increase in VO_2peak with HIIT was almost twice the increase gained from MICT. In addition, although there is limited data, HIIT appears to be well-tolerated and safe.

Efficacy

HIIT increased VO_2peak by 5·4 ml/kg/min (19·4%) compared to 2·6 ml/kg/min (10·3%) with MICT. It has been shown that a 1-MET (3.5 ml/kg/min) increase is associated with a 10-25% improvement in survival.(280) Indeed, the protective role of fitness occurs even in the presence of established cardiovascular disease risk factors.(26) Cardiorespiratory fitness is an easily modifiable risk factor, therefore identifying the most effective way to improve VO_2 peak should be a health priority.

Some of the studies investigated mechanisms explaining the greater improvement of VO_2peak with HIIT. Peripheral adaptations leading to improvements in cardiorespiratory fitness were investigated by measuring PGC-1alpha and Ca^{2+} reuptake into sarcoplasmic reticulum in skeletal muscles. Mitochondrial dysfunction is central to most chronic diseases and could play a vital role in the reduced cardiorespiratory fitness that is experienced by these populations. Improvements in mitochondrial biogenesis have been demonstrated by increases in PGC-1alpha after a single bout of low volume HIIT.(287) Studies by Tjonna et al. and Wisloff et al. identified significant increases in PGC-1alpha (138% and 47% respectively), indicating mitochondrial biogenesis in the HIIT group.(192, 193) Wisloff et al. found the increase in PGC-1alpha to be strongly correlated with improved VO_2peak ($r=0{\cdot}72$, $p<0{\cdot}01$), supporting the influence of mitochondrial function on exercise capacity.(193) Mitochondrial biogenesis is essential in maintaining structural integrity of skeletal muscle. Daussin et al. concluded that fluctuations in adenosine tri-phosphate turnover in interval training, which is different to usual steady state conditions of adenosine tri-phosphate production, activates the signalling pathways which leads to increases in PGC-1alpha.(288) The increase in PGC-1alpha identified by Wisloff et al. and Tjonna et al. may translate into improvements in both aerobic and functional capacity. Both studies also significantly increased the maximal rate of Ca^{2+} reuptake in the sarcoplasmic reticulum by 50- 60%.(192, 193) The increase in calcium cycling reduces skeletal muscle fatigue and would contribute to improvements in muscle function and therefore cardiorespiratory fitness. The findings by Fu et al. demonstrate repeated bouts of deoxygenation in HIIT but not MICT, which may contribute to the observed adaptations to the muscles oxidative capacity.(197)

Improvement in VO_2peak with HIIT can also be explained mechanistically by central factors. Findings from one of the studies indicate that HIIT improved ejection fraction in heart failure patients to the same degree as treating with angiotensin converting enzyme inhibitors and β-blockers, outlining the potential for further left ventricular remodelling with HIIT. Improvements in cardiac remodelling are supported by the findings of Fu et al., with significant reductions in brain plasma natriuretic peptide levels.(197) HIIT may also contest modern medical treatments in improving systolic and diastolic blood pressure, with significant decreases of 12 mmHg and 8 mmHg respectively, found in hypertensive patients.(191) It was also found that myocardial contractile function was improved with HIIT in terms of stroke volume, mitral annular excursion, ejection velocity, and systolic mitral annular velocity. Besides a small reduction in left ventricular filling pressure with MICT, the

above mentioned improvements in systolic and diastolic function with HIIT were not found with MICT.

Conversely, Roditis et al. (284) demonstrated similar improvements in VO_2peak for both HIIT and MICT groups. Furthermore, the findings suggest that phase II of oxygen kinetics (indirect measurement of muscle oxidative capacity), showed superior improvements after MICT. The use of 30 second Wingate sprint intervals in this study may highlight the need for longer duration of intervals, such as those used in four of the other studies reviewed.(192-194, 283) Although 30 second intervals are a more time efficient option than endurance training (similar improvements shown with ~90% less volume), 30 seconds may not be long enough to induce *superior* health-enhancing benefits.(278) It appears from the studies reviewed that HIIT elicits many superior benefits to MICT, in a slightly shorter time period (median 38 vs. 46 minutes, respectively). It seems reasonable to suggest, gaining similar benefits would require either a longer period of MICT, or an even shorter period of HIIT-supporting the ideology of HIIT being a more time efficient option. Future studies should identify the minimum amount of HIIT needed to elicit adaptations similar to MICT. Another study that did not find a greater benefit of HIIT over MICT, may be due to the method of establishing intensity by assessing blood lactate levels.(195)

The HERITAGE study has established the influence genetic components have on the trainability of VO_2max in certain individuals, resulting in 'non-responders' to exercise training.(289) The consistently large improvements in VO_2max seen in a variety of cohorts in HIIT studies, questions whether this type of training can ameliorate the familial dampening of VO_2 in these 'non-responders'. Only 1 study in 1986 has examined the relationship between HIIT and hereditary trainability.(290) Although aerobic capacity wasn't assessed in this study, it was found that genotype *was* associated with anaerobic capacity after a HIIT training intervention, suggesting the effectiveness of HIIT is affected by hereditary factors.(290) Indeed, in the studies reviewed in the meta-analysis, the SD of the change in VO_2peak in the HIIT group was 26% (relative to the mean change), whereas the MICT group had only an 8% variance. Future studies are needed to further compare the effects of HIIT and MICT on VO_2peak trainability.

In addition to cardiorespiratory fitness, efficacy can also be assessed by the effect on cardiometabolic risk factors. The studies reviewed found reductions in risk factors in HIIT notwithstanding differences in body mass, BMI or weight loss between HIIT and MICT.

These findings are supportive of the theory of 'fitness over fatness'- in that it is more beneficial to health to improve physical fitness than focus solely on weight loss. This is supported by Terada et al. (2013) who found similar reductions in body fat percentage in HIIT and MICT groups after a 5 day/week, 12 week program.(291) Although, weight loss was not different between groups, one study found a reduction in fatty acid transport protein-1 and fatty acid synthase levels in the HIIT group, suggesting suppression of fatty acid uptake and lipogenesis. No studies measured visceral adiposity, however increased circulating adiponectin levels found with HIIT and not MICT is suggestive of decreased intra-abdominal obesity.

Due to the augmented PHR and cardiac output that is experienced in HIIT, it is likely that pulsatile and shear stress are influencing vasculature during periods of intense exercise.(292) Interval exercise has been shown to reduce arterial stiffness through increased distensability of the vessels.(252, 292) A recent study by Smart (2012) demonstrated no difference in fitness and cardiac function between moderate training and intermittent training groups matched by volume of work.(293) However, the intervals were not high intensity, suggesting that to elicit changes, intervals need to increase heart rate to a certain level. Arterial stiffness is a key contributor to a number of cardiovascular conditions that influence mortality through cardiovascular disease.(294) Two studies demonstrated higher endothelial derived nitric oxide production in the HIIT group.(192, 193) It is suggested that shear stress through an increase in pulse and frequency pressure promotes vasodilatory nitric oxide release, thereby increasing arterial compliance.(295) This is further supported by Wisloff et al. and Tjonna et al. with greater increases in flow mediated dilatation found in HIIT than in MICT.(192, 193) The improvements in endothelial function are supported by the findings from Fu et al.(197) The study's findings demonstrate an increase in perfusion and oxygen utilization between cerebral and muscular tissues which occurs through increases in shear flow and metabolic stress. The increased perfusion to the vastus lateralis and frontal cortex would promote increased flow and shear stress, and therefore the release of nitric oxide.

Safety

The reporting of adverse events enables the safety of the intervention to be inferred to the population being studied. Four of the ten reviewed studies reported no adverse events as a result of the exercise training.(193, 194, 198, 283) One study that did not mention adverse

events did explain that follow-up testing was not performed on five patients due to either orthopaedic-related problems *or* inconvenient program timetable.(284) Other reasons cited for loss of patient follow-up were comorbidities (not cited which group),(283) ankle fracture in the HIIT group and knee injury in the MICT group.(194) One participant in a MICT group died from cardiac causes, however it was stated that this was unrelated to exercise training.(193)

A recent study by Rognmo et al. (2012) aimed to assess the safety of HIIT compared to MICT, both during and after exercise, of coronary heart disease patients in cardiac rehabilitation.(296) The study of 4846 participants indicated that the risk of a cardiovascular event occurring is low in both MICT and HIIT. During this study there was one fatal cardiac event during the MICT and two non-fatal cardiac events during the HIIT. The calculated event rates were one per 129 456 hours of moderate intensity exercise and one per 23 182 hours of high intensity exercise. Moreover, when vigorous intensity exercise was energy matched with moderate intensity exercise, greater cardioprotective benefits were elicited. To accurately assess a cardiac event occurring during training, the study required a sample size of >20 500 patients. Despite the study being underpowered,(297) the results explore the beneficial dose-response relationship associated with HIIT and the negligible risk that is involved.

The safety of HIIT has been controversial amongst health professionals treating high risk populations. This has perhaps been created through a common avoidance of anginal episodes, which can sometimes be triggered by high intensity exercise. Although commonly thought to be a dangerous by-product of exercise, episodes of controlled ischaemia may actually be beneficial in preventing secondary conditions in coronary artery disease via ischemic preconditioning.(298) Exercise induced coronary collateral formation is based on vascular endothelial growth factor and this adaptation is correlated with exercise intensity. Lu et al. (2008) found that exercise-induced intermittent ischaemia promoted coronary angiogenesis by vascular endothelial growth factor.(299) The favourable effects of intermittent exercise on angiogenesis is further supported in a meta-analysis on peripheral arterial disease.(300) A normal cardiac troponin I in the study by Lu et al. indicated that no myocardial damage occurred when ischaemia was applied. Lu and co-authors state that HIIT is safe even for high risk chronic disease patients with stable angina. Rather than promoting the instigation of angina in HIIT, this information is intended to highlight the safety of HIIT in high risk populations. Caution should be applied on a case-by-case basis depending on the participants

cardiac history. Indeed, despite the patients in the studies reviewed being a high risk cohort, patients were only included if their diseases were relatively well-controlled, or stable. Therefore, these findings may not be applicable to some patients in these disease groups. Table 7.4 provides a suggested guide of contraindications for participation in HIIT in high risk patients. In summary, when used with appropriate screening, and communication with the patients doctor, HIIT can be a safe option for high risk patients.

Table 7.4 Suggested contraindications to HIIT

- Unstable angina pectoris
- Uncompensated heart failure
- Recent myocardial infarction (<4 weeks)
- Recent coronary artery bypass graft or
- Percutaneous coronary intervention (<12 months)
- Heart disease that limits exercise (valvular, congenital, ischemic,
- Hypertrophic cardiomyopathy)
- Complex ventricular arrhythmias or heart block
- Severe chronic obstructive pulmonary, cerebrovascular disease or uncontrolled peripheral vascular disease
- Uncontrolled diabetes mellitus
- Hypertensive patients with blood pressure >180/110 (or uncontrolled)
- Severe neuropathy

Limitations

Although this review provides the basis for further clinical trials testing of HIIT in rehabilitation of chronic disease patients, all studies analysed experienced methodological limitations. Although three of the studies used a different training protocol to the other seven, the heterogeneity of the VO_2peak measure was low (Chi^2=9·90, I^2=9%, p=0·36).(197, 198, 284) Due to the relatively recent interest with HIIT in chronic disease, HIIT training guidelines have not yet been created. Consequently, current research consists of a variety of intervals, intensities and program durations. Furthermore, comparison of studies proves difficult when volumes of HIIT and MICT are equated in different ways. There are also

discrepancies in the percentage of heart rate used. Some of the studies used a higher definition of moderate intensity (75%) to the general intensity of moderate exercise suggested by Norton et al (55-70% MHR) and ACSM (60-70% MHR).(63, 193, 194, 222) Not all studies included assessor blinding of VO_2peak, which may have influenced the testing results. Also, studies in cardiac rehabilitation programs may have been guided by other confounding factors such as dietary advice or smoking status. A limitation of this review, is that 6 of the 10 studies reviewed are from the same research group. It is important that these findings are replicated in future studies at other institutions.

The studies reviewed consisted of small sample sizes, with one study including an unequal ratio of males to females.(193) Current literature is lacking in HIIT studies on all the chronic diseases. This systematic review on cardio-metabolic lifestyle-induced chronic diseases would have benefited from studies with other lifestyle diseases such as Type II diabetes and CKD. Due to the limited research in this area, a variety of diverse diseases were included under the title cardio-metabolic disease for this meta-analysis. However, the pathophysiology of the varied diseases may mean HIIT will have different influences on the parameters being compared amongst different studies. Research on more chronic diseases will allow specific prescription guidelines to be recommended.

Protocol Recommendations

Future research is needed to establish the optimal protocol of HIIT. However, based on the findings from this review, recommendations in Table 7.5 are provided. It is important to note that the effectiveness and adherence to different HIIT protocols will always be highly individualised.

Table 7.5 Protocol recommendations for HIIT

Frequency	3x/week
Duration	40 minutes
Modality	Treadmill/hill, cycle ergometer. Increasing speed or incline
Intensity	Interval = 85%-95% PHR
	Rest = Passive – 70% PHR
Interval times	4x4 minute intervals
	3x3 minute recovery
Warm-up	10 minutes at 60% PHR
Cool-down	5 minutes at 50% PHR

PHR=peak heart rate

Mode and Intensity: The duration of intervals can differ greatly in various HIIT protocols. Two studies which used shorter duration intervals of 30 seconds both showed no improvements and smaller improvements in VO_2peak respectively.(198, 284) Studies which demonstrated the biggest changes in VO_2peak after a HIIT intervention used the 4x4 approach (4 intervals at 4 minutes each at between 90 and 95% PHR).(191-194, 196, 283) The recommendation of the 4x4 approach (~40 minutes duration) in Table 7.5 does not preclude shorter duration HIIT protocols as being potentially more appropriate. However, more research is needed to compare a shorter duration of HIIT with the current recommendation. Furthermore, future research comparing HIIT and MICT should ensure that the MICT is kept consistent with current definitions of moderate exercise.

Recovery: The intensity and duration of the recovery period in HIIT may play just as integral role in HIIT as the interval. Past research has suggested that an active recovery is recommended in order to effectively aid the process of lactate removal.(301) However, a study by Dupont et al. indicated that during the active rest period there was less replenishment of oxygen in myoglobin and haemoglobin and a reduced rate of resynthesis of phosphocreatine from the previous work phase.(302) Meyer et al's study on chronic heart failure patients found that short intervals (30s) with passive recovery provided the most benefit (longest time to exhaustion in an exercise test).(303) This study also determined that including a passive recovery instigated a lower RPE as indicated on the BORG scale, despite having higher oxygen pulse, which is dependent on stroke volume and arterio-venous difference.(303) Logistically it may be more practical and convenient to include an active

recovery for the purpose of timing and equipment control, albeit as low intensity as possible. To successfully employ HIIT, recovery intensities need to be achieved. If the heart rate is not reducing to the required intensity by the end of the recovery period, the interval work rate should be adjusted accordingly.

Feasibility

Indisputably, the success of an exercise intervention and maximising long term benefits relies directly on adherence to the exercise program. Central to adherence is enjoyment of the activity being prescribed. Bartlett et al found that ratings of perceived enjoyment after HIIT were higher when compared to MICT.(304) It was identified in this study that rating of perceived exertion had a positive correlation with enjoyment, through a likely combination of stimulation and accomplishment. Wisloff et al's study addresses the feasibility of HIIT being performed independently.(193) During home sessions, participants were provided with heart rate monitors that recorded heart rate data. These monitors were placed so that they could not be seen by the participant. The patients achieved the correct heart rate zones without feedback from the heart rate monitor. The design of this study supports the feasibility of incorporating HIIT into a real-world situation, where a home HIIT program can be successfully prescribed after a familiarization period. This is further supported by Moholdt et al., who demonstrated adherence to a home program of HIIT was as suitable as usual cardiac residential care.(305)

Future Directions

Given the small number of studies conducted thus far there are still important questions that need to be addressed. Similar to current issues with the dose response of less intensive exercise, much more work is needed to determine if the recommendations provided here are the optimal approach for future studies. Furthermore, it is realistic that most people will combine HIIT with MICT, therefore determining the optimal ratio of *both* types of training over longer periods of time is also important. Similarly, current guidelines for all populations recommend inclusion of resistance training in an exercise program, yet the interaction between HIIT and resistance training is unknown. As it is likely that most people will include HIIT and resistance training in the same session, future studies should identify the ratio of HIIT and resistance training needed for optimal benefits.

Analogous to the clinical trial phases in the development of a new pharmaceutical product, the evaluation of HIIT training for patients with cardiometabolic diseases would be in phase 2 (assessing efficacy). Indeed, guidelines for clinical populations should use the same approach and criteria as other therapies (e.g. drugs) with eventual recommendations based on large randomized control trials and not on small feasibility and efficacy studies alone.
Another important question is regarding adherence after the supervised program. Future studies should determine whether this approach is sustainable in a non-supervised, home-based environment.

Consistency of Terminology

The terminology used to describe HIIT varies across research groups. The Norwegian research group that has conducted the majority of studies in this area has preferred the term 'aerobic interval training (AIT)'. Other prominent groups have used 'SIT' to describe all-out supra-maximal (>100% VO_2max) intervals in studies with healthy individuals (278, 306), or low-volume HIIT when short intervals (~30 seconds) with an intensity <95% are used.(307, 308) We suggest that the classification scheme shown in Figure 7.3 is used to standardise terminology in future studies. This approach is based on 1) the widespread media use of the term 'HIIT' rather than 'AIT' for near-maximal intensity (80-100% PHR) intervals, 2) the additional information regarding the intensity of the interval provided by HIIT compared to AIT and 3) the need to separate all-out supra-maximal sprint interval training from HIIT as there are concerns for the safety of this all-out approach in clinical populations. Indeed, only Roditis et al. has performed SIT in chronic disease populations. For safety reasons, Gibala's group in Ontario have modified their low-volume SIT program for a cohort with Type II diabetes using a peak intensity of ~90% PHR (rather than an 'all-out' effort) for 10x60 second work bouts. Based on the above recommended terminology, this therefore becomes HIIT, rather than SIT.(308) It is also suggested that in comparative studies, the term 'moderate intensity continuous training' (MICT) should be used, where appropriate.

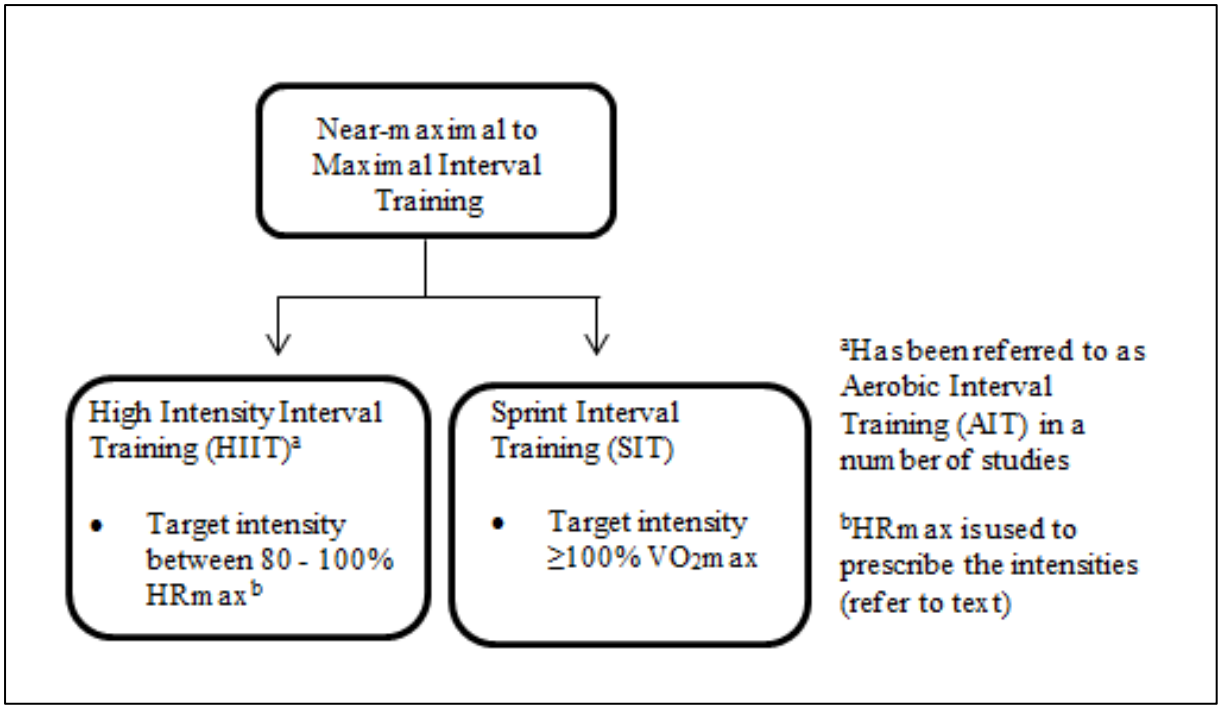


Figure 7.3 Suggested classification scheme for interval training based on exercise intensity

Conclusions

This meta-analysis has identified that HIIT has more physiological benefits than MICT in lifestyle-induced chronic disease patients. HIIT significantly improved cardiorespiratory fitness by almost double that of MICT (19·4% vs 10·3%). This should translate into greater decreases in risks of morbidity and all-cause mortality. Incorporation of HIIT into a rehabilitation program may be a more achievable way for people with chronic disease to reach a level of exercise that promotes health enhancing benefits. These findings suggest that future exercise guidelines for lifestyle induced chronic disease should incorporate HIIT. There are various logistical considerations that contribute to the feasibility of a HIIT program, however if participants are adequately screened and the program is prescribed and supervised by appropriately trained and qualified individuals then it should be an achievable training option.

Chapter 8.
The feasibility of high intensity interval training vs. moderate intensity continuous training

8.1 Abstract

Background: High intensity interval training **(**HIIT) has been previously shown to improve fitness and decrease cardiovascular disease risk in both healthy and chronic disease populations. The impact of HIIT has not previously been studied in the CKD population. The aim of this pilot study was to compare the feasibility and safety of HIIT with moderate intensity continuous training (MICT) in patients with CKD.

Methods: Twenty-one participants with stage 3-4 CKD (eGFR MDRD 25-60 ml/min/1.73m^2) were recruited for this randomized control trial (RCT). Participants were divided into either HIIT or MICT for three sessions of supervised exercise training per week for 12 weeks. In each session, the HIIT group completed 4 intervals of 4 minutes duration at 85-95% maximum heart rate (MHR), interspersed with 3 minutes of active recovery (65% MHR). The total duration of exercise time was 40 minutes for the MICT group and 33 minutes for the HIIT group. Feasibility was based on completing the prescribed high intensity intervals and safety was assessed by asking participants at the end of every session if they had any injuries or incidents to report. Additional measures included cardiorespiratory fitness (VO_2peak), exercise capacity (estimated METS), haemodynamics (blood pressures, pulse wave velocity [PWV] and augmentation index [AIx]), body composition (DEXA) and standard clinical measures. These were assessed prior to and following the 12 weeks of training. An average rating of perceived exertion (RPE) for the session was reported after each session. Exercise enjoyment (Physical Activity Enjoyment Scale) was measured post intervention.

Results: Out of 71 eligible CKD patients approached, 21 agreed to participate and were randomized to either HIIT or MICT. Fourteen of these participants (HIIT n=9, MICT n=5) completed follow-up testing. Out of a possible 36 sessions, the HIIT group attended, median[IQR], 33[7] sessions and the MICT group attended 34[3] sessions. No adverse cardiovascular events or musculoskeletal injuries occurred as a result of the training sessions. Exercise capacity improved in both groups (HIIT= 0.8±1.2, MICT= 1.3±1.6 METs). The average RPE of each session was higher in the HIIT than the MICT group (HIIT= 14.6±2.4, MICT= 13.5±2), however participants in the HIIT group reported higher exercise enjoyment (HIIT= 71±10, MICT= 63.5±21.4). However, none of the changes in either group were statistically significant.

Conclusions: This pilot study identified that HIIT is a feasible and safe for patients with CKD. It was also identified that HIIT was more enjoyable than MICT despite a higher reported RPE during the HIIT.

8.2 Introduction

Exercise therapy in the CKD population has been shown to improve cardiovascular disease risk factors and therefore has a likely impact on health outcomes.(38, 234, 309) Yet, there has been little work comparing the effectiveness of different exercise prescriptions in this population. HIIT has been shown to be superior to MICT in improving fitness and decreasing cardiovascular disease risk in both healthy and chronic disease populations.(188, 282, 310, 311) However, this type of training is yet to be evaluated in CKD patients.

HIIT may be an ideal way to improve the low cardiorespiratory fitness which is prevalent in the CKD population. This type of training involves alternating short bursts of high intensity exercise with active recovery at a lower intensity. Interval training allows individuals to work harder than otherwise would be possible at steady state, thereby increasing cellular and systemic stress and subsequent training adaptations.(312) Indeed, data pooled on 112 coronary heart disease patients identified exercise intensity as a significant predictor of an increase in VO_2peak.(313) This type of training may also be ideal for improving cardiorespiratory fitness in patients with low exercise tolerance who have difficulty performing continuous exercise.(312)

HIIT may be a more enticing option than the prospect of continuously exercising for an extended duration. Indeed, Jung et al. (2014) found that 44 inactive adults who completed both a HIIT and MICT training session reported greater enjoyment of the HIIT sessions, with similar task self-efficacy between HIIT and MICT.(314) In this study HIIT was also the preferred exercise modality over MICT. The enjoyment of HIIT in chronic disease populations has not yet been studied. Indeed, the self-efficacy of sedentary CKD patients with low exercise capacity completing HIIT may be dissimilar to what is seen in healthy populations. However, informal comments from patients with metabolic syndrome in the study by Tjonna et al. (2008) reported HIIT to be motivating due to the varied procedure and MICT to be ‘quite boring’.(192) The greater enjoyment may be pertinent for increasing adherence in this highly sedentary population.(315) The safety of high intensity training in

coronary heart disease patients has been assessed by Rognmo et al. (2012).(296) The findings from this study indicate the risk of a cardiovascular event occurring is similar between HIIT and MICT in 4846 coronary heart disease patients.

The aim of this study was to investigate the feasibility and safety of HIIT in patients with CKD. It was hypothesised that HIIT would be feasible for CKD patients, with no untoward events occurring as a result of exercise training. It was also hypothesised that HIIT would be more enjoyable than MICT.

8.3 Methods

Study design

Twenty-one patients with stage 3-4 CKD (eGFR MDRD 25-60 ml/min/1.73m^2) were recruited for this pilot RCT. Participants were recruited from the LM3 cohort on completion of their involvement in the study. Testing was undertaken at the St Lucia campus, The University of Queensland. Exercise training was undertaken in equipped gyms at the University of Queensland, The Princess Alexandra Hospital, Logan Hospital and Browns Plains Community Centre, Brisbane, Australia. Appendix 11.4 details the study patient information and consent form. Randomization was stratified by two levels- sex and diabetes status. Allocation was concealed to the assessors, as participant details were emailed to a person external to the study for randomization. Participants were randomized in a 1:1 ratio into HIIT or MICT. Measurements were taken at baseline and 12 weeks (Figure 8.1).

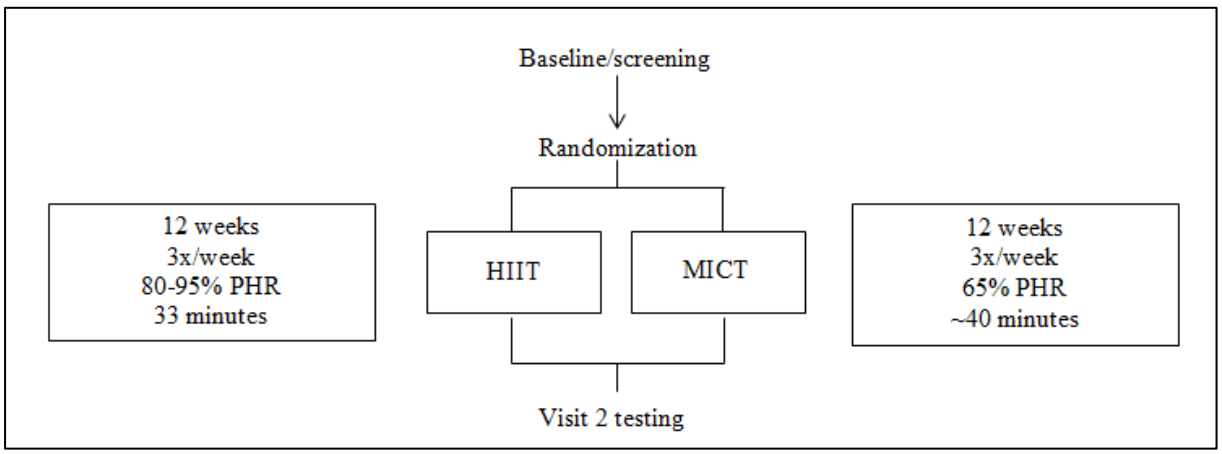


Figure 8.1 Study design

Inclusion and exclusion criteria

As participants were recruited from the LM3 study- the same inclusion criteria were in place. Furthermore, patients with an exercise stress test positive for ischaemia identified by their last LM3 visit, or significant cardiac history, were assessed by their cardiologist prior to enrolment. Participants with musculoskeletal disease that precluded high intensity exercise were also excluded.

Intervention

Participants undertook three sessions of exercise training per week for 12 weeks, supervised by an Accredited Exercise Physiologist. Exercise intensities for each group were based on their MHR achieved in the initial maximal stress test. The high intensity group were eventually completing a 4x4 protocol that consisted of 4 intervals of 4 minutes duration interspersed with 3 minutes of active recovery. The intervals were progressed between 80-95% of MHR over the 12 weeks and the recovery intervals were set at 65% MHR. The MICT group trained at an intensity of 65% MHR for approximately 40 minutes per session. The time spent on the treadmill for the MICT group was approximately isocalorically matched to the HIIT group based on ACSM's estimated energy expenditure calculations for walking.(222) Both HIIT and MICT groups performed a warm-up at 50-60% of MHR for five minutes and a cool down at the same intensity for three minutes. Table 8.1 shows the progression of target heart rates and interval times over the 12 week program, and how it compares to time spent training in the comparison MICT group. The initiation at 80% MHR for 3 minute intervals in the HIIT group allowed patients to ease into the program, as some participants (those who had been allocated to the control group of LM3) had no history of exercise training. By week 9 all HIIT subjects were training 4x4 minutes at 95% MHR. A treadmill was the primary modality of exercise, however one participant completed the training on a cycle ergometer due to musculoskeletal limitations. The speed and inclination of the treadmill was continually adjusted to ensure that the heart rate was being maintained at the desired target. Typically, subjects preferred their intervals to be increased by incline walking rather than running. As such, there would be a small increase in the speed and a significant increase in the gradient for each interval. On average it would take participants approximately 2 minutes to reach their desired heart rate. The speed and incline would be adjusted to ensure participants heart rate stayed at this desired rate (within ~3 beats/minute). A maximum ratio of 2 participants per 1 Accredited Exercise Physiologist ensured accurate maintenance and adjustment of treadmill control to maintain the

desired heart rate. After familiarization with the training, participants were more autonomous in making these changes; however the Exercise Physiologist was always supervising. Typically, the target recovery heart rate would be reached two minutes into the recovery. A heart rate (HR) monitor was used to assess training intensity in patients not on beta-blockers (n=11). Participants on beta-blockers (n=4) used BORG's RPE scale to assess intensity rather than heart rate (Appendix 11.6).(316) The MICT group and the HIIT recovery interval was prescribed an RPE of ~12-14, whereas the high intensity interval was prescribed an RPE of ≥16.(63)

Table 8.1 Progression of target heart rates and interval times over the 12 week training period. The total training time includes a five minute warm-up and three minute cool-down.

Week	Interval time (mins)	Interval intensity (% MHR)	Recovery time (mins)	Recovery intensity (% MHR)	Total training time (mins)	MICT training time (mins)
1-2	4x3:00	80	3x2:00	65	26	30
3-4	4x3:00	85	3x2:00	65	26	31
5-6	4x3:00	90	3x2:00	65	26	38
7-8	4x4:00	90	3x3:00	65	33	40
9-12	4x4:00	95	3x3:00	65	33	40

MHR=maximum heart rate; MICT=moderate intensity continuous training

Outcome measures

Outcome measures were performed at baseline and 12 weeks. Measurements were taken prior to randomization and within one week of completing the training intervention. Patients were asked to abstain from taking their beta-blocker medication on the morning of testing. Detailed protocols for the outcome measures that are the same as those in the LM3 study are reported in Chapter 2.

Safety

Patients were asked at the end of each session whether there were any concerns or problems which had occurred during the exercise session. Patients were also instructed to inform the research staff if there were any medical issues which had occurred during the intervention period. Adverse events were reported by research staff in an incident report form.

Fitness and physical activity

Cardiorespiratory fitness was assessed by peak oxygen uptake (VO_2peak) using indirect calorimetry and the peak 15 second average VO_2 during the final minute of exercise (Parvo Medics TrueOne 2400, UT, USA). The test protocol (Bruce, Balke or Naughton) was determined by the Duke Activity Status Index (141) and the same protocol was replicated post intervention. Exercise capacity (METs) was assessed by the treadmill software based on the time on the test (CASE V6.51, GE Medical Systems, Milwaukee, WI, USA). As the assessor also conducted the exercise training sessions, they were not blinded to the group allocation of participants performing the post exercise stress test. However, motivation and encouragement was kept consistent for every participant. The self- reported Active Australia questionnaire was used to evaluate average weekly physical activity levels from the preceding six months. Baseline total physical activity was calculated from the addition of walking, moderate intensity and vigorous intensity time in an average week in the preceding six month period.

Enjoyment and perceived exertion

Exercise enjoyment was assessed by the Physical Activity Enjoyment Scale (Appendix 11.5). The seven-point Likert scale questionnaire was administered to participants on completion of the intervention at 12 weeks. The overall perceived intensity of the training sessions was measured by RPE on completion of each training session and the mean RPE across all sessions was calculated.

Body composition

DEXA, using whole body composition analysis was used to determine body fat percentage and lean mass (Hologic QDR 4500A Version 12.6, Massachusetts, USA). Lower limb lean mass is reported as the sum of the lean mass in both legs.

Blood biochemistry

Fasting blood samples were performed prior to any other testing. Serum vacutainers (BD vacutainers, Franklin Lakes, NJ, USA) were used to collect 10 ml venous blood samples following an overnight fast. Creatinine was measured by the Jaffe method on a Beckman DxC800 general chemistry analyser. Blood biochemistry was assessed through venipuncture after an overnight fast.

Haemodynamics and vascular function

Applanation tonometry was used to measure central PWV (SphygmoCor 8.1, AtCor Medical, Sydney, Australia). Central blood pressure and AIx were determined through pulse wave analysis (PWA). PWV and PWA measures were taken in duplicate and the average value was reported. Peak exercise blood pressure was measured during the last stage completed in the maximal treadmill test using a manual mercury sphygmomanometer. Blood pressure was also measured prior to exercise training and on completion of the training session.

Statistics

Mean±standard deviation (SD) was used to describe normally distributed baseline characteristics, with percentages used to describe frequencies for categorical variables. Normality was assessed by the Shapiro-Wilk test. Comparison between groups was assessed by an independent t-test of the delta from baseline to 12 weeks. Delta data is reported as mean±SD or median[IQR] based on normality of the delta values. Mann-Whitney U test was performed on not-normally distributed variables. Differences between groups for categorical variables were analysed using Pearson's Chi Square test. Within group differences were assessed by paired t-tests for normally distributed and log transformed variables. Wilcoxon-Sign rank test was used for not normally distributed variables. All statistical analyses were performed on IBM SPSS Statistics 22. Power calculations were performed on PS Power and Sample Size Calculations Version 3.0. Statistical significance was set at $p \leq 0.05$.

8.4 Results

Table 8.2 outlines the reasons participants declined involvement in the study. Out of the 50 eligible CKD patients approached who declined participation, nearly half (46%) cited a lack of time as a reason for not taking part. Twelve participants were randomized into the HIIT group and nine to the MICT group (Figure 8.2). After seven withdrawals, there were nine participants who completed the HIIT intervention and five who completed the MICT intervention. Two participants from each group withdrew from the study due to a reported lack of time. One patient who withdrew from the HIIT group completed the intervention, however was unable to complete the follow-up testing due to a bout of rheumatoid arthritis.

One participant withdrew mid-way through the MICT due to osteoarthritis knee pain which was deemed too painful to continue exercising. The second MICT patient to withdraw was due to a hip dislocation which occurred at home. Technical errors occurred in the VO_2peak post-test of one of the HIIT participants and as such pre and post VO_2peak relative, VO_2peak absolute and respiratory quotient (RQ) data is only reported on eight HIIT participants.

Table 8.2 Reasons given by patients from the LM3 study for not taking part in the current study

Reason for not participating (n=50)	**(n,%)**
Lack of time	23(46)
Already does enough exercise	2(4)
Does not like exercise	4(8)
Lack of transport	4(8)
Too far to drive to gym	4(8)
Currently injured	2(4)
Too old	1(2)
Maybe- then unresponsive	5(10)
No reason given	4(8)
Doesn't like VO_2 mouthpiece	1(2)

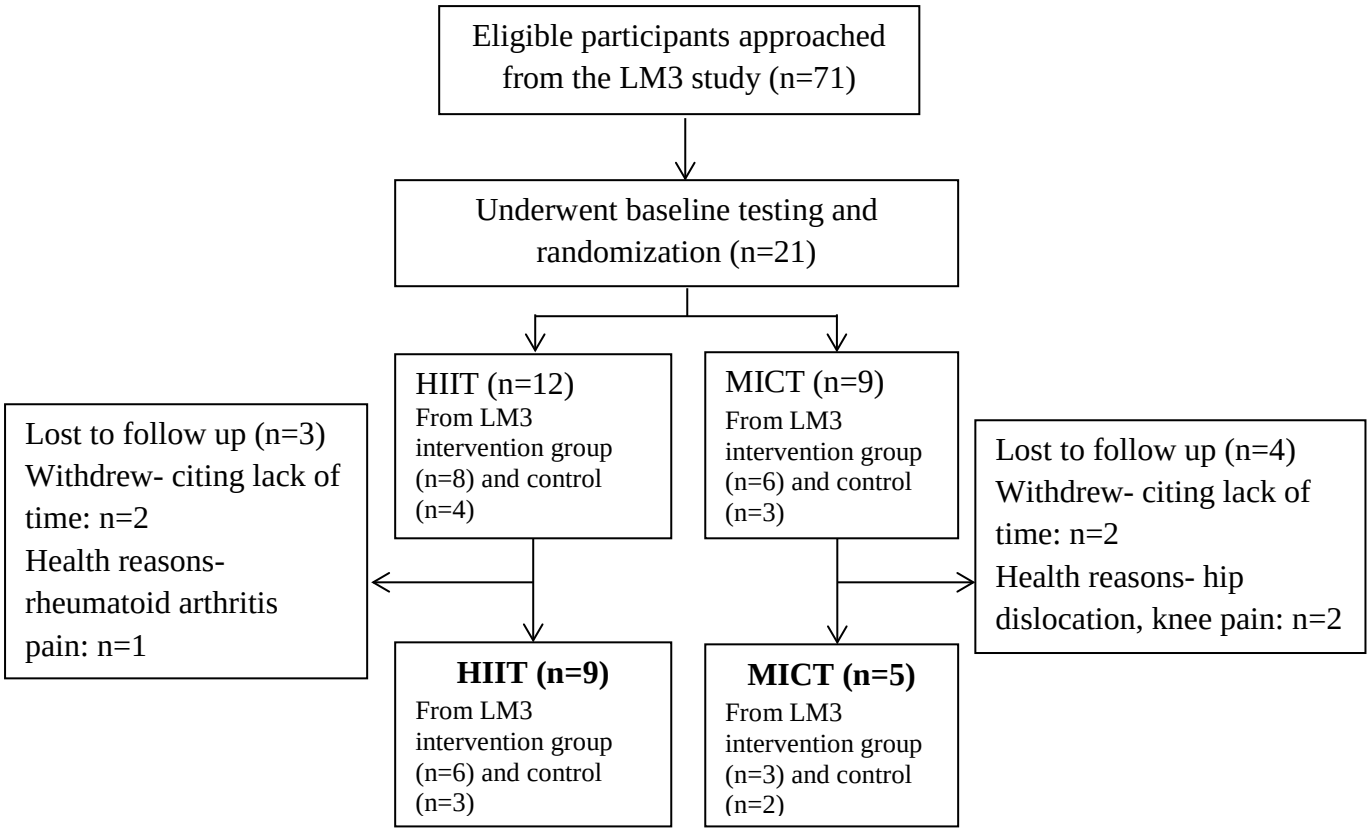


Figure 8.2 Consort diagram

Patient characteristics

Table 8.3 shows no significant differences in patient characteristics between the HIIT and MICT groups. However, there were a greater number of participants in the MICT group on insulin. On average, patients had moderate CKD with an eGFR of 34 ml/min/1.73m^2 and were considered obese with an average BMI of 31 kg/m^2. There were an equal number of patients with diabetes in both groups. The HIIT group reported performing higher levels of physical activity (3.5[4.7] hours) per week at baseline, compared to the MICT group (1.5[4.0]).

Table 8.3 Patient characteristics

Variable	HIIT (n=9)	MICT (n=5)	p value
Age (years)	60.9±6.3	62.8±10.5	0.67
Female sex	3(33.3)	1(20)	0.60
eGFR (ml/min/1.73m^2)	33.8±10.8	34.8±8.2	0.86
Diabetes status	4(44.4)	4(80)	0.20
BMI (kg/m^2)	31.7±5	29.9±6.2	0.57
Previous physical activity levels (hours/week)	3.5[4.7]	1.5[4.0]	0.15
History			
Hyperlipidaemia	4(44.4)	4(80)	0.20
Myocardial infarction	1(11.1)	1(20)	0.65
Peripheral vascular disease	1(11.1)	0	0.44
Hypertension	9(100)	5(100)	
Stent	0	1(20)	0.16
Medication			
Ace-inhibitor	5(55.6)	4(80)	0.36
β-blocker	2(22.2)	2(40)	0.48
Thiazide	1(11.1)	1(20)	0.65
Statin	5(55.6)	3(60)	0.87
Insulin	0	3(60)	**<0.01**
Allopurinol	4(44.4)	0	0.08

Data is presented as mean±SD. Not normally distributed variable presented as median[IQR]. Categorical data is presented as n(%)

Feasibility and safety

Both groups completed a similar number of exercise sessions (Table 8.4). All patients in the HIIT group achieved their target heart rate for each interval. No adverse events occurred as a result of exercise training. However, pre-existing osteo-arthritis of the knee did deter one participant in the MICT group from continuing with the program.

Fitness measures

Both groups had improvements in cardiorespiratory fitness and exercise capacity but there were no significant group differences in relative or absolute VO_2peak in both groups (Figure 8.3a,c).

Anthropometry

The HIIT group had no change in weight and the MICT group experienced minor weight loss (-1.3±1.9 kg). There were no changes in both groups for body fat percentage. There was also no change in lean mass in the HIIT group, however the MICT group had a reduction in lean mass, although this change was not significant.

Table 8.4 Baseline and changes in primary and secondary outcome measures

Variable	HIIT			MICT			
	Baseline	**Change**	**Relative change (%)**	**Baseline**	**Change**	**Relative change (%)**	**p value**
Fitness measures							
Estimated METs	9.8±3.4	0.8±1.2	7.6	8.8±3.4	1.3±1.6	12.9	0.52
VO_2peak (ml/kg/min)	22.3±7.6	0.1±2.4	0.6	21.7±6.1	1.6±2.3	6.9	0.31
VO_2peak (L/min)	1.97±0.6	0.03±0.3	1.5	1.80±0.4	0.06±0.1	3.2	0.86
RQ	1.08±0.1	0.02±0.1	1.8	1.1±0.1	-0.04±0.1	-3.8	0.40
Anthropometry							
Weight (kg)	86.2±9.6	0.1±2.0	0.1	84.9±25.3	-1.3±1.9	-1.6	0.22
Body fat (%)	37.7±8.2	0.1±1.5	0.3	35.1±5.6	0.2±2.8	0.6	0.92
Lower limb lean mass (kg)	18.0[3.0]	-0.1[0.4]	0.6	16.9[12.3]	-0.5[4.1]	-2.9	0.68
Haemodynamics and vascular							
Systolic BP (mmHg)	124.6±15.0	0.9±15.5	0.7	126.0±12.	3.0±9.9	2.3	0.89
Diastolic BP (mmHg)	80.6±6.7	-0.9±7.9	-1.1	76.3±7.8	-10.5±31.8	-16	0.42
Peak systolic BP (mmHg)	176.7±25.0	-3.6±26.6	-2.0	171.6±14.	-14.4±14.5	-9.2	0.42
Peak diastolic BP (mmHg)	77.8±11.5	6.4±16.3	7.6	72.2±14.2	-11.6±12.9	-19.1	0.06
Central systolic BP (mmHg)	118.3±12.7	1.8±16.4	1.5	118±22.5	-3.6±18.6	-3.1	0.62
Central diastolic BP (mmHg)	78.4±13.1	3.1±14.3	3.8	76.2±7.8	-3.4±9.9	-4.7	0.44
Resting heart rate (bpm)	66.3±10.5	-2.7±7.6	-4.2	70.7±14.2	-0.3±7.4	-0.4	0.61
Peak heart rate (bpm)	142.1±28.8	3.1±13.0	2.2	141.0±33.	-7.4±24.8	-5.5	0.36
Pulse wave velocity (m/s)	9.0[4.2]	-1.7[2.3]	-23.3	*	*	*	*
AIx (%)	26.6±9.3	0.9±6.8	3.3	20.6±17.3	3.9±11.0	15.9	0.57
Average systolic BP (mmHg)[#]	131.6±9.2	-3.3±7.3	-2.6	130.9±6.9	-2.2±8.6	-1.7	0.83

Average diastolic BP (mmHg)[#]	75.9±3.7	-1.1±2.8	-1.5	72.9±11.4	-1.7±2.4	-2.4	0.73
Post intervention		**12 weeks**			**12 weeks**		
Physical activity enjoyment		71±10.0			63.5±21.4		0.54
Sessions attended		33.0[7.0]			33.5[3.3]		0.53
Rating of perceived exertion		14.6±2.4			13.5±2.0		0.49

*Only n=2 participants had PWV performed in the MICT group, therefore no comparisons were performed. Data is presented as mean±SD. Not normally distributed variable presented as median[IQR]. The p value is of the delta value between groups. Change data is calculated by the 12 week value minus the baseline value. [#]Values reported in the baseline column are the average of the blood pressures recorded before each exercise session. The change value reported is the average change in blood pressure following each session. BP= blood pressure; bpm= beats per minute

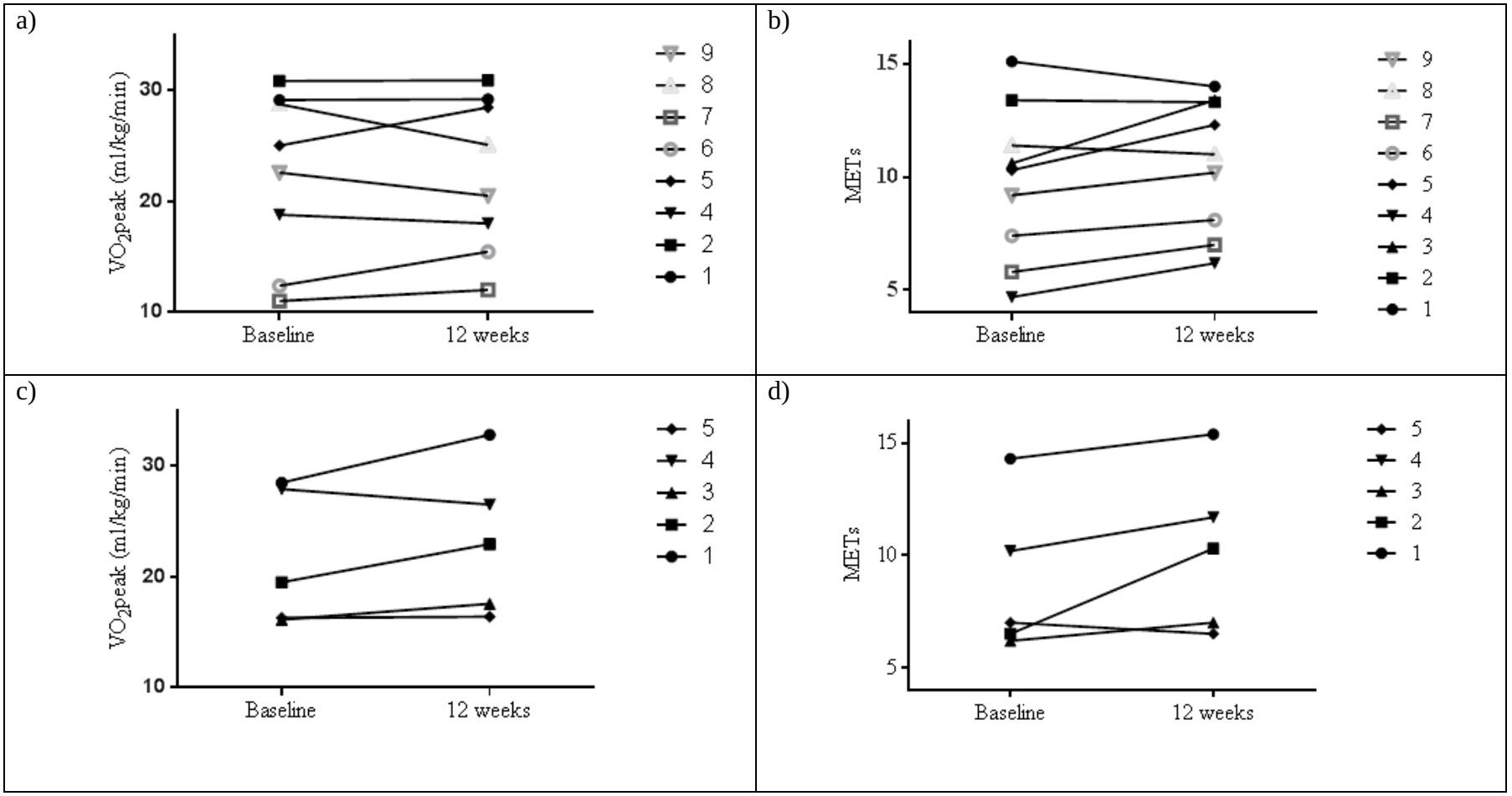


Figure 8.3 a) change in VO_2peak in each participant in the HIIT group. b) change in METs in each participant in the HIIT group. c) change in VO_2peak in each participant in the MICT group. d) change in METs in each participant in the MICT group

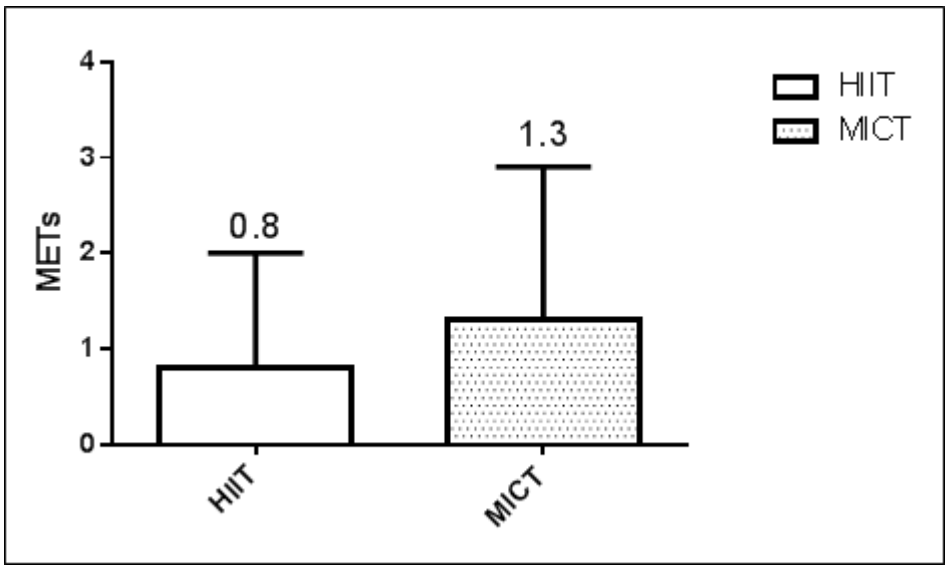


Figure 8.4 Change in estimated METs for both HIIT and MICT groups. Data is presented as mean and SD

Enjoyment and perceived exertion

The physical activity enjoyment scale identified that participants in the HIIT group enjoyed the exercise intervention more than participants in the MICT group but this was not statistically significant (Table 8.4 and Figure 8.5). There were also no significant differences between HIIT and MICT in any of the 12 questions separately (Figure 8.5). Participants in the HIIT group also reported slightly higher average RPE over the 12 weeks compared to the MICT group.

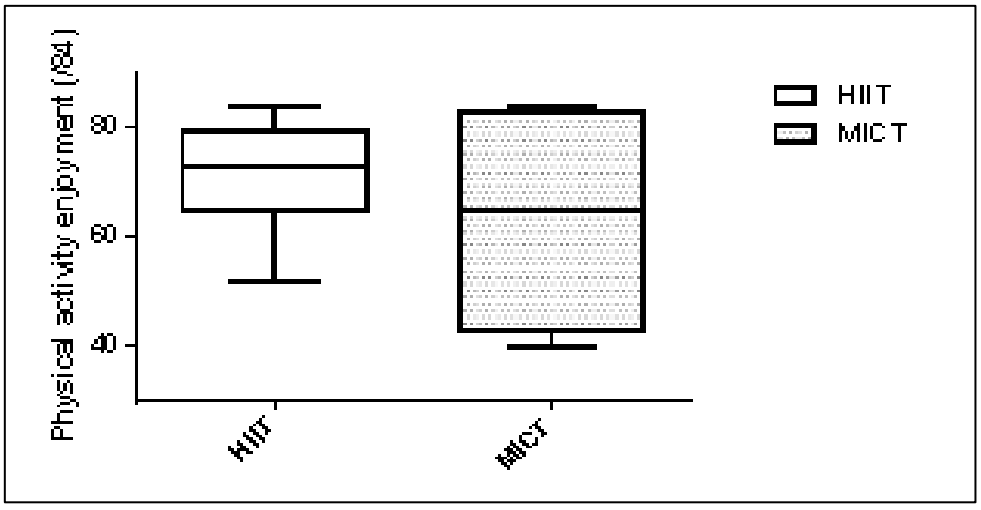
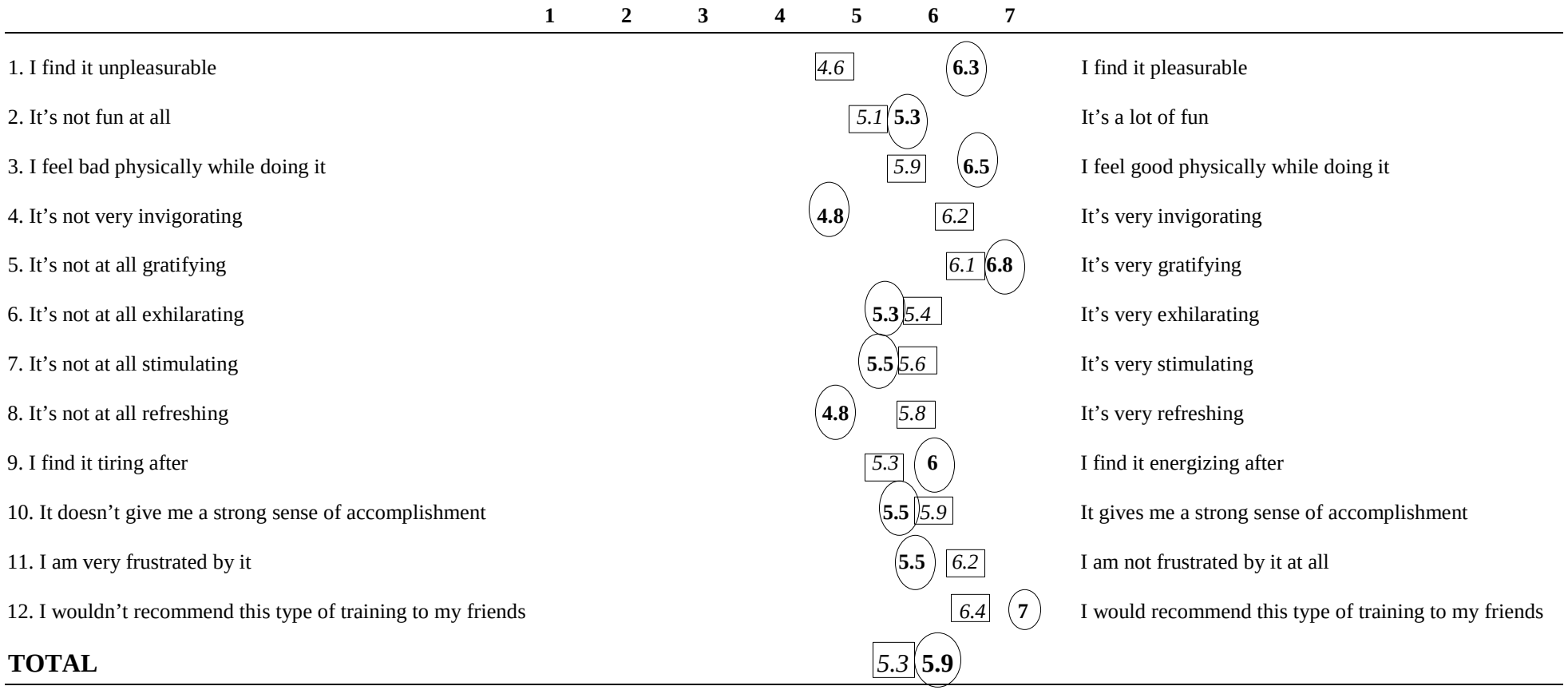


Figure 8.5 Scores from the physical activity enjoyment scale for HIIT and MICT groups. Physical activity enjoyment questions are on a 1-7 Likert scale with a maximum possible score of 84. Data is presented on a boxplot with median, first and third quartiles and minimum and maximum error bars

Figure 8.6 Physical activity enjoyment scale in HIIT and MICT



HIIT is represented in bold figures in a surrounding circle. MICT is represented italic figures in a surrounding rectangle.

Haemodynamic and vascular function

None of the haemodynamic or vascular function variables had significant between or within group differences (Table 8.4). Although, resting HR decreased in the HIIT group and remained the same in the MICT group. Systolic blood pressure was reduced after training in both groups, however the change seen in the HIIT group was slightly greater than in the MICT group. The reduction in diastolic blood pressure after each session was similar between groups. Both central systolic blood pressure and diastolic blood pressure increased in the HIIT group and decreased in the MICT group. Due to measurement difficulties only two participants in the MICT group had the PWV measurement completed. PWV in the HIIT group decreased after 12 weeks. There was a greater increase in AIx in the MICT group than in the HIIT group.

8.5 Discussion

This is the first study to investigate the effects of HIIT in patients with CKD. The main findings are; 1) HIIT is a feasible option for some CKD patients, 2) no adverse events occurred as a result of either HIIT or MICT and 3) the improvements in exercise capacity and cardiorespiratory fitness were greater after MICT than HIIT.

Feasibility of HIIT

The findings from this study indicate that HIIT is a feasible training option for patients with CKD. Participants in the HIIT group attended a median of 33 out of 36 sessions and all participants reached the desired heart rate in the high intensity intervals. CKD patients have a high degree of co-morbidities and general lethargy and whether this impedes the ability of these individuals to successfully complete HIIT has not previously been studied. The feasibility of HIIT in patients with low exercise capacity and significant co-morbidities, such as in this population, is supported by Wisloff et al. (2007). Wisloff et al. identified that patients age 75.5±11.1 years with stable heart failure post-infarction with an average VO_2peak of 13±1.6 ml/kg/min were able to successfully complete HIIT and improve cardiovascular disease risk factors.(193) It seems that the malaise experienced by CKD patients does not limit the ability to participate in HIIT.

In this study, the majority of patients performed uphill walking for their high intensity intervals. However, there were some participants who preferred to complete their training using running or cycling intervals. This was entirely dependent on the patient's preference and any musculoskeletal conditions. There are a number of exercise modalities which can be used to perform HIIT, such as swimming, rowing and resistance training. Aamot et al. (2013) found that participants in Norway performing HIIT at home in their own chosen modality, whether it be cross-country skiing, cycling or cross-training, still met their target HR using a HR monitor.(317) Furthermore, a study by Lunt et al. (2014) used the 4x4 protocol in outdoor group sessions.(318) Even though the authors found improvements in VO_2peak to be slightly lower than previously observed in other studies, this study recognized the feasibility of HIIT in a real world setting.

Safety of HIIT

The findings from this study indicate that HIIT is safe for CKD patients, with no adverse events occurring as a result of the exercise training. As previously suggested in Chapter 7 (Table 7.4), HIIT is appropriate for part of the population. It is suggested that patients considered 'high risk' seek medical clearance to identify any underlying cardiac conditions which may be exacerbated by HIIT. As participants in this study had finished the LM3 study, they all had at least 2-4 exercise stress tests, supervised by a Nephrologist and reviewed by a Cardiologist. They also underwent extensive blood work and review every 6 months. This 12 week study also had both pre and post exercise stress tests supervised by a Nephrologist. Consequently, there were no underlying diseases detected that were likely to be aggravated by high intensity exercise. In routine practice, Exercise Physiologists working with CKD patients may not have access to this level of screening. Therefore, adequate medical clearance from the treating physician will assist in identifying the suitability of a patient for HIIT. Nevertheless, the risk of a cardiovascular event occurring has reported to be similar between HIIT and MICT in 4846 coronary heart disease patients.(296) Furthermore, a study in patients with heart failure with reduced ejection fraction showed no change in cardiac troponin, C-reactive protein (CRP), brain natriuretic peptide, or significant arrhythmias after either HIIT or MICT.(319) More detailed studies in CKD patients specifically are necessary to adequately assess the safety of HIIT.

Efficacy of HIIT

The findings from this pilot study indicate that HIIT induces similar benefits in exercise capacity as MICT. The increase in METS in both groups (HIIT= 0.8±1.2, MICT= 1.3±1.6) are clinically significant as it has been reported that for every 1 MET increase there is a 17% reduced risk of mortality.(26) The differences were not statistically significant; however this is a pilot study that does not have the required sample size to assess changes in fitness. Indeed, based on the observed mean difference in VO_2peak between the two groups (1.5 ml/kg/min) and high standard deviation of the mean difference (2.35 ml/kg/min), the power of this study to detect a significant difference (α=0.05) was only 15%.

The observation that the MICT group had slightly greater improvements in exercise capacity and cardiorespiratory fitness was likely due to having considerably lower physical activity levels and fitness at baseline. This study did not show the same improvements in cardiorespiratory fitness as other cardiometabolic populations.(188, 320) This could be due to a combination of the small sample size and heterogeneous nature of the CKD population. Indeed, McCann et al. (2012) identified a significant variation in response to HIIT in patients with type 2 diabetes, which was independent of compliance. Furthermore, any physical activity performed outside of scheduled exercise sessions may also explain the lack of difference between groups. The current study identified three patients in the HIIT group to decline in VO_2peak, despite compliance to the exercise sessions and heart rates. The significant *improvement* in exercise capacity in two (patient #4 and #9) of these patients who declined VO2peak (and only a minor decline in exercise capacity in the third patient [patient #8]),(Figure 8.3) may indicate a resistance to physiological changes in cardiorespiratory fitness. This finding is similar to the findings from the main LM3 study. Non-responders to changes in cardiorespiratory fitness with exercise training is discussed in more detail in Chapter 6. This trend of increasing exercise capacity without a parallel increase in cardiorespiratory fitness in this CKD cohort warrants further investigation.

Although 12 weeks has been shown to be sufficient in improving cardiorespiratory fitness, a longer duration intervention may have elicited greater improvements in the current study.(283) The progression of the intensities over the 12 weeks was designed to gradually increase confidence and self-efficacy with this style of training. Perhaps if week one of training commenced at a higher percentage of MHR for 4 minutes rather than 80% MHR for 3 minutes, greater improvements may have been observed. The ability of the patients to effectively complete 4x4 minute intervals at 95% MHR indicate that starting at this intensity in future studies may be possible.

Vigorous exercise has been shown to cause greater transient decreases in blood pressure post-exercise than moderate or low intensity exercise in hypertensive males.(321) The findings from our

study indicate a drop in systolic blood pressure from pre to post exercise training in both exercise groups, with a greater decrease seen in the HIIT group. However, these findings were not statistically significant. Brito et al. (2014) also identified a greater post-exercise hypotensive response in high intensity resistance exercise (80% 1RM) than moderate intensity resistance exercise in elderly hypertensive patients.(322) These findings are further supported by Whyte et al. (2010) who found a transient decrease in systolic blood pressure 24 hours post two week sprint interval training intervention, which was not maintained at 72 hours.(323) This study also found a reduction in forearm vascular resistance within the first minute of recovery. These acute decreases in blood pressure post-exercise highlight the potential for chronic improvement in blood pressure if regular exercise is maintained. Our findings also demonstrated a considerable improvement in PWV in the HIIT group. Furthermore, AIx was found to increase slightly in the HIIT group, with a greater increase seen in the MICT group. The concurrent occurrence of post-exercise hypotension and decreased arterial stiffness as measured by PWV after interval training is similarly identified by Tordi et al. (2009).This is likely indicative of the shear stress that occurs during HIIT which increases elasticity of the vessel walls, thereby creating a greater stimulus for adaptation.(292)

The findings from our study indicate an improvement in weight in the MICT group, with no change in the HIIT group. Although HIIT has been shown in other studies to be superior in improving cardiorespiratory fitness, MICT is often reported to be superior for weight loss.(324) In light of this evidence, prescribing exercise should be focused around the desired outcome. Both reduced cardiorespiratory fitness and increased weight are cardiovascular disease risk factors. This finding supports a combination approach of HIIT, MICT and resistance training (to ameliorate muscle atrophy) to provide the best health outcomes in CKD patients.

Exercise enjoyment

The greater reported enjoyment in the HIIT group is important as it has implications for exercise adherence and long-term behaviour changes. It has previously been shown that participants are more likely to continue to perform high intensity exercise compared to moderate intensity training after their respective intervention,(325) likely due to a higher reported enjoyment of the high intensity sessions. To our knowledge, a study by Oliveira et al. (2013) has been the only study to compare enjoyment *during* HIIT or MICT.(326) The authors reported negative feeling scale responses in HIIT compared to MICT. However in this study the work interval was set at 100% VO_2peak for 2 minutes, with passive rest periods. Half of the participants could not complete the

HIIT session, therefore the reduced self-efficacy of not being able to complete the sessions likely affected the enjoyment of the exercise. Future studies should investigate enjoyment of the exercise during traditional aerobic intervals of 85-95% MHR and shorter sprint intervals ≥100% VO_2max as defined by our group in Chapter 7 (Figure 7.3)(188) to confirm these findings.(188) The benefit of using the Physical Activity Enjoyment Scale post intervention is the low burden to participants. However, despite a higher burden, this study may also have benefited from including measures of enjoyment and self-efficacy at each training session. Indeed, in addition to assessing the reflective feelings of enjoyment after completing the intervention, gauging the enjoyment of the exercise immediately after the session may provide some indication as to the likeliness of adherence to this type of training in a real world setting. Furthermore, the Exercise Task Self Efficacy scale can be utilized upon completion of the exercise session to assess participants confidence in their ability to repeat that exercise session at various time frequencies.(327) For example, this scale asks questions such as- *how confident are you that you can complete an exercise training session such as the one performed today 3 times per week for the next 4 weeks?* Participant responses to these questions would also be helpful in assessing the likelihood of HIIT being adhered to in an unsupervised real-world environment. The Feel Scale can be used to measure general affective valence.(328) This would have been helpful in assessing the feelings of patients (ie. pleasure/displeasure) pre, immediately post and 20 minutes post exercise training.(314) Use of qualitative measures of the HIIT intervention may also have provided some greater insight into the feasibility of this type of training. Indeed, semi-structured interviews pre and post intervention may have explored motivators, enablers and barriers to participation in the study.

Barriers to exercise participation

It is interesting to note the reasons for not partaking in the current study from approached patients from the LM3 study. These findings may provide insight into barriers to exercise participation in the chronic disease population. Namely, “lack of time” was cited as the most common reason for not being involved in the study. These findings suggest that using a shorter duration HIIT approach may be necessary to encourage exercise participation in CKD patients. The 4x4 HIIT protocol is not considerably shorter in time than the MICT protocol. However, the protocol is considered time-efficient, as improvements in VO_2peak are double the improvements that occur with MICT.(188) Therefore, to see the same increases in fitness, a significantly greater amount of time would need to be spent completing MICT. Although, considering lack of time is cited as the biggest barrier to exercise participation, perhaps the greater increases in VO_2peak should be sacrificed for similar

improvements in fitness with considerably less exercise time. The low participation rate also occurred in the study by Terada et al. (2013).(291) The researchers from this study found that from 126 patients who showed interest in joining the exercise study, only 15 were randomized and completed the program. However, the patients who did commence the study attended either their HIIT or MICT training 5 days/week for 12 weeks with 97.2% attendance rate with no drop outs.

Assessing intensity of exercise

McCann et al (2012) used HRR ((maximum HR – rest HR) + rest HR) to measure intensities of training in patients with diabetes.(329) This might be an ideal alternative to MHR when measuring intensity in some CKD patients, due to the regular occurrence of high resting HR and low MHR. In these individuals, calculating a percentage of MHR allows only a small difference in work rate between the interval and recovery intensities. Furthermore, the 65% of MHR used in the MICT group is often too close to the resting HR to expect a reasonable change to occur. If this situation occurred in the current study participants were advised to maintain an exercise intensity between 12-14 on the RPE scale. An individualized approach using either HRR *or* MHR may provide more achievable targets and therefore greater outcomes. On the other hand, using a percentage of VO_2peak, as opposed to HR, may provide more accurate measurement of energy expenditure and metabolic strain. Indeed, high intensity intervals performed at 85-90% VO_2peak would provide a fixed work rate, without the limitation of haemodynamic variability using MHR during the training sessions. However, identifying the practicality of using MHR was important for this feasibility study, due to the ease of HR monitoring in real-world settings.

The low RPE reported by the HIIT group (14.6±2.4) may also explain why the improvements in HIIT were not as great as expected. Patients were asked to rate their perceived exertion over the whole exercise session, therefore including rest intervals. However, as high intensity HR's should correlate with an RPE of 16-18, it was expected that the overall RPE would be higher than what was reported. These low RPE values were being reported despite all patients reaching their target heart rate percentage. This finding may indicate that basing intensity on a percentage of MHR may not be ideal in CKD patients. In fact using target heart rates may limit the capacity of the patient to train at a high intensity. It has been suggested by Guiraud et al. (2010) that manipulating walking or running velocity to maintain heart rate in a target zone may lead to a decreased VO_2max as a result of cardiovascular drift.(127) Indeed, using an RPE rather than heart rate target may be desirable for some CKD patients.

The optimal dose of HIIT

The time effectiveness of certain HIIT protocols is one of its primary advantages. However, with the 4x4 protocol the time difference with MICT is minimal. This HIIT protocol was selected due to its superior benefits in health outcomes compared to MICT in similar chronic disease populations.(188) Indeed, the reported two-fold improvements in fitness with HIIT compared to MICT in a similar time duration means the 4x4 is a time efficient approach. However, if lack of time was proving to be a barrier to exercise participation, a shorter duration protocol which allowed similar improvements in fitness to MICT may be warranted. As such, it is unknown whether the duration of this type of training would be a deterrent in real-world settings. However, more recently a study by Tjonna et al. (2013) found that 1x4 minute intervals 3x/week improved VO_2max by 10%, compared to a 13% increase in healthy men who undertook 4x4 minute intervals 3x/week.(330) The study by Tjonna et al. provides important foundations for future time efficient *and* efficacious aerobic HIIT protocols to be explored, however this needs to be confirmed in chronic disease populations. Alternatively, a modified 4x4 protocol may provide a more time efficient strategy. Perhaps, if the recovery interval was shortened, the time benefit may prove more enticing to patients.(318) Whether this would evoke the same physiological adaptations would need to be investigated. It has been suggested that HIIT of less than 1 minute may not be feasible in older adults.(331) Although low volume HIIT is enticing from a time efficiency perspective, the effort required may be impractical for many individuals.(332) Indeed, short work intervals would require the participants to reach the desired HR rapidly. The benefit of the 4 minute intervals is that it gradually increases the HR for approximately the first two minutes and then maintains the HR for the following two minutes. It has been suggested that high intensity intervals between 2-4 minutes could be difficult or unsafe for patients and could lead to non-compliance of the training program.(127) The ability of the patients in the HIIT group in the current study to adhere to this type of training with high attendance rates and successful achievement of the HR intensities, indicate that this is not the case. Substantially more research is needed on the dose-response relationship of HIIT in achieving the greatest adaptations with the least amount of time. A balanced training approach is important and future studies should also identify the effectiveness of incorporating HIIT *and* MICT with resistance training in future exercise studies in CKD patients.

Including HIIT in exercise guidelines

The benefits of aerobic exercise and resistance training in CKD patients have previously been reported, with an emphasis on moderate intensity exercise.(75) Before current exercise guidelines in CKD patients can include HIIT as standard clinical practice there needs to be large scale RCT's looking at patient safety, prognosis and compliance with HIIT. Given the promising results found in other clinical populations, further investigation of this type of training is essential. Furthermore, future research should investigate HIIT in conjunction with MICT and resistance training to replicate real world exercise prescription. Due to the heterogeneous nature of the CKD population it is likely the dose and ratio of exercise types should be individualized to promote the best possible health outcomes. As suggested by Arena et al. (2013) to support the clinical application of HIIT into standard practice, research needs to demonstrate physiologic benefit, functional and quality of life benefit, low adverse event rate, superiority to current clinically accepted exercise programs, high level of patient compliance, cost efficacy and decrease in morbidity and mortality.(333)

Limitations

There are a number of limitations to this study. Firstly, the sample size was not sufficient to detect a mean difference in fitness outcomes between HIIT and MICT groups (study power 15%). The change in SD of VO_2peak in our cohort was significantly larger than the assumed VO_2peak SD previously reported in cardiometabolic patients.(188) Secondly, there was a lack of blinding of the technicians in the exercise tests, due to limited resources. Regardless, the same instructions and encouragement were given to each patient to avoid any bias. Thirdly, although the baseline characteristics of this cohort were older adults, obese, had low cardiorespiratory fitness and appear to be typical of a CKD patient, the generalizability of the participants may be limited. Potentially, the individuals who volunteered to participate in a high intensity training study may be more motivated to make a significant lifestyle change. Indeed, the previous physical activity levels were high compared to the general population of the CKD patients from the LM3 study at baseline. Furthermore, although Table 8.2 demonstrates that 50 LM3 patients declined participation in this study, this may not be a true representation of the CKD population willing to participate in an exercise study. The participants had already demonstrated some level of compliance and high self-efficacy, evident by their participation in the previous LM3 study. As such, caution should be taken in extrapolating the high adherence rates in this specialized cohort to the general CKD population. It is also likely that the long duration of the LM3 study may have resulted in study fatigue and contributed to the high decline rate in the HIIT study. It would have been ideal to recruit participants with limited physical activity levels at baseline, however due to the difficulty in

recruiting CKD participants for exercise studies, the small sample size would have made the study unfeasible. Indeed, the intervention may have had a greater impact and benefit in patients who were not as physically active prior to participation in the study. However, the current study was still worthwhile in the studied cohort, as despite their reasonably high levels of baseline physical activity levels, their cardiorespiratory fitness were still low. Thus, it is likely that their exercise routines would benefit from optimization. Fourthly, monitoring the dietary intake of participants in both groups may have elucidated the differences in weight loss between groups. Lastly, blood pressure measures were performed before and after each session on all patients purely for safety reasons. Therefore, a strict protocol on time and rest period was not adhered to before taking the measurements. Rather, reporting of this measure was a post-hoc analysis and designed to give a general indication of the change in blood pressure as would occur in the normal supervised exercise setting. Generally the same protocol was followed each time, with patients instructed to rest for a few minutes before both pre and post exercise blood pressures were taken.

Conclusions

This pilot study identified that HIIT is feasible and safe for patients with CKD. It was also identified that HIIT was more enjoyable than MICT despite a higher reported RPE. The exercise capacity and cardiorespiratory benefits were greater in the MICT group, however this may be attributable to the higher baseline levels of activity and fitness in this group. Nonetheless, the study was underpowered to detect statistical differences between groups. Based on these findings, both HIIT and MICT are feasible options for CKD patients and exercise prescription should be based on patient history and interest. The higher enjoyment may indicate that adherence to HIIT may be greater than MICT. This pilot study provides evidence of the potential benefit of HIIT in the CKD population.

Chapter 9.
Conclusions and future directions

9.1 Summary

This thesis has contributed significantly to a range of areas relevant to exercise training in patients with CKD. The review of the literature in Chapter 1 identified the high prevalence of cardiovascular disease risk factors in patients with CKD. By exploring previous exercise trials in CKD patients it was identified that aerobic, and a combination of aerobic and resistance exercise training, significantly improves cardiorespiratory fitness and exercise capacity. Resistance training studies also appear to increase muscle strength and cross-sectional area. Based on the positive findings from the reviewed studies, it seems likely that exercise training plays an important role in the prognosis of patients with CKD. However, the review of the literature identified that further research is required, as most exercise studies in the CKD populations have excluded patients with cardiovascular disease, have small sample sizes or are not randomized control trials (RCT). Furthermore, only 1 RCT has investigated the effects of combination aerobic *and* resistance training, despite this being the recommended approach to exercise training by Exercise and Sports Science Australia. Moreover, no studies have investigated the effects of high intensity interval training (HIIT) in CKD patients.

The LM3 study, reported in Section 1, is the largest exercise trial in CKD patients to date and provides important practical findings for the renal community. Reduced strength has previously been reported in CKD patients; however this is the first study to elucidate the extent of this impairment. Indeed, it was identified that 82% of patients had grip strength below age-predicted normative values (Chapter 3). It was also identified that elevated oxidative stress was independently associated with reduced strength. The findings from this study provide insight into the potential for more targeted treatments (eg. antioxidants or strength training) in restoring the reduced strength and lean mass losses seen in this population. Chapter 4 identified the feasibility of CKD patients participating in higher intensity exercise. Participants who reported completing higher intensity exercise had the greatest exercise capacity after 12 months of exercise training compared to patients reporting moderate intensity and those not meeting guidelines. Furthermore, by identifying baseline haemoglobin levels as a significant predictor of participation in higher intensity exercise, patients with suitable haemoglobin levels can be recognized and targeted for this type of training. The paucity of research in exercise in CKD patients have precluded inferences to be made on the agreement of kidney function measures after an exercise intervention. Chapter 5 is the first study to assess the agreement between cystatin-C and creatinine based eGFR measures before and after a combination aerobic and resistance, home-based exercise training program. It was identified that cystatin-C eGFR was considerably lower than creatinine based estimates. However, this

discrepancy was consistent after 12 months of exercise training. This finding may suggest that an exercise training program as commonly prescribed to CKD patients does not influence either cystatin-C *or* creatinine based eGFR measures. Although, the difference between the measures may indicate a combination of cystatin-C and creatinine eGFR measure could provide the most representative measure of kidney function. The primary finding from this thesis is the feasibility of a long-term lifestyle intervention (LI) in significantly increasing physical activity levels and exercise capacity in patients with moderate CKD (Chapter 6). The improvement in arterial stiffness seen in the LI group also suggests that this model is effective in reducing cardiovascular disease risk. This is an important finding considering the high prevalence of cardiovascular co-morbidities and obesity in this population. The findings from this thesis provide support for the prescription of exercise training in standard nephrological care.

The LI in study 1 utilized a multi-disciplinary approach to promote long-term behaviour change. Other health professionals involved in this study included a nurse practitioner, dietitian, exercise physiologist as well as a diabetic educator and psychologist if needed. This thesis specifically focussed on the contribution of exercise training to health outcomes. Although it is reasonable to suggest that exercise training explicitly contributed to improvements in physical activity and fitness measures, the influence on haemodynamics, biochemistry and vascular cardiovascular risk factors is unknown. Although, the LI group showed no change in weight or body fat percentage, which may indicate the dietary advice was not as effective in improving outcomes as expected. Furthermore, encouragement of physical activity by the other health professionals may have also contributed to improvements in physical activity adherence and fitness measures. Although this collaborated approach is ideal for multi-disciplinary care, for the purpose of this thesis it does make it difficult to delineate the specific impact of the exercise training on health outcomes. Indeed, multi-disciplinary encouragement of physical activity in standard nephrological care may be beneficial in maintaining positive exercise behaviour. On the other hand, the psychologist in this study was only utilized as needed for patient concerns unrelated to exercise. Perhaps if a psychologist was also involved in promoting lifestyle change, greater improvements in physical activity and diet adherence may have been perceived. Indeed, the collaboration of an exercise physiologist with a psychologist may encourage long-term behaviour change. Furthermore, the findings from Chapter 4 and Chapter 6 indicate that the patients with higher baseline fitness levels had the greatest changes in fitness measures after the intervention. The whole cohort had strength and fitness below age-predictive normative values, was obese and had multiple co-morbidities, so adherence to the LI in the patients who are considered ‘fitter’ is still worthwhile for improving health outcomes. However, patients with lower fitness and perhaps greater disease state may have the most to benefit from a LI.

Strategies to target these patients is vital in improving health outcomes in the entire CKD cohort. Again, working closely with behavioural psychologists may be helpful in optimizing the LI to see the greatest outcomes in *all* patients. These patients may also require closer supervision and support and may benefit from increasing accountability through instruments such as regular food and exercise diaries.

The meta-analysis in Chapter 7 (Section 2) has identified that HIIT has more physiological benefits, and has nearly twice the improvement in cardiorespiratory fitness than MICT in lifestyle-induced chronic disease patients. The meta-analysis did not include any studies of patients with CKD, as this thesis is the first to report on the effects of HIIT specifically in the CKD population. This study was also the first to provide important considerations, contra-indications and protocol recommendations for HIIT training in chronic disease patients. Chapter 8 identified that HIIT is a feasible option for patients with CKD and was reported to be more enjoyable than MICT. Although the improvements in cardiorespiratory fitness were similar between the groups, the greater enjoyment may suggest that HIIT may be better adhered to in this sedentary population. Considering the lack of statistical changes in the HIIT study due to the small sample size, the addition of more quantitative and qualitative analysis to establish self-efficacy, affect and adherence may have provided more useful information in this feasibility study.

Considerations for exercise training CKD patients

There are number of factors to consider when exercise training or testing patients with CKD. It is pertinent for exercising CKD patients to be educated on the occurrence of post-exercise hypotension, which may be exacerbated if patients are taking anti-hypertensive medications such as α-blockers, calcium channel blockers or vasodilators. If the exercise is taking place in the gym setting, ACSM suggests blood pressure should be assessed before commencement of an exercise session.(222) Relative contraindications to exercise testing is systolic blood pressure >200 mm/Hg and/or diastolic blood pressure >110 mm/Hg. Patients with exaggerated blood pressure responses to exercise may also require regular assessment of blood pressure during an exercise session. ACSM suggests maintaining systolic blood pressure below 220 mm/Hg and/or diastolic blood pressure <105 mm/Hg during exercise. Although, for maximal exercise testing it is reasonable to continue exercise up until systolic blood pressure 250 mm/Hg and/or diastolic blood pressure 115 mm/Hg. It is also recommended that regular checking of blood pressure and ECG monitoring occurs during exercise, particularly when electrolyte abnormalities are present. If patients have diabetes then it is

important to check blood glucose levels before and after every training session. Assessing post-exercise blood glucose levels in patients with diabetes is particularly important if they are on beta-blockers, as the adverse effect on thermoregulatory function may not only increase the predisposition to hypoglycaemia, but may also mask the manifestations. Patients with diabetic nephropathy should be checked regularly for other microvascular complications, such as the presence of any foot ulcers. Such patients should also be instructed and educated on appropriate breathing during resistance exercise to ensure intraocular pressure is not significantly elevated. Due to the high prevalence of obesity in CKD, a significant number of patients suffer from osteoarthritis. As such, exercise prescription should be aimed at accommodating suitable, as well as non-painful, exercises to ensure exercise adherence and enjoyment. Patients should also be educated on the deleterious effects non-steroidal anti-inflammatory medication can have on kidney function.

An important consideration when exercising patients with CKD is to ensure appropriate hydration status. Dehydration is said to occur when >2% of body weight is lost from water deficit.(334) Short term dehydration and reduced blood volume can decrease renal perfusion and can therefore cause reductions in eGFR,(335) which may place a comprised CKD patient at risk for acute renal failure. It may be important for patients with CKD to begin exercise at a euhydrated state. Of course this recommendation is dependent on patient's individual fluid requirements and whether or not they are taking diuretics. In most cases if significant sweating occurs, lost fluids should be replaced during exercise or as soon after as possible.

9.2 Future recommendations

Further studies are necessary to extend the current findings in this thesis and previous research. Arguably, the two most important future areas to be investigated are; 1) the effects of a long-term exercise intervention on cardiovascular morbidity and mortality, and 2) the effects of exercise training on different eGFR measures in adequately powered studies (n=1000). Study 1 (LM3) and Study 2 (HIIT) from this thesis have the potential to further contribute to the literature, as reporting of some outcome measures were outside the scope of this thesis. Such measures to be analysed and published in the future are reported below.

The muscle wasting which occurs with CKD is an important therapeutic target for not only providing physiological improvements in health, but also improvements in psychological well-being and quality of life. It seems reasonable to suggest that a combination of aerobic *and* resistance training is the most applicable to real-world exercise prescription and addresses the scope of CKD

related detriments. Therefore, assessing mitochondrial function and muscle atrophy markers through muscle biopsy after combination training programs are warranted. Whether the influence of oxidative stress contributes to the impairment of muscle function and structure as assessed by more sophisticated measures such as magnetic resonance imaging (MRI) and muscle biopsy should be investigated. In particular, HIIT has shown to be effective in improving mitochondrial function in other chronic disease populations, and as such a combination training program which also includes HIIT should be investigated. Furthermore, future exercise studies should investigate whether a combination of HIIT, MICT, resistance *and* flexibility training has the most comprehensive benefits and whether the variety in training modalities is central to exercise adherence. The effectiveness of the LI reported in Chapter 6, provides groundwork for future studies to demonstrate whether a similar LI results in a reduced burden on the health care system, which would then support the inclusion of exercise physiology in standard practice. Studies should perform an economic analysis which assesses the cost effectiveness of the LI model compared to the expected reduced cost associated with hospitalisation and co-morbidities. Furthermore, the cost of this type of predominantly unsupervised LI model should be assessed against more supervised programs which have the potential for greater physiological adaptations.

Recommendations for future analyses from the LM3 study

Only baseline oxidative stress (F2-isoprostanes and protein carbonyls) and anti-oxidant measures (glutathione peroxidase and total anti-oxidant capacity) have been measured and reported in Chapter 4. Our group are currently analysing the change in oxidative stress measures after the 12 month LI in patients reported to have high levels of F2-isoprostanes at baseline (≥250 pg/ml). Further, exploring these measures every 6 months (7 visits) in *all* patients would provide interesting insight into whether the change in oxidative stress levels are related to the decline in grip strength. Currently, baseline and 12 month serum cystatin-C measures have been analysed from the LM3 study. However, analysis of cystatin-C samples at every 6 month visit should also be measured. Comparisons of both creatinine and cystatin-C eGFR measures over 7 visits would be helpful in confirming the findings in Chapter 5, and help elucidate the change in eGFR seen in Chapter 6. Furthermore, future exercise training studies comparing cystatin-C and creatinine based eGFR measures in specific hypertrophy and high intensity training programs are warranted to confirm the findings in Chapter 5.

Recommendations for future analyses from the HIIT study:

Patients in the HIIT study also underwent muscle biopsies at baseline and 12 weeks. We have collected baseline muscle tissue on 19 participants, of which post exercise muscle biopsies were performed on 12 of the participants who completed the HIIT (n=6) and MICT (n=5) protocols. In the future our group we will be measuring PGC-1α, PGC-1α4, myostatin, mTOR, p70s6k, rpS6, 4E-BP1, MuRF, 14 kDa actin fragment and heat shock proteins in these samples. A baseline comparison of muscle function in these patients will be compared to physical activity matched healthy controls. This cross-sectional analysis will provide further insight into the pathogenesis of muscle wasting in CKD patients. Furthermore, the pre and post analysis of muscle tissue in HIIT vs. MICT will identify which intensity of aerobic exercise stimulates the greatest improvements in muscle function. Reporting of these measures were outside the scope of this thesis, however once the muscle analysis is complete, manuscripts of the baseline and intervention data will be prepared for peer reviewed journals.

9.3 Conclusions

This thesis provides novel findings on a range of issues affecting CKD patients. The primary issues addressed have been; mechanisms of reduced strength, predictors of higher intensity exercise, appropriate kidney function measures after an exercise intervention, the translation of a LI to a generalizable CKD cohort and feasibility and efficacy of high and moderate intensity aerobic training. By ascertaining the mechanisms and predictors of exercise training, appropriate strategies to optimize exercise induced adaptations and adherence can be proposed. The findings from these studies indicate that regular exercise training should play an integral part in CKD patients' lives and must be supported in standard clinical practice. Furthermore, to support the success of physical activity behaviour change, treating Nephrologists should ensure that exercise professionals are involved in rehabilitating appropriate patients. The findings presented in this thesis will assist Exercise Physiologists in targeting their exercise prescription to provoke the greatest improvements in health pertaining to individual patient needs. This thesis provides convincing evidence as to the feasibility and benefits of combination aerobic and resistance home-based exercise training, HIIT and MICT in patients with CKD. The findings from these studies will significantly contribute to health outcomes of CKD patients.

Chapter 10. References

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Chapter 11. Appendices

11.1 Patient information and consent form for LM3

PATIENT INFORMATION SHEET -VERSION 7

STUDY: Landmark 3 – The impact of aggressive risk factor and lifestyle modification in patients with CKD utilising a nurse led co-ordinated care approach.

Reason for the Study

The Departments of Renal Medicine and Cardiology at the Princess Alexandra Hospital are studying ways of improving the health and management of patients with CKD.

Patients with CKD have an increased risk of heart disease. We would like to assess the usefulness of a new way of providing care to patients who often have complex health issues. The LANDMARK TRIAL PROGRAM will be made up of a multidisciplinary team working together with your usual kidney doctor to reduce risk factors which contribute to progression of both kidney and heart disease. These risk factors include cholesterol, high blood pressure, high blood sugars, anaemia, abnormal blood chemistry (high calcium and phosphate), and smoking, overweight and poor physical fitness.

The LANDMARK TRIAL team will include a specialist nurse, diabetes educator, dietician, pharmacist, exercise physiologist, psychologist and social worker. Their task is to help work out the best way of tackling the risk factors that you have with the aim of improving your kidney and heart health.

The team will focus on:

- A co-ordinated approach led by specialist nurses to get risk factors to target. Patients with CKD will often be on treatment for risk factors but may be finding it difficult to 'get to target'.
- Healthy lifestyle - working towards healthy diet and weight, improving fitness. Stopping smoking.
- Education about heart and kidney disease, medications and why reducing risk factors will improve your health

This study is being done to work out if a multidisciplinary clinic improves heart and kidney health for patients. If you agree to participate, you will be randomised to one of two groups, that is, half the patients will be allocated to the **usual care group** and half to the **trial clinic group**. If allocated

to the usual care group you will continue to receive care in the general nephrology clinic exactly as you do now. If allocated to the trial clinic group you will receive care under a nurse led multidisciplinary team in the CKD clinic, plus your medical appointments (the appointments may not need to be so often).

This will allow us to compare the treatments without bias and study what really happens- not just what we expect to happen.

In the usual care group your treatment will be prescribed (according to current guidelines) by your usual doctor. If your doctor thinks that you need to see any additional health professionals (e.g. dietician) they will refer you as usual.

In the trial clinic group, you will be seen by the specialist nurse who will assess how you are well you are 'getting to target'. They will then organise for you to see members of the team that will give you additional advice and help work out a plan for you to achieve your risk factor goals. Diet and exercise are also very important in improving and maintaining your overall health. Healthy weight and fitness can improve blood pressure control, reduce cholesterol and possibly decrease the progression of heart and kidney disease. Therefore, after assessment by your doctor, trial clinic patients will undergo a **Diet, Exercise and Lifestyle program** to target weight loss and fitness goals. The Diet and Lifestyle program includes weekly education sessions, over a four-week period with a dietician, exercise physiologist and psychologist, focusing on lifestyle changes and a sustainable diet designed especially for CKD patients. After this, an individualised maintenance program will be developed for you to especially meet your needs. (See Attachment 1)

The Exercise program will be available to you with a qualified exercise physiologist who will tailor a program to meet your individual exercise requirements, and help you with motivation and behaviour change. (See Attachment 2).

In caring for patients with kidney disease, kidney specialists in conjunction with heart specialists routinely do tests to assess a patient's heart and blood vessels. We would like tests to be more able to predict patients with heart problems. This study aims to investigate other tests, which have only recently become available at this hospital. If these new tests prove more accurate in predicting which patients will develop heart problems, we will use them routinely.

The following tests will be for both groups:

Heart Tests

After assessment by your doctor, you will undergo an exercise stress echocardiogram to assess the likelihood of heart disease being present. This involves taking an ultrasound picture of the heart before and immediately after exercise which will involve you walking on a treadmill. The picture is obtained by pressing an ultrasound probe on the chest; this transmits sound waves and collects the

reflected waves to make a picture of the underlying structures. The test involves attaching you to ECG monitoring equipment, taking a picture of the heart at rest and then repeating the picture after you have exercised. **The procedure will be stopped if you develop any chest pain or heart rhythm disturbances while exercising or if you are unable to continue for any other reason.** If you are unable to exercise, a dobutamine stress echo will be performed instead. This involves taking an ultrasound picture of the heart before and immediately after the heart is stressed by giving a drug called dobutamine. Dobutamine is a medicine that increases the heart's workload (similar to exercise) when you are resting. The test involves attaching you to ECG monitoring equipment, taking a picture of the heart at rest, inserting an intravenous line in order to give the medicine and repeating the picture after the medicine has taken effect. This procedure is usually well tolerated, and significant side effects (such as heart rhythm disturbances or severe chest pain) occur in about three in a thousand patients. The laboratory is fully fitted out with monitoring and safety equipment. The procedure is performed under intensive monitoring by a doctor with a nurse and/or echo technician, and takes about an hour; we will attempt to arrange it to coincide with other hospital appointments so that you do not need to make an extra trip.

If your heart test shows any signs of significant heart disease, you will be notified and a follow up appointment with a cardiologist will be made.

If it is not medically appropriate to perform an exercise stress echocardiogram OR a dobutamine stress echocardiogram, a standard echocardiogram will be performed, at the start of the study, at 24 months and the end of the study.

We will also test the degree of blood vessel damage by tests, which examine the carotid (neck) and brachial (upper arm) arteries by ultrasound. The carotid study involves taking a picture of the artery by placing a transducer over the neck – the procedure takes about 20 minutes and is painless. The brachial artery study involves the same type of picture (this time, of the arm) at baseline, followed by blocking of the vessel by inflating a blood pressure cuff for 4 minutes and repeating the images after the cuff is deflated. The test is repeated after taking a tablet of nitroglycerine under the tongue. Serious complications have not been reported with either of these studies, although inflation of the blood pressure cuff may cause pins and needles or arm pain, and nitroglycerine use may cause dizziness or headache – but both are temporary. **These imaging studies are performed purely for research purposes and obtained at the start of the study and then every 12 months until the end of the study. The cost of these tests will be covered by the Centre of Clinical research Excellence.**

<u>Blood Tests</u>

We collect approximately 50mls of blood at each visit. Most of these tests will be part of your normal medical care, but some of these tests are not routinely used. Some of your blood will be stored and tested for a range of proteins that may be associated with an increased risk of heart disease and we wish to study them in detail to determine if they are useful in predicting who will develop heart disease in the future. If this is the case, then we may be able to use them routinely in the future. There is NO storage of DNA or genetic material.
Your usual clinic bloods tests may also be taken at this time so you will only have one needle.

Urine tests

At baseline only, we will ask you to collect your urine in a special container over a 24-hour period. This will help us to measure the components of your urine, and helps to assess how your kidneys function. You will be asked to return the collection to your nearest Queensland Health facility for analysis.

We collect approximately 50mls of urine at baseline and all other visits. This is part of your normal medical care, but some of your urine may be stored and tested for a range of proteins that may be associated with an increased risk of heart disease and we wish to study them in detail to determine if they are useful in predicting who will develop heart disease in the future.

Body Composition

As part of the nutritional assessment data we will gather at your visits, we would also like to use a test to look at your body composition. This test is called DEXA (dual x-ray absorptiometry) and involves using non-invasive x-ray imaging to assess abdominal and subcutaneous fat distribution and lean body mass. It is a simple procedure which requires you to lie flat on a bed for about 30 minutes whilst imaging takes place. This procedure will allow us to gain more exact information about your body composition. These will be performed at Baseline, 12months, 24 months and 36 months.
At baseline only, the amount of water in your body will also be measured using a bioelectrical impedance analysis technique. You will not be asked to undertake this test if you have a pacemaker or metal implants. This procedure will take 5 to 10 minutes. You will be required to lie flat while we place small electrodes on your hands and feet, which sends a very small electric current. You will feel slight tingling at the site of the electrodes. It is a safe and simple technique that is often used in practice.

Lateral Lumbar X-Ray

To further assess the health of your blood vessels we will do an X-ray of the major vessel in your abdomen, the aorta. This helps us identify whether any calcium is present, which is a sign of blood vessel damage. This will involve one non-invasive X-ray image being taken while you are standing. It only takes a few seconds. This will be done at baseline and 36 months. This research involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this study is about 1 mSv. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk is believed to be very low.

Quality of Life Questionnaires and Interviews

As we are looking at the best way to provide care to individuals with CKD, you will also be asked to complete quality of life questionnaire every 6mths whilst you are enrolled in the study. The questionnaire asks your opinion of your quality of life as related to your kidney disease and helps us to understand your experience. The questionnaire will take approximately 10 minutes to complete. Your responses on this questionnaire will be kept strictly confidential.

You may also consent to take part in the 'Quality of Life Substudy' where we will ask you to participate in an interview that will explore further your expectations of treatment and experiences in living with CKD. We plan to interview approximately 30 patients in each group. You will be asked to sign a separate consent section if you choose to participate in these interviews and another patient information sheet regarding the interview will be provided.

Confidentiality

At all times your confidentiality will be respected. No information regarding your medical history will be divulged and the results of any tests involving you, which may be published, will be done in such a way that your anonymity will be preserved.

Participation in this study is **voluntary** and you may withdraw your consent to be involved in the study at any time without affecting your treatment. Furthermore, consent to participant in the trial will not affect your right to obtain compensation in the event of injury that was proven to be related to the research.

Length of the Study

Whichever group you are assigned to, we will follow you for 3 years.
Your general practitioner and other treating practitioners will be notified of your involvement in the study and any significant results arising from it.

Further information or queries can be directed to:

Dr Nikky Isbel Department of Renal Medicine
Princess Alexandra Hospital
Phone: **3240 5080** or 3240 2111

Professor Tom Marwick University Department of Medicine
Princess Alexandra Hospital
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11.1.1.1

The Princess Alexandra Hospital Research Ethics Committee Princess Alexandra Hospital
Ipswich Road**,** Woolloongabba
Phone: **3240 5856**

TREATMENTS IN THE FOCUS GROUP

NUTRITION AND LIFESTYLE PROGRAM- Attachment 1

If you are allocated to the focus group, a 4-week lifestyle program focusing on a healthy sustainable diet, improving fitness and behaviour changes necessary for maintaining change, will be run by the Dietitian, Exercise Physiologist, Psychologist and also include Social Work input.

The groups will meet once per week for 2hrs for 4 consecutive weeks. We aim to have 12 or less people in each group.
A subset of patients will be asked to complete a 7 day food diary, approximately 45 in each group. This helps us understand better what your dietary intake is like and will give us a good guide as to where we can make some changes if needed during your 4 week program. You will need to complete the diary at your baseline, 1 year and 3 year visit.

Lifestyle Program Structure

Week 1 – Goal Setting, Guide to a Healthy Diet, Self-Monitoring– Dietitian/Psychologist
Week 2 – Cholesterol, Fats, Sugars, Salt, Healthy Meal Plan - Dietitian
Week 3 – Motivating change - understanding Eating and Exercise Behaviours, Triggers, Rewards, Problem Solving, Self-Esteem, Barriers to Change –Psychologist
Week 4 –Food Label reading, Recipe Modification, - Dietitian

You will also be given a workbook which will contain information on the above topics, self-monitoring exercises, homework and evaluation.

Individual Dietary Reviews:
After the group sessions are completed, you will be seen on an individual basis for the remainder of the trial, as determined by the dietician and as negotiated with you depending on your needs. Reviews will either be in person or via telephone. This allows a more flexible and sustainable structure of care.

Group Meetings:

If possible, every 6 months group reunions will be held for approx 1 hour until through out the trial.

EXERCISE AND LIFESTYLE PROGRAM- Attachment 2

Exercise Training for CKD patients in Landmark 3.

A full exercise program as outlined below will be available to you. You can meet with your exercise physiologist to discuss the best plan for you, this maybe all, some or at least a little of what is being offered.

Full Program

The exercise training intervention will be provided in two stages – an initial, eight-week supervised, gym-based training program with additional home-based training (stage one), followed by home-based training with regular telephone exercise counselling and gym refresher sessions (stage two).

You will begin by attending a 30 minute consultation session with an accredited exercise physiologist. During this session you can provide details on your exercise history, discuss likes and dislikes, barriers and opportunities to exercise. This will help us design an individualized exercise program for you. The program will consist of both moderate-intensity cardiorespiratory exercise and resistance training, and will be designed according to fitness and any health problems you may have.

During stage one, it will be recommended that you attend the **Lifestyle Clinic** at the Princess Alexandra Hospital as well as doing home based exercise. The number of sessions/week will depend on your individualized exercise program taking into account your medical history. The cardiorespiratory training will be chosen based on your preference. A variety of options will be given to you so that you may choose with the help of your trainer.

After the completion of the first stage of your individual intervention program, you will continue to exercise at home. We will continue to provide support via the phone weekly for three months, fortnightly for three months and monthly thereafter for the remainder of the study or as required. The telephone exercise counselling will aim to monitor and record your levels of exercise and physical activity, as well as ensure you can achieve, maintain and where possible, increase the

amount of exercise you do through motivation, changing the exercise prescription where necessary, and implementing strategies to account for disruption to the exercise routine.

As part of evaluating how your training is going, we will measure your central blood pressure. This will be done at the same time as you attend the echo department for you hearts tests.

Central blood pressure will be measured whilst you are lying down, seated and standing, as well as during very light exercise similar to activities of daily living (cycling on a bike for about 10 minutes).This is measured by placing a probe lightly on the surface of your skin at the artery in your wrist, neck and upper thigh. Upper arm blood pressure will also be recorded by the usual method of a cuff around the upper arm. This is a non-invasive measure of your central blood pressure but very useful.

11.2 Active Australia questionnaire

ACTIVE AUSTRALIA SELF-REPORT PHYSICAL ACTIVITY MEASURE *The following questions are about the physical activity you did IN THE LAST WEEK:*

1A. IN THE LAST WEEK how many times have you walked continuously, for at least 10 minutes, for recreation/exercise or to get to or from places? ___*times*

1B. What do you estimate was the total time that you spent walking in this way IN THE LAST WEEK? ___*hrs* ___*mins*

2A. IN THE LAST WEEK how many times did you do any vigorous gardening or heavy work around the yard which made you breathe harder of puff and pant? ___*times*

2B. What do you estimate was the total time that you spent doing vigorous gardening or heavy work around the yard IN THE LAST WEEK? ___*hrs* ___*mins*

The next question excludes household chores or gardening or yardwork

3A. IN THE LAST WEEK, how many times did you do any vigorous physical activity which made you breathe harder or puff and pant? (e.g. jogging, cycling, aerobics, competitive tennis, etc.) ___*times*

3B. What do you estimate was the total time that you spent doing this vigorous physical activity in THE LAST WEEK? ___*hrs* ___*mins*

The next question excludes household chores or gardening or yardwork

4A. IN THE LAST WEEK how many times did you do any other more moderate physical activity (e.g. gentle swimming, social tennis, golf etc.) ___*times*

4B. What do you estimate was the total time that you spent doing these activities IN THE LAST WEEK? ___*hrs* ___*mins*

The next three questions are about your average WEEKLY level of activity IN THE LAST SIX MONTHS

5A. On average, IN THE LAST SIX MONTHS how much time did you spend each week walking for recreation/exercise or to get to or from places? (This is walking continuously for at least 10 minutes) ___*hrs* ___*mins*

The next question excludes household chores or gardening or yardwork

5B. On average, IN THE LAST SIX MONTHS how much time did you spend each week doing vigorous physical activity which made you breathe harder or puff and pant? (e.g. jogging, cycling, aerobics, competitive tennis, etc.) ___*hrs* ___*mins*

The next question excludes household chores or gardening or yardwork

5C. On average, IN THE LAST SIX MONTHS how much time did you spend each week doing any other more moderate physical activity that you haven't already reported. (e.g. gentle swimming, social tennis, golf, etc.) ___*hrs* ___*mins*

11.3 Duke activity status

Name: _____ CCRE ID: _____

DOB _____ UR: _____

	Can You…	Yes, with no difficulty.	Yes, with some difficulty.
1	Take care of yourself, that is, eating, dressing, bathing, and using the toilet?	2.75	-
2	Walk indoor, such as around the house?	1.75	-
3	Walk a block or two on level ground?	2.75	-
4	Climb a flight of stairs or walk up hill?	5.50	-
5	Run a short distance?	8.00	-
6	Do light work around the house like dusting or washing dishes?	2.70	-
7	Do moderate work around the house like vacuuming, sweeping floors, carrying groceries?	3.50	-
8	Do heavy work around the house like scrubbing floors, or lifting or moving heavy furniture?	8.00	-
9	Do yard work like raking leaves, weeding or pushing a power mower?	4.50	-
10	Have sexual relations?	5.25	-
11	Participate in moderate recreational activities, like golf, bowling, dancing, doubles tennis, or throwing baseball or football?	6.00	-
12	Participate in strenuous sports like swimming, singles tennis, football, basketball or skiing?	7.50	-

Total Score _____

Protocol Naughton <18.37
Balke 18.38-34.65
Bruce >34.66

11.4 Patient information and consent form for Study 2

THE UNIVERSITY OF QUEENSLAND

Participant Information Sheet

Title: The effects of high intensity interval training on exercise capacity in patients with CKD.

Principal Investigator: Kassia Weston, PhD student,
School of Human Movement Studies, The University of Queensland.
Email: kassia.weston@uqconnect.edu.au
Phone: 3176 7650

Supervisor: Professor Jeff Coombes,
School of Human Movement Studies, The University of Queensland.
Email: jcoombes@uq.edu.au
Phone: 3365 6767

This document has been designed to invite you to voluntarily participate in a research study looking at the effects of exercise training on CKD. Please feel free to discuss your participation, or anything in this document you are unsure of, with your GP, specialist or one of the study doctors.

Aim:

CKD (CKD) is associated with an increased risk of death from cardiovascular disease (CVD). This is largely due to the likelihood of CKD to be a associated with lifestyle related cardiovascular risk factors such as high blood pressure, diabetes and high cholesterol. Furthermore, patients with CKD endure significant muscle wasting which greatly impacts quality of life. As the disease progresses, so does the muscle breakdown. For this reason, sufferers of CKD experience muscle cramping, fatigue and reduced exercise capacity.

Exercise therapy, in particular aerobic exercise, in the CKD population has been shown to decrease these cardiovascular risk factors and subsequent CVD, however the most effective training intensity is yet to be identified. Short bursts of high intensity exercise has been shown to be superior to long duration moderate exercise in improving fitness and decreasing cardiovascular risk in both healthy populations and other chronic diseases, however is yet to be studied in the CKD population. As exercise capacity is widely known to be associated with cardiovascular risk, the effects of the different training intensities will be assessed through a maximal exercise stress test. Additionally, the effects of training intensity on muscle breakdown will be assessed through whole body x-ray and muscle biopsies.

Subject involvement:

Participation in this study will require 4 visits to the University of Queensland (UQ), St Lucia for testing, 2 visits before the training program and 2 after. You will also be required to attend the UQ gym, Princess Alexandra Hospital, Logan Hospital, or Browns Plains Community Clinic, for 3x/week training sessions. During your testing visits at UQ you will undertake a maximal treadmill test and measures of your heart through looking at the pulse in your neck, hip and wrist. Fat mass and muscle mass will also be looked at through a body composition x-ray. We will also require a small muscle biopsy to be taken from your right thigh with the aid of a local anaesthetic, to look at the effects of exercise on reducing muscle breakdown.

Additionally, your daily levels of activity will be measured in two ways on four occasions; initially, at week 6 and 12 of the protocol and 6 weeks after you finish the program. Firstly, you will be required to wear an accelerometer (a small unobtrusive device that is worn on the hip on an elastic belt) and inclinometer (worn on the thigh attached with and adhesive patch) to objectively measure physical activity over 7 consecutive days. You will be given the monitors at the time of attending the University for testing or training and will be required to complete a log to record when the devices are worn over the 7-day period. Secondly, you will be required to complete two telephone interviews with a project investigator where you will be asked to recall your activities over the last 48 hours. This will be conducted on two occasions on either a Sunday or a Monday. The interview will take approximately 45 minutes and will be scheduled at a time that is convenient to you.

After the initial testing, you will be randomly selected to go into one of 2 groups- a high intensity interval exercise group or a moderate continuous exercise group. The exercise intervention groups will undertake 12 weeks of either short bursts of high intensity training interspersed with periods of light exercise, or moderate intensity longer duration exercise. All training will be undertaken on a treadmill and intensity will be increased by increasing the incline of the treadmill, simulating uphill walking. Exercise will be appropriately progressed through the 12 weeks. Free parking is provided

at all locations for all visits involved with the study. At the end of the intervention both groups will complete their second visit for testing to compare the differences. All training groups will be supervised by a qualified Exercise Physiologist. The exercise intervention groups will complete 3 supervised training sessions per week. There will be 10 people randomly selected to go into each of the 2 groups. On completion of the study, you will receive your individual results as well as the group findings.

Information on muscle biopsy:

Muscle biopsy is a commonly performed procedure in both research and medical diagnosis of muscle disorders. As CKD is associated with a breakdown of muscle, we aim to find out the causes of this muscle wasting and whether exercise can improve this decline. The procedure will be performed by a medical doctor trained in muscle biopsies.

The muscle over your lower thigh on your right leg will be thoroughly cleaned before a local anaesthetic will be injected into the skin. You may feel a momentary stinging sensation, as with all needles. The use of anaesthetic minimises any discomfort, however during the procedure you may feel slight pressure. A biopsy needle will be inserted through a small incision (approximately 4-5mm) and a small piece of muscle (~100 mg) will be removed. The biopsy itself only takes a few seconds. Your leg will be iced and a compression bandage applied to minimise the chance of bruising. When the anaesthetic wears off you may feel a tightness in the muscle as if you have bumped your leg.

Potential Risks:

The risk of an allergic reaction to the anaesthetic is low at less than 1 in 1 000 000.

The risk of a skin infection is less than 1 in 1 000 and can be minimised by keeping the injection site clean and dry until it heals. However, if redness or swelling does occur after the procedure, contact the attending physician immediately.

The chance of numbness over the site is approximately 1 in 5000, however it would not be enough to affect daily activities and would resolve within a year.

A 4-5 mm scar may also occur as a result of the biopsy.

Other risks involved with the study:

CKD is associated with an increased risk of cardiovascular disease so there is a small risk to the subjects of a cardiac event occurring during exercise. Supervising Exercise Physiologists are well trained to recognise and prevent any early signs and symptoms of a cardiac event occurring.

As with any exercise there is an associated risk of musculoskeletal injuries. If randomized to an exercise intervention, you will undergo a thorough warm-up procedure before each exercise session to reduce the chance of any injury occurring.

An x-ray scan is used to determine fat mass and muscle mass and involves emission of low dose radiation. However, approximate dosage received from one scan (62 mSv) equates to less than 9 days of natural background radiation and the risk level of associated cancers is classed as extremely low.

Injuries related to participation in the study:

If you think you have any side effects that are related to the muscle biopsy or any other aspect of the study, you should immediately notify Professor Robert Fassett. He may be reached at the Department of Renal Medicine, Royal Brisbane and Women's Hospital. Phone: 0419399571 (24 Hour contact number).

If you have a study-related illness, the investigator and the study staff will make sure that you receive necessary treatment. The University of Queensland provides indemnity insurance coverage for all potential damage or loss that may be sustained as a result of negligence carried out in the course of performing this activity.

Benefits:

This study will answer a number of questions relating to the effects of exercise training on CKD. Furthermore, we are looking to see the effects different training intensities have on the deterioration of kidney function and the symptoms associated with this disease. By determining the best mode of exercise we will be able to better treat and improve the lifestyles of people with CKD. If this study is shown to improve kidney function, cardiovascular risk factors, muscle strength and cramping then it may be implemented as standard hospital care in the future.

Confidentiality:

Personal information gained from the study such as muscle volumes, fitness and cardiovascular measures will be recorded but not identified to any one individual. Once all the measures are completed and the study outcome is verified, subject data will be de-identified and filed in a locked cabinet within the supervisors office in the Princess Alexandra Hospital. To ensure longevity of the data, results will also be kept in a password locked computer with access granted only to the primary investigators. Data will be kept for a minimum of 5 years at the university before being shredded.

Inclusion criteria:

Aged 18-80

Stage 3 or 4 CKD (eGFR >25ml/min or <60ml/min)

Exclusion criteria:

Pregnant

Current involvement in other research studies

Organ transplant

History of significant coronary artery disease

Inability to provide fully informed written consent

Ethical considerations:

This study has been reviewed and approved by the University of Queensland and Princess Alexandra, Human Research Ethics. Should you wish to discuss the study with someone not directly involved, in particular in relation to matters concerning study policies, information about conduct of the study or your rights as a participant, or should you wish to make independent complaint, you can contact:

The Coordinator or Chairperson, Human Research Ethics Committee, The University of Queensland, St Lucia QLD 4072, phone 07 3365 4584 or The HREC Coordinator, Metro South Hospital and Health Service Human Research Ethics Committee (EC00167), Centres for Health Research, Princess Alexandra Hospital, Ipswich Road Woolloongabba QLD 4102, phone 07 3443 8049, email: PAH_ethics_research@health.qld.gov.au

If you wish to discuss this study with someone directly involved in this research you are free to discuss your participation with the principal investigator- Kassia Weston on (07) 3176 7650.

It is a requirement of the Investigator that the informed consent process has taken place with the subject and that the subject:

meets all study inclusion criteria;

was appropriately consented (as described above);

understands the requirements of the study; and

has received a copy of the informed consent document.

PARTICIPATION IS ENTIRELY VOLUNTARY AND SUBJECTS ARE FREE TO WITHDRAW FROM THIS STUDY AT ANY TIME WITHOUT PENALTY.

Participant Consent Form

Title: The effects of high intensity interval training on exercise capacity and cardiovascular risk in CKD.

The investigators of this study conform to the principles governing the ethical conduct of research, and will protect the safety, interests and well being of subjects at all times. This form, the information sheet has been given to you in the interest of your own protection. They contain an outline of procedures and possible risks involved. By signing this consent form you are indicating that:

You have read and understand the information sheet for this study and are aware of the risks involved.

To the best of your knowledge you do not meet any of the criteria for exclusion from the study.

You acknowledge that participation in this study involves you undergoing a muscle biopsy, body composition x-ray and maximal treadmill test; and wearing an accelerometer and inclinometer to measure physical activity.

You are aware that you are able to withdraw from the study at any time without prejudice from either the University of Queensland or Princess Alexandra Hospital.

You understand that due to limited funding you are unable to receive any compensation for your participation, besides parking remuneration.

You understand that the data you provide to the researchers is confidential and can be identifiable only to the primary investigators even if the study is published.

You have been given the opportunity to discuss the study contents with one of the investigators prior to starting the study and that all questions you have asked have been satisfactorily answered.

I agree to participate in the procedures outlined in the patient information sheet to the study: **The effects of high intensity interval training on exercise capacity and cardiovascular risk in CKD.**

_____ _____ _____

Name of Participant Date Signature

Date of Birth: _____/_____/_____

Address:_____

_____

Declaration by researcher: I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

_____ _____ _____

Kassia Weston Date Signature

Chapter 11 Appendices

Emergency Contact

Name	Relationship	Contac

11.5 Physical activity enjoyment scale

This questionnaire is designed to assess your enjoyment of the exercise training that you undertook (either high intensity interval training or moderate intensity continuous training) for the 12 weeks at the University of Queensland. Please answer honestly about your feelings regarding your enjoyment *during* the exercise sessions. With these answers we are looking to compare the enjoyment of high intensity interval training versus moderate intensity continuous training, which may affect adherence to particular exercise programs. Please circle the number on the scale which reflects how you feel for each of the 12 statements.

1. I find it pleasurable	1	2	3	4	5	6	7	I find it unpleasurable
2. *It's no fun at all	1	2	3	4	5	6	7	It's a lot of fun
3. I feel good physically while doing it	1	2	3	4	5	6	7	I feel bad physically while doing it
4. *It's not very invigorating	1	2	3	4	5	6	7	It's very invigorating
5. It's very gratifying	1	2	3	4	5	6	7	It's not at all gratifying
6. *It's not at all exhilarating	1	2	3	4	5	6	7	It's very exhilarating
7. It's very stimulating	1	2	3	4	5	6	7	It's not at all stimulating
8. *It's not at all refreshing	1	2	3	4	5	6	7	It's very refreshing
9. I find it energizing after	1	2	3	4	5	6	7	I find it tiring after
10. *It doesn't give me a strong sense of accomplishment	1	2	3	4	5	6	7	It gives me a strong sense of accomplishment
11. I am not frustrated by it at all	1	2	3	4	5	6	7	I am very frustrated by it
12. *I wouldn't recommend this type of training to my friends	1	2	3	4	5	6	7	I would recommend this type of training to my friends

11.6 Rating of perceived exertion scale

rating	description
6	NO EXERTION AT ALL
7	EXTREMELY LIGHT
8	
9	VERY LIGHT
10	
11	LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD (HEAVY)
16	
17	VERY HARD
18	
19	EXTREMELY HARD
20	MAXIMAL EXERTION

11.7 Cardiorespiratory fitness is associated with autonomic function in patients with chronic kidney disease

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This manuscript is currently being prepared for submission to Nephrology Dialysis Transplantation. This work is by a Summer Scholarship student under the supervision of Kassia Beetham and Jeff Coombes. The data was collected by Kassia Beetham and Erin Howden but was analysed and reported by Rachel Colbran.

Financial Disclosure

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Keywords:

Physical fitness, exercise, autonomic nervous system diseases, CKD

ABSTRACT

Background: CKD (CKD) patients have reduced cardiorespiratory fitness which is associated with a higher incidence of cardiovascular disease and mortality. In healthy individuals the function of the autonomic nervous system (ANS) is positively related to cardiorespiratory fitness, however this has not been investigated in CKD patients. This study aimed to determine the relationship between cardiorespiratory fitness and ANS function in CKD patients. A further aim was to compare ANS function of CKD patients with and without diabetes mellitus (DM) and the relationships with cardiorespiratory fitness.

Methods: Seventy-two patients with stage 3 or 4 CKD were included in the study (eGFR 25-60 mL/min//1.73 m^2). Twenty-seven patients without DM [CKD DM-], 45with DM [CKD DM+]) and 22 healthy controls were investigated. Autonomic function was assessed through measurement of time, frequency and non-linear components of heart rate variability (HRV) obtained through 5-minute supine electrocardiography recordings. Cardiorespiratory fitness (VO_2peak) was assessed via a treadmill exercise stress test.

Results: Patients with CKD exhibited significantly ($P<0.05$) lower HRV and VO_2peak compared with healthy controls. Similarly, the majority of HRV parameters and VO_2peak in CKD patients with diabetes were significantly ($p<0.05$) lower compared to CKD patients without diabetes. There were significant ($p<0.05$) relationships between VO_2peak and a number of measures of HRV for all CKD patients (standard deviation of RR intervals [SDNN], r=0.34; root mean square of successive RR interval differences [RMSSD], r=0.30; high frequency [HF], r=0.28; low frequency [LF], r=0.30; short term variability [SD1], r=0.31; long term variability [SD2], r=0.32) and those without diabetes (SDNN, r=0.29).

Conclusion: Patients with CKD exhibited compromised HRV that was related to cardiorespiratory fitness, suggesting a link between autonomic dysfunction and cardiorespiratory fitness in CKD patients. The additional presence of DM resulted in lower HRV and cardiorespiratory fitness in CKD patients, with cardiac autonomic dysfunction possibly a result of combined uremic and glycaemic neuropathy. Further studies are needed to examine and identify an optimal exercise program for CKD patients to improve cardiorespiratory fitness and cardiac autonomic function.

INTRODUCTION

Diabetes mellitus (DM), CKD (CKD) and cardiovascular disease (CVD) together account for approximately one quarter of the total disease burden in Australia, and two-thirds of total deaths.(336) These diseases (DM, CKD and CVD) are intricately linked, with DM the most common cause of CKD. The most common cause of death in patients with DM or CKD is CVD,(337, 338)which could in part be due to autonomic nervous system (ANS) dysfunction, in particular cardiac autonomic neuropathy. ANS dysfunction, as measured by heart rate variability (HRV) has been linked to increased cardiovascular associated mortality.(339) Examination of ANS dysfunction has been conducted in other chronic disease populations (340, 341) with HRV providing a simple, non-invasive, reproducible and cost-effective marker of ANS function.(342)

The pathophysiology of CKD and DM has been suggested to contribute to ANS dysfunction, albeit through separate mechanisms.(343, 344) Renal impairment may result in an accumulation of neurotoxic solutes in the blood, leading to uremic neuropathy and autonomic dysfunction.(343) Alternatively, hyperglycaemia in DM may result in glycaemic neuropathy and subsequent ANS dysfunction.(344) Regardless of the mechanism, the disease burden of both CKD and DM is significant,(345) with both conditions associated with reduced cardiorespiratory fitness compared to healthy controls.(346, 347) Establishing the relationship between cardiorespiratory fitness and heart rate variability is important as both contribute independently to high mortality rates.(62) Fitness represents a potentially modifiable risk factor, thus if a relationship exists exercise training may be a suitable countermeasure to improve autonomic dysfunction. Previous studies have demonstrated HRV parameters to be related to cardiorespiratory fitness,(348) however this relationship has not been investigated in patients with CKD.

The aim of this study was to determine the relationship between HRV and cardiorespiratory fitness in CKD patients. We also sought to compare the HRV and cardiorespiratory fitness of CKD patients with and without DM. It was hypothesized that HRV parameters would be significantly associated with cardiorespiratory fitness for all CKD patients, with CKD patients exhibiting greater ANS dysfunction than healthy controls. Furthermore, we expected that CKD patients with DM would have lower HRV parameters than CKD patients without DM.

PARTICIPANTS AND METHODS

This study was a cross-sectional analysis of the 'LANDMARK 3' (Longitudinal Assessment of Multiple Discrete Atherosclerotic Risk Factors in Kidney Disease) study that investigated the effects of a lifestyle intervention on cardiovascular risk factors in CKD patients. Seventy-two participants aged 18 to 75 years with stage 3-4 CKD (estimated glomerular filtration rate (eGFR) between 25 and 60 ml/min/1.73m^2) were included in this study. Inclusion criteria: Aged 18 to 75 years and at least one of the following risk factors – blood pressure or lipids not at target; overweight (BMI >25); and poor diabetic control (haemoglobin A1c >7%). Exclusion criteria were: intervention for, or, symptomatic coronary artery disease (within 3 months), current heart failure (New York Heart Association class III and IV) or significant valvular heart disease, pregnant or planning to become pregnant and life expectancy or anticipated time to dialysis or organ transplant < 6 months. The CKD patients with diabetes group consisted of patients with type 1 and type 2 diabetes. The study protocol was approved by the Princess Alexandra Human Research Ethics Committee (HREC 2007/190), and was registered at www.anzctr.org.au (Registration Number ANZCTR12608000337370). Patients provided written and informed consent. Results for a group of healthy, age-matched adults (controls, n=22) were obtained from a sub-population of a previous study (349) for comparison with the current study population.

Heart rate variability

Patients rested in the supine position for at least 5 minutes in a quiet room with no disturbances. Measures were performed after an overnight fast followed by a light meal approximately 30 minutes before testing. All caffeinated products, smoking and alcohol were abstained from for at least 12 hours prior to testing. A standard 3-lead electrocardiograph was recorded continuously for 5 min. R-R intervals were determined automatically from the 3 lead ECG recording using Sphygmocor 8.2 (AtCor Medical, Sydney, Australia) and exported for analysis. The R-R interval tachogram was then visually inspected for any artifacts or ectopic beats that were then replaced with linearly interpolated values calculated from the preceding and proceeding beats. Entire recordings of RR intervals were detrended using a smoothness priors regularization procedure before the time, frequency and non-linear domain HRV parameters outlined in Table 1 were calculated (Kubios HRV v2.0 software, 2008, Finland).(349)

Chronotropic incompetence

Heart rate (HR) was measured immediately after the termination of the exercise stress test at one, two, and three minutes into the cool-down period. HR at each of these time-frames was subtracted from peak HR of the exercise stress test. Heart rate recovery (HRR)-1, HRR-2, and HRR-3 are used to describe the overall HRR at those respective time points. Chronotropic incompetence was determined by calculating the percent HR reserve (%HR reserve). The following equation used in this study was taken from the Framingham Heart Study(350) with the recommendation that patients are deemed chronotropically incompetent with a %HR reserve of <80:

$$\%\ \mathrm{HR} - \mathrm{Reserve} = \frac{(\mathrm{HR\ stage} - \mathrm{resting\ HR})}{(\mathrm{APMHR} - \mathrm{resting\ HR})} x\ 100$$

'HR stage' refers to the HR corresponding to the end of the exercise stress test stage. In this study, stage 2 is used to calculate %HR reserve, meaning that patients must have at least completed two stages on the exercise stress test. APMHR was calculated as 208 - (0.7 x age).

Cardiorespiratory fitness

Cardiorespiratory fitness, exercise capacity, PHR and peak BP were obtained during a treadmill exercise stress test using the Bruce protocol. Cardiorespiratory fitness was measured by peak oxygen uptake (VO_2peak) and was determined by expired gas analysis (Vmax29c, SensorMedics, CA, USA) using the peak 20 second average of the final minute. Exercise capacity was measured by estimated metabolic equivalent tasks (METS)(General Electric Case, Wisconsin, USA) at peak exercise based on treadmill speed, inclination and time spent in the respective stage at the time of completion.

Additional measures

During rest and exercise, BP was recorded manually from the right brachial artery using an aneroid dial and mercury sphygmomanometer and stethoscope. Arterial stiffness measures were obtained after 10 minutes of supine rest using tonometry of the femoral and carotid pulses (Sphygmocor,AtCor Medical, Sydney, Australia). Measures of LDL, HDL, TC, TG, urea and eGFR were assessed from blood samples collected via venipuncture and standard laboratory techniques following an overnight fast. Patients on B-blockers withheld their B-blocker medication for at least 24 hours prior to testing. The Modification of Diet in Renal Disease-175 formula was used to

calculate eGFR.(14) Data on patient demographics, comorbidities and current medications were obtained at the baseline visit. Physical activity levels were determined using the self-report Active Australia Questionnaire.(137)

Data Analysis

Data was analysed using GraphPad Prism (GraphPad, La Jolla, USA). Normality of distribution was assessed using the Kolmogorov-Smirnov test. Parametric one-way ANOVA with a Tukey post-hoc test was used to compare normally distributed variables between the control, all CKD patients, CKD with diabetes mellitus (DM+) and CKD without diabetes mellitus (DM-) groups. The CKD DM+ and CKD DM- makes up the all CKD group, in order to demonstrate differences between the healthy control and CKD patients, before analyzing differences between diabetes and no diabetes in CKD. Non-parametric Kruskal-Wallis tests with Dunn's post-test were used to compare skewed variables. Correlations between variables were assessed using Pearson correlation or Spearman's Rho coefficients. Values were presented as mean ± SD with alpha set at ≤ 0.05.

RESULTS

Baseline characteristics

Clinical characteristics of the CKD patients and controls are displayed in Table 2. All CKD patients had significantly ($p<0.05$) higher BMI resting blood pressure, and significantly lower VO_2peak compared to the healthy control group. There were 27 patients with CKD and clinically diagnosed DM (CKD DM+), and 45 patients with CKD alone (CKD DM-) participating in this study. Compared to CKD DM- patients, CKD DM+ patients displayed significantly ($p<0.05$) lower VO_2peak, estimated METS, resting diastolic blood pressure, peak diastolic blood pressure, and significantly higher pulse wave velocity (PWV), and incidence of hyperlipidaemia (Table 2).

Heart rate variability parameters between groups

The HRV parameters for all groups are displayed in Table 3. Compared to the control group, all CKD patients exhibited lower HRV for 6 out of the 11 parameters including RMSSD, pNN50, HF, LF, SD1 and SD2. Comparisons between CKD patients indicated that HRV for CKD DM+ patients

was significantly ($p<0.05$) lower compared to CKD DM- patients for 7 out of the 11 HRV parameters investigated: SDNN, RMSSD, pNN50, HF, LF, SD1 and SD2.

Heart rate variability and cardiorespiratory fitness

Table 4 shows a number of significant ($p<0.05$) correlations between VO_2peak and HRV (i.e. SDNN (Figure 1), RMSSD, HF, LF, SD1 and SD2) in all CKD patients. When CKD patients were separated based on diabetic status, SDNN was significantly ($p<0.05$) associated with VO_2peak for the CKD DM- subjects. There were no significant associations between VO_2peak and HRV parameters for the control or CKD DM+ groups. Weekly physical activity was not significantly ($p>0.05$) associated with $\dot{V}O_{2peak}$ for any of the CKD groups, but was significantly associated with SDNN (Figure 1), SD2 ($r = 0.25$, $p<0.05$) and LF($r = 0.28$, $p<0.05$) for all CKD patients. There were no significant associations between chronotropic incompetence measures and SDNN.

DISCUSSION

This is the first study to investigate the relationship between HRV and cardiorespiratory fitness in CKD patients. The main findings were that: 1) CKD patients have compromised HRV which was related to low cardiorespiratory fitness; 2) CKD patients with DM have decreased HRV and cardiorespiratory fitness compared to those without DM; 3) physical activity levels were not associated with cardiorespiratory fitness but were related to HRV for all CKD patients. Together, this data suggests that improving cardiorespiratory fitness and physical activity in CKD patients may lead to an improvement in heart rate variability. Future longitudinal studies are needed to test this hypothesis.

The current study demonstrated that CKD patients both with and without DM had significantly decreased HRV parameters compared to healthy control subjects. This supports previous studies,(115, 259) which indicate autonomic dysfunction in renal patients, suggested to be attributable to uremic neuropathy. The uremic environment associated with renal dysfunction may result in generalized axonal loss and demyelination of neurons, resulting in subsequent autonomic dysfunction.(351)

Whilst the findings demonstrated CKD patients to have lower HRV compared to healthy controls, most HRV parameters were significantly lower for CKD DM+ patients compared to CKD DM-

patients. This is consistent with previous research.(352) More recently, Myonopoulou *et al.* (2010) and Chandra *et al.* (2012) (353) reported that the majority of 24-hour HRV measures from Holter ECG recordings were significantly lower for CKD DM+ patients compared to CKD DM- patients. This lower HRV and decreased autonomic function may result from the combined effects of glycaemic and uremic neuropathies on cardiac innervation. In patients with DM, hyperglycaemia has been observed to contribute to autonomic dysfunction by first decreasing the activity of parasympathetic nerves (leading to sympathetic augmentation) and then later affecting the sympathetic nerves.(344, 354) The significant decrease in both LF and HF measurements in the current study provides further support for the damage to both parasympathetic and sympathetic modulation possibly due to advanced autonomic denervation.(339) The exact mechanistic pathways for the impact of uremic induced neuropathy on ANS activity remains to be clarified and warrants further investigation, including trialling approaches known to enhance ANS activity such as regular exercise.(355)

Regular exercise has been reported to enhance cardiorespiratory fitness in a range of clinical populations (75, 356) with cardiorespiratory fitness regarded as an independent predictor of mortality.(26, 357) It is well reported that cardiorespiratory fitness is lower for CKD patients compared to healthy controls.(309) In the current study, CKD patients with DM exhibited decreased cardiorespiratory fitness and HRV compared to those without DM. Furthermore, the relationship between HRV and cardiorespiratory fitness was only apparent for the CKD patients without DM. A similar association between HRV and cardiorespiratory fitness has also been noted previously in healthy populations.(358) It may be reasonable to suggest that damage to the ANS by uremic and importantly glycaemic neuropathy may result in decreased cardiorespiratory fitness through chronotropic incompetence, a restriction in the ability to raise heart rate during exercise.(359) However, this study found no association between chronotropic measures and the global measure of HRV, SDNN. This may indicate that autonomic dysfunction is influencing cardiorespiratory fitness through mechanisms other than chronotropic incompetence. Previous studies have identified chronotropic incompetence due to cardiac autonomic neuropathy in diabetic patients,(360, 361) although the exact mechanisms for this change remain unclear. The decreased cardiorespiratory fitness observed in CKD patients is likely multifactorial and identifying the mechanisms through which autonomic dysfunction leads to reduce cardiorespiratory fitness warrants further investigation.

It is interesting to note that both cardiorespiratory fitness and weekly physical activity levels were significantly associated with HRV (SDNN) but they were not related to each other in the present study. A lack of association between physical activity and measures of cardiorespiratory fitness

have previously been reported.(362) This may reflect the importance of exercise intensity (e.g. moderate or high intensity exercise) in improving cardiorespiratory fitness. Furthermore, the correlation between cardiorespiratory fitness and physical activity with HRV may indicate that physical activity is influencing cardiac autonomic function in patients with CKD. Exercise intensity has been suggested to play an important role in HRV adaptations (355) with renal patients reported to enhance HRV with regular exercise training (117, 260) although this is not always observed.

Interestingly, the findings from this study also demonstrated that PWV was significantly greater for CKD DM+ patients compared to CKD DM- patients. PWV is an indicator of arterial stiffness and has been shown to predict cardiovascular mortality in patients with DM.(363) A previous study has shown PWV to be significantly and independently correlated with autonomic neuropathy.(363) Several reasons for this have been suggested, including that arterial stiffness may contribute to autonomic neuropathy (aortic and carotid wall stiffness may impair baroreceptor function). It has been suggested that damage can occur to the autonomic nerves passing signals from the baroceptors to the medulla oblongata, and from the medulla oblongata to the cardiovascular system, which may result in dysfunction of the baroreceptor reflex.(364) In contrast, it has also been suggested that autonomic neuropathy may influence the elastic properties of the arteries.(365) Increased arterial stiffness in CKD DM+ patients may decrease the degree of stretch of the baroreceptors in the aortic arch and carotid sinus, thereby decreasing baroreceptor activation and impairing blood pressure regulation.(366) Regardless of aetiology, increased arterial stiffness appears to be linked with autonomic dysfunction, and could be involved in the decreased cardiorespiratory fitness of CKD DM+ patients compared to CKD DM- patients. For example, recent research has suggested that the HRV measure of LF may reflect baroreceptor reflex modulation and not sympathetic tone as previously thought.(367) Therefore, the significantly decreased LF observed in CKD DM+ patients in the current study may indicate decreased activity of the baroreceptor reflex. Impairment of the baroreceptor reflex may contribute to exercise intolerance, as blood pressure changes associated with exercise could not be corrected as quickly or efficiently as in a healthy individual. Increases in rate and force of cardiac contraction in response to decreased blood pressure would be impaired, resulting in chronotropic incompetence.

There were several strengths to the current study, including the large sample size across different populations and the use of non-linear measures of HRV. These HRV measures have been reported to be superior to conventional time and frequency domain HRV measures.(368) The current study also possessed some limitations. Firstly, this study was a cross-sectional analysis of the CKD population and therefore could not definitively deduce causality. Secondly, 5-minute recordings of HRV were used instead of 24-hour Holter monitoring that may provide greater details of longer-

term ANS function. Nonetheless, the current HRV results were significant and provide a unique examination of HRV and cardiorespiratory fitness in CKD patients. The use of the Active Australia questionnaire is well validated in the older population,(137) however without an objective measure of physical activity such as use of a pedometer or accelerometer, the questionnaire may have resulted in over-reporting by the patients. In an attempt to limit over-reporting, the questionnaire was administered by an Exercise Physiologist, with each question carefully explained with examples. The use of negative chronotropic drugs, such as B-blockers, may also influence heart rate variability. Although participants were asked to refrain from taking this medication at least 24 hours prior to testing, some residual medication may still influence heart rate. Finally, not all variables were available for the control group, with future studies potentially providing greater comparisons.

In summary, our study was the first to investigate the relationship between HRV and cardiorespiratory fitness in CKD patients. The main findings were that CKD patients exhibited compromised HRV compared to healthy controls, which positively correlated with cardiorespiratory fitness in CKD DM- patients only. Additionally, CKD patients with DM exhibited lower HRV and cardiorespiratory fitness compared to those without DM. Further studies are needed to examine and identify if exercise training is able to impact HRV in patients with DM and CKD.

Transparency Declarations

None to declare.

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Table 1 Heart Rate Variability (HRV) parameters

Frequency domain HRV measures		**Unit**	**Description**
LF	Low frequency power (0.04 - 0.15Hz)	ms^2	Correlated to both parasympathetic and sympathetic modulations.
HF	High frequency power (0.15 - 0.4Hz)	ms^2	Indicator of parasympathetic modulations.
LF/HF	Ratio of LF/HF	No units	Indicator of sympathovagal balance.
Time domain measures of HRV		**Unit**	**Description**
pNN50	Ratio between the number of pairs of adjacent NN intervals differing by more than 50ms and the total number of NN intervals	%	Reflects high frequency heart rate variations due to parasympathetic modulation.
SDNN	Standard deviation of all NN intervals	ms	Indicator of overall HRV
RMSSD	Square root of the mean of the squares of successive R-R interval differences	ms	Reflects high frequency heart rate variations due to parasympathetic modulation. Indicates short-term HRV components.
Non linear measures of HRV		**Unit**	**Description**
SD1	Short axes of the Poincare plot	ms	Reflects short-term instantaneous RR modulation and parasympathetic activity.
SD2	Long axes of the Poincare plot	ms	Reflects long-term RR modulation and overall HRV.
SampEn	Sample entropy		Estimates the predictability or

		randomness (i.e. complexity) of the data
Alpha1	Short-term fractal scaling exponent, based on four to 16 beats.	Quantifies the presence or absence of fractal-like correlations. Scaling exponent of 1 indicates normal cardiac health, 0.5 indicates random dynamics or white noise, while 1.5 is highly correlated but of non power law or brown noise.
Alpha 2	Long-term fractal scaling exponent, based on 16 to 64 beats.	

Table 2 Clinical characteristics of control adults and CKD patients with and without diabetes mellitus.

	Control (n = 22)	**All CKD (n = 72)**	**CKD with diabetes mellitus (n = 27)**	**CKD without diabetes mellitus (n = 45)**
Demographics				
Age (years)	64.5 (4.5)	63.0 (4.3)	64.0 (4.5)	60.1 (6.9)
Sex				
Female (%)	36.4	48.4	48.1	42.2
Body Mass Index (kg/m^2)	25.5 (5.6)	31.1 (4.7)*	33.2 (3.4)*	30.7 (4.3)*
Current smokers (%)	0	15.7	7.4	20.9
Comorbidities				
Hyperlipidaemia (%)	-	69.6	88.5	58.1#
Hypertension (%)	31.6	95.7*	100.0*	93.2*
Peripheral Vascular Disease (%)	-	16.2	23.1	11.9
Past Myocardial Infarction (%)	0	20.3	30.8*	14
Blood biochemistry				
HDL cholesterol (mmol/L)		1.1 (0.3)	0.9 (0.5)	1.2 (0.4)
LDL cholesterol (mmol/L)		2.4 (0.3)	2.2 (0.3)	2.6 (0.3)#
Total cholesterol (mmol/L)		4.4 ± 1.2	3.9 ± 1.5	4.7 ± 0.9#
Triglycerides (mmol/L)		1.6 (0.5)	1.6 (0.4)	1.6 (0.6)
eGFR (mL/min/1.73m2)		38.2 ± 12.6	36.7 ± 10.3	39.0 ± 13.8

Urea(mmol/L)		9.6 (2.4)	9.8 (3.0)	9.3 (2.2)
Hemodynamic values				
Resting SBP (mmHg)		130.0 (13.3)	137.0 (11.0)	126.0 (14.0)
Resting DBP (mmHg)		73.2 ± 9.8	69.1 ± 9.9	75.6 ± 9.0[#]
Peak SBP (mmHg)		170.0 (10.5)	170.0 (10.0)	170.0 (14.0)
Peak DBP (mmHg)		80.0 (10.0)	80.0 (0.0)	89.0 (1.5)[#]
Resting heart rate (bpm)	62.8 ± 8.2	79.6 ± 13.1	83.3 ± 15.7	77.3± 10.7
Peak heart rate (bpm)		147.0 ± 19.7	142.2± 19.1	149.9 ± 19.7
Pulse wave velocity (m/s)		0.55 (1.30)[#]	10.60 (2.90)	7.85 (2.45)[#]
Medications				
Beta blockers (n,%)	14.3	39.7	15, 53.8	16, 31.0
ACE inhibitors (n,%)	44.4	50	16, 57.7	25, 45.2
Metformin (n,%)		5.8	4, 15.4	0, 0[#]
Insulin (n,%)		24.6[#]	17, 65.4	0, 0[#]
Sulfonylurea (n,%)		1.4	1, 3.7	0, 0
Thiazolidinedione (n,%)		9.7[#]	7, 26	0, 0[#]
Statins (n,%)	37.5	70.6	25, 88.5[*]	30, 59.5
Exercise Parameters				
$\dot{V}O_{2peak}$ (mL/kg/min)	34.7 (5.6)	21.4 (3.8)[*]	19.2 (2.5)[*]	23.5 (3.6)[*#]
Respiratory Quotient		1.05 (0.08)	1.00 (0.14)	1.06 (0.07)
Weekly physical activity (mins)		80.0 (130.0)	45.0 (93.8)	125.0 (115.0)
Estimated METS		7.2 ± 3.1	6.0± 2.4	8.0± 3.1[#]

LDL: Low-density lipoprotein; HDL: high-density lipoprotein; eGFR: estimated glomerular filtration rate; DBP: diastolic blood pressure; SBP: systolic blood pressure; ACE: angiotensin converting enzyme.

Normally distributed quantitative variables are reported as mean ± standard deviation (SD). Not normally distributed variables are reported as median (IQR). Categorical variables are reported as % (percentage of patient group positive for that characteristic).

*Significantly ($p<0.05$) different to control

Significantly ($p<0.05$) different to CKD patients with diabetes mellitus

Table 3. Comparison of heart rate variability (HRV) parameters between CKD patients with and without diabetes mellitus

	Control (***n*** **= 22)**	**All CKD** **(n= 72)**	**CKD with diabetes mellitus** (***n*** **= 27)**	**CKD without diabetes mellitus** (***n*** **= 45)**
Time domain parameters				
SDNN (ms)	28.3 (3.9)	27.0 (8.0)	17.5 (7.4) *	30.4 (6.7) #
RMSSD (ms)	24.6 (2.5)	17.4 (5.4)*	10.5 (8.7) *	19.4 (5.9) #
pNN50 (%)	4.4 (2.7)	1.2 (2.3)*	0.0 (2.0) *	1.5 (4.1) #
Frequency-domain parameters				
HF (ms^2)	201 (57)	96 (83)*	40 (47)*	144 (67)#
LF (ms^2)	411 (173)	131 (96)* #	41 (85)*	168 (135)* #
LF/HF ratio	1.9 (1.8)	1.6 (1.0)	1.5 (1.0)	1.6 (1.2)
Non-linear measures				
SD1 (ms)	17.5 (1.9)	12.3 (3.8)*	7.3 (6.3) *	14.1 (3.8) #
SD2 (ms)	54.3 ± 17.6	34.6 ± 16.7* #	24.3 ± 14.0 *	40.8 ± 15.1 * #
Alpha1	1.137 ± 0.299	1.148 ± 0.252	1.108 ± 0.282	1.171 ± 0.233
Alpha 2	0.949 (0.153)	1.059 (0.092)	1.086 (0.171)	1.045 (0.098)
SampEnt	1.428 ± 0.272	1.423 ± 0.304	1.440 ± 0.345	1.413 ± 0.280

Variables are reported as mean ± standard deviation (SD).

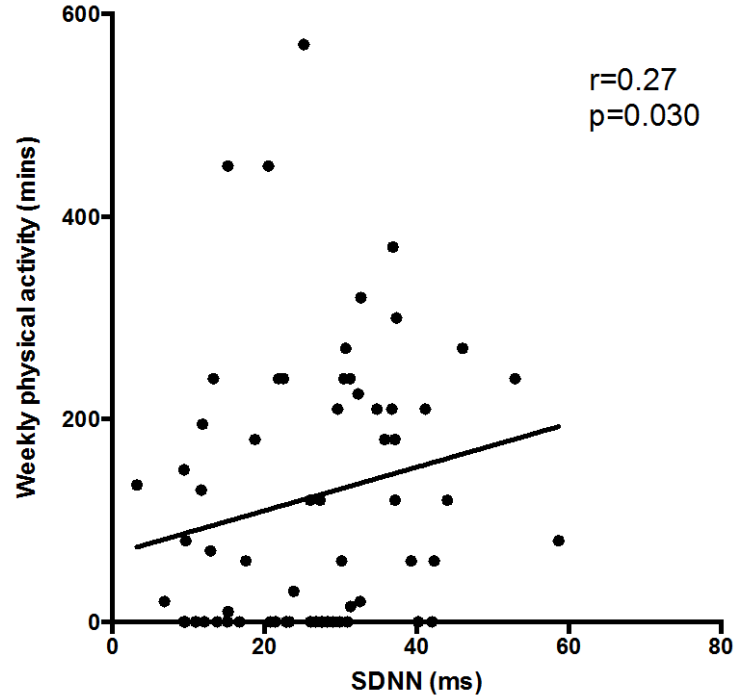
* Significantly ($p<0.05$) different to control

Significantly ($p<0.05$) different to CKD (CKD) patients with diabetes mellitus

Table 4 Correlations between VO_2peak and heart rate variability parameters

	Controls (n=22)	All CKD (n=72)	CKD with diabetes mellitus (n=27)	CKD without diabetes mellitus (n=45)
Time-domain				
SDNN (ms)	0.33	0.34**	-0.08	0.29*
RMSSD (ms)	0.02	0.30*	0.03	0.19
pNN50 (%)	0.00	0.23	-0.03	0.19
Frequency-domain				
HF (ms^2)	0.19	0.28*	0.04	0.11
LF (ms^2)	0.33	0.30*	0.02	0.17
LF/HF ratio	0.24	0.01	-0.03	-0.04
Non-linear				
SD1 (ms)	0.09	0.31*	0.03	0.20
SD2 (ms)	0.39	0.32*	-0.08	0.24
Alpha1	0.02	-0.07	-0.17	-0.13
Alpha 2	-0.2	-0.05	0.09	-0.03
SampEnt	-0.12	0.08	0.24	0.04

Parametric (r value) and non-parametric (rho value) correlations between $\dot{V}O_{2peak}$ and heart rate variability shown. * $p<0.05$, ** $p<0.005$.



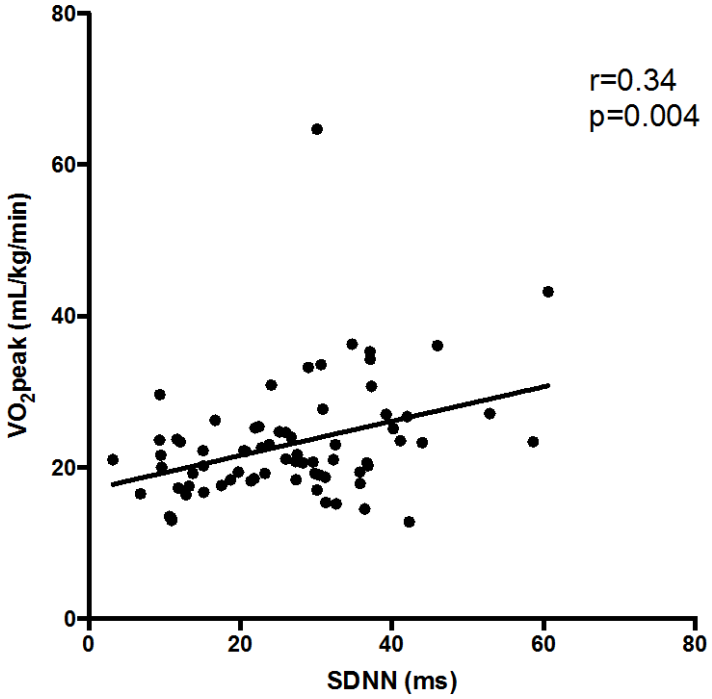


Figure 1 Relationships between VO_2peak and weekly physical activity levels (WPA) with SDNN in all CKD patients.

11.8 Oxidative stress is associated with decreased heart rate variability in patients with chronic kidney disease

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Abstract

Background: Elevated oxidative stress is a hallmark of patients with CKD (CKD). Reduced heart rate variability (HRV) is also prevalent in CKD and is associated with increased morbidity and mortality. The relationship between oxidative stress and HRV has not been investigated in the CKD population.

Methods: 78 patients with stage 3-4 CKD (MDRD eGFR 25-60 mL/min/1.73 m^2) provided blood for the assessment of plasma total F2-isoprostanes, glutathione peroxidase activity and total antioxidant capacity. Time and frequency HRV parameters were collected after five minutes in a supine position. Additional blood measures, such as lipids and inflammatory markers were also obtained.

Results: Participants with elevated F2-isoprostanes had a reduced HRV as measured by the standard deviation of the normal-normal intervals (SDNN). A number of HRV parameters were found to be inversely correlated with F2-isoprostanes, including SDNN (r= -0.337; *p*= 0.003), very low frequency (r= -0.281, *p*= 0.013), low frequency (r= -0.315, *p*= 0.005) and total power (r= -0.288, *p*= 0.01). Multiple linear regression found F2-isoprostanes to be an independent predictor of SDNN (β= -0.272, *p*= 0.01). F2-isoprostanes and male sex explained 28.7% of the variance in SDNN (*p*<0.01).

Conclusion: Oxidative stress is significantly and independently associated with HRV in patients with CKD.

Keywords: autonomic dysfunction; CKD; heart rate variability; oxidative stress

INTRODUCTION

Elevated oxidative stress is common in patients with CKD (CKD).(161) Oxidative stress occurs when there is a disruption in redox signalling and control pathways.(159) Elevated oxidative stress may contribute to a multitude of disease pathways including a variety of cardiovascular conditions (369), the mortality rates of which are increased in the CKD cohort.(370, 371) The gold standard for the measurement of oxidative stress injury is plasma total F2-isoprostanes, the by-product of lipid peroxidation.(372)

Patients with CKD also exhibit a reduction in heart rate variability (HRV).(373) HRV is a measure of the constantly deviating R-R interval and, thus the balance between the dual sympathetic/parasympathetic stimulation of the pacemaking sino-atrial node in the heart.(374) A reduction in HRV is indicative of cardiac autonomic dysfunction and associated with increased morbidity and mortality.(114, 375) Indeed, sudden cardiac death is a significant problem reportedly contributing to up to 60% of cardiovascular mortality in dialysis patients, increasing with each stage of CKD.(376) It has been suggested that impaired baroreflex effectiveness through autonomic dysfunction can initiate fatal arrhythmias and sudden cardiac death.(377, 378) Despite many time and frequency parameters that can be attained from a single heart rate variability measure, standard deviation of normal-normal intervals (SDNN) is used as a global measure of HRV.(379) A reduced SDNN value indicates reduced HRV and therefore autonomic dysfunction.(380)

Previous studies have investigated the relationship between HRV and elevated oxidative stress in diseases such as hypertension (340, 381) and diabetes.(382, 383) Pavithran et al. (2008) found that heart rate variability was significantly lower in hypertensive patients compared to the control group;(340) a finding that was replicated by Thiyagarajan et al. (2013) in pre-hypertensive subjects.(381) In like manner, Ziegler et al. (2004) found that oxidative stress biomarkers were increased and antioxidant levels decreased in diabetic patients with peripheral neuropathy.(383) They also found that oxidative stress was even more marked in patients who had dual peripheral and cardiac neuropathy. What remains unclear, is the relationship between CKD and oxidative stress in the pathogenesis of cardiac autonomic neuropathy. Investigating the relationship between oxidative stress and HRV is of particular importance as it can assist in further developing our understanding and, therefore, treatment of poor clinical outcomes in CKD patients. Therefore, the purpose of this study was to determine the association between oxidative stress and HRV in the CKD population. It was hypothesised that elevated plasma F2-isoprostanes would be associated with reduced heart rate variability within patients with stage 3-4 CKD.

SUBJECTS AND METHODS

The data from this study is a cross-sectional analysis of the 'LANDMARK 3' study (Longitudinal Assessment of Multiple Discrete Atherosclerotic Risk Factors in Kidney Disease), looking at the effects of a lifestyle intervention in CKD. 78 subjects with stage 3 or 4 CKD (MDRD eGFR 25-60 mL/min/1.73 m^2) were included in this study. Patients were aged between 18 to 75 years and had at least one of the following risk factors – blood pressure or lipids not at target; overweight (BMI >25 kg/m^2); or poor diabetic control. Exclusion criteria were life expectancy less than 6 months, pregnancy, enrolment in another research study or organ transplant recipient.

The study protocol was approved by the Princess Alexandra Human Research Ethics Committee (HREC 2007/190), and was registered at www.anzctr.org.au (Registration Number ANZCTR12608000337370). Patients provided written and informed consent.

Routine Blood Biochemistry

Blood measures of lipids, haemoglobin, phosphate, creatinine, CRP, magnesium, albumin, glucose and insulin were conducted using standard laboratory techniques through venepuncture after an overnight fast. Insulin resistance was computed using the HOMA-IR method.(152) Estimated glomerular filtration rate (eGFR) was determined using the Modification of Diet in Renal Disease-175 formula.(14) Insulin resistance was computed using the HOMA-IR method.(152) Patients were asked to refrain from taking their β-blocker medication at least 12 hours before testing.

Inflammation

Inflammation markers interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and interferon gamma (INF-ɣ) were measured by an electrochemiluminescence technique using Human Pro-inflammatory 4-plex Ultra-sensitive Kit with the Sector Imager 6000 from Meso Scale Discovery (Gaithersburg, USA). The assays were performed according to the manufacturer's instructions with coefficients of variation less than 20% being considered acceptable, as previously described.(384)

Heart rate variability

After 5 minutes of supine rest HRV was recorded from a supine 5 minute 3 lead ECG recording that was conducted in a quiet room with no disturbances (such as talking or coughing). The R-R interval tachogram was visually inspected and any ectopic beats or artefacts were removed by linear interpolation of the preceding and proceeding beats. Software (Kubios version 2.1, Kuopio, Finland) was used to detrend the data using a smoothness priors regularization procedure before calculating the time, frequency and non-linear domain HRV parameters. A number of time and frequency parameters of HRV were assessed. Time parameters included the standard deviation of RR intervals (SDNN), the root mean square of differences in successive RR intervals (RMSSD), the number of successive RR intervals greater than 50ms (NN50), and the proportion of NN50 intervals in the entire recording (pNN50). Frequency parameters included a spectral power analysis incorporating very low frequency (VLF; ≤0.04 Hz), low frequency (LF; 0.04-0.15 Hz), high frequency (HF; 0.15-0.4 Hz) and total power (TP). All HRV measures are described in table 1. The LF/HF ratio was also computed using these values. SDNN was used in regression analyses as it is recognized as a robust global marker of HRV.(379) Patients with a pacemaker were excluded from analysis.

Plasma F2-isoprostanes

Samples were analysed in duplicate. Total F2-isoprostanes were analysed using a gas chromotography mass spectrometry protocol developed in our laboratory.(153) The coefficient of variation for this assay is 4.5%. Patients were divided into two groups based on their plasma F2-isoprostane levels: normal (<250 pg/ml) and elevated (≥ 250 pg/ml). This was based on using a value 1.5 standard deviations from mean values from a previous study on apparently healthy 18-30 year old males and females from our laboratory.(154)

Plasma Glutathione Peroxidase Activity

Samples were measured in duplicate using an automated method adapted from Wheeler et al. as the rate of oxidation of NADPH at 340nm in a coupled reaction, cycling oxidised glutathione to reduced glutathione using glutathione reductase.(156) These measures were performed on an autoanalyser (Cobas Mira, Roche Diagnostica, Switzerland). The laboratory coefficient of variation for this assay is 2.4%

Plasma Total Antioxidant Capacity

Total antioxidant capacity was measured using a modified version of an assay previously described(385) and adapted for a Cobas Mira autoanalyser (Cobas Mira, Roche Diagnostica, Switzerland.). Briefly, plasma was incubated with met-myoglobin and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS). After incubation, hydrogen peroxide was added and the sample incubated again. Absorbance was measured spectrophotometrically to determine total antioxidant capacity. The laboratory coefficient of variation for this assay is 1.9%.

Statistics

Mean ± standard deviation (SD) was used for the description of normally distributed baseline characteristics. Log transformed and not normally distributed variables were reported as median (IQR). Frequencies were used to describe categorical variables. Study variables that did not exhibit normal distribution were transformed using the natural logarithm. These included: F2-isoprostanes, VLF ms^2, LF ms^2, HF ms^2, LF: HF, TP ms^2, TG, IFN and CRP. A series of Independent Sample T tests were used to compare variables between the normal and elevated F2-isoprostanes group. Non-parametric tests were performed on not normally distributed variables that did not normalise after log transformation. Mann-Whitney-U tests were used for the remaining variables that were not normally distributed, which included: age, NN50, pNN50, HDL, TC, HOMA, Mg, IL6, TNF, albumin, HbA1c. A Pearsons Chi square test was used for categorical variables. Bivariate correlations between plasma F2-isoprostanes and HRV variables were assessed using Pearsons correlations for normally distributed data and Spearmans Rho for data that was not normally distributed or categorical. A multiple linear regression using the enter method was undertaken with SDNN as the outcome measure against all variables that exhibited a significant univariate association with SDNN. Significance for all tests was assumed at the $p<0.05$ level. The statistical program used for the analysis was SPSS 20 (IBM, New York, USA).

RESULTS

The characteristics, medications, heart rate variability parameters and biochemical measures of the study participants are presented in Table 2. 16 Patients were identified as having elevated levels of F2-isoprostanes. Patients with elevated F2-isoprostanes had significantly ($p<0.05$) reduced HRV measures of SDNN, VLF and LF. Compared to historical healthy control data from a similar age

category from our group, the elevated F2-isoprostanes group had significantly reduced SDNN, RMSSD, pNN50(%), HF, LF and LF:HF ratio.

Table 3 shows F2-isoprostanes of all patients to be inversely correlated with SDNN, STD HR, VLF, LF and TP. Mean RR, RMSSD and HR also had an inverse correlation which was approaching significance.

Patients were asked to abstain from taking their β-blocker medication at least 12 hours prior to HRV testing. However, to ensure residual β-blocker medication was not influencing the findings, a sub-analysis on patients not on β-blockers was also performed (normal F2-isoprostanes n=39, elevated F2-isoprostanes n=14). In addition to SDNN LF this analysis also identified the LF:HF ratio and STD HR to be lower in the elevated F2-isoprostanes group. In the sub-analysis VLF was trending towards significance (p=0.083) (table 4). The sub-analysis on patients *not* on β-blockers also identified the same significant correlates with F2-isoprostanes (STD HR, SDNN, VLF, LF and TP) (table 5).

A multiple linear regression model (Table 6) found F2-isoprostanes to be an independent predictor of SDNN (β=-0.272, p=0.01), along with sex (β=-0.263, p=0.014). A model that included the significant bivariate associates (HbA1C, diabetes status, F2-isoprostanes and sex) explained 28.7% of the variance in SDNN (p<0.01).

DISCUSSION

The findings from this study indicate that CKD patients with elevated plasma F2-isoprostanes have evidence of autonomic dysfunction. Time parameters of HRV, SDNN, and frequency parameters, VLF and LF were significantly reduced in patients with high levels of plasma F2-isoprostanes. An analysis of all patients identified significant inverse relationships between F2-isoprostanes and a number of HRV parameters including SDNN, STD HR, VLF, LF and TP. Mean RR, RMSSD and HF were approaching significance. A sub-analysis of patients *not* on β-blockers confirmed 1) the difference in HRV parameters between normal and elevated F2-isoprostanes groups and 2) and the inverse correlation between HRV parameters and F2-isoprostanes. Furthermore, a multiple linear regression identified plasma F2-isoprostanes to be an independent predictor of global HRV measure, SDNN.

Due to the complexity of HRV there are a number of time and frequency parameters that should be considered. Generally, time domain parameters are thought to give a global measure of autonomic

activity, whereas frequency parameters provide an indication of autonomic balance.(386) Our study found significant reductions in a number of both time and frequency parameters in the patients with elevated plasma F2-isoprostanes. The reduction in SDNN indicates an overall decrease in autonomic function. Furthermore, the frequency data showed that reductions in VLF and LF were associated with increased plasma F2-isoprostanes. It is commonly accepted that HF relates to parasympathetic function and that VLF and LF are more representative of the sympathetic contribution.(379) Although not statistically significant, HF was also considerably lower in the elevated F2-isoprostanes group, suggesting global autonomic dysfunction of both parasympathetic and sympathetic nervous systems. When the HRV values in the elevated F2-isoprostanes group were compared to the historical healthy control data previously recorded in our group, it was found that CKD HRV values were significantly lower. Despite no normative values for HRV existing, this comparison highlights the impairment of HRV in CKD patients with elevated F2-isoprostanes. This study also identified no significant differences between GPX and TAC in the normal and elevated F2-isoprostanes groups. The disconnect between F2-isoprostanes and GPX and TAC in our findings may be suggesting that an increase in oxidants is occurring without a compensatory increase in antioxidants.(168) This imbalance may be important in the pathogenesis of oxidative injury and therefore autonomic dysfunction in CKD patients.

In the case of CKD, it is understood that elevated oxidative stress is present relatively early during the disease progression. Kidney disease is associated with a decrease in antioxidant scavenging enzymes and increase in the production of ROS, thereby leading to oxidative stress.(387) Furthermore, previous studies have found that the level of oxidative stress continues to increase as CKD progresses.(168, 388) A study by Dounousi et al. (2006) found a significant correlation between increasing oxidative stress levels and declining kidney function, as measured by eGFR.(388)

A number of studies have suggested a role of oxidative stress in the development of the autonomic neuropathy in other populations. Oxidative stress has been associated with a decrease in cardiovagal modulation in pre-hypertensive subjects.(381) Additionally, reduced HRV has been associated with air pollutant-induced oxidative stress.(389, 390) A study similar to the current investigation, in patients with diabetes, found that oxidative stress levels are enhanced prior to the development of polyneuropathy and remain high within this cohort.(383)

The relationship between oxidative stress in CKD and cardiac autonomic function has not been well studied. Although the precise mechanistic pathways through which reactive oxygen species cause autonomic damage remains unclear, it has been hypothesised that oxidative stress leads to neuronal

injury in a variety of different ways. For example oxidative stress can cause damage to proteins and lipids that contribute to axonal transport.(391) It has also been well documented that cellular stressors, such as ROS, can lead to mitochondrial damage causing damage to the electron transport chain and upregulating the release of pro-apoptotic proteins, such as cytochrome C. This, in turn, activates the caspase cascade system, which ultimately leads to apoptosis of neurons.(392, 393) Therefore, large-scale neuronal apoptosis could be considered a potential hypothesis for the observed decrease in autonomic stimulation.(382) It should also be noted that a build-up of uremic toxins, as seen in CKD, may be a source of ROS, although the role that uric acid plays in the genesis of ROS remains unclear.

Clinically, a greater understanding of the pathological processes leading to decreased HRV can provide a potential therapeutic target. Oxidative stress has been shown to lead to the proliferation of various pathologies and we have shown that it has a relationship with reduced autonomic function. Thus, it can serve as a potential therapeutic target with the aim of restoring cardiac autonomic function. Some studies have suggested possible anti-oxidant therapies to specifically target ROS (e.g. α-tocopherol, vitamin C, *N*-acetylcysteine), however, a lack of a clinically valid global marker for oxidative stress means that the current evidence of their efficacy is lacking.(182) Other studies have established that regular exercise can protect against oxidative stress by promoting up-regulation of anti-oxidant enzymes in response to the acute increases in ROS which occur with exercise training.(179)

Limitations

Due to the cross-sectional design of this study and the corresponding analysis, a direct causative link between oxidative stress and reduced heart rate variability cannot be inferred. However, it is evident that a relationship between these two factors exists and further study in this area is needed. An additional confounder arises from the fact that the HRV data was extracted from a 5 minute ECG reading. Ideally, a 24 hour ECG would provide greater accuracy in determining the patient's level of heart rate variability due to the natural physiological variation of the heart. The use of negative chronotropic drugs such as β-blockers may influence HRV. Participants were asked to refrain at least 12 hours prior to testing, however some residual medication may still be in effect. However, the normal F2-isoprostanes group were actually the ones with greater β-blocker use (which would lower HRV), so it does not appear that the difference in β-blocker medication between the groups would be influencing the current findings.

Conclusion

In summary, CKD patients with elevated plasma F2-isoprostanes also display reduced HRV parameters. It was also identified that F2-isoprostanes independently predicted SDNN, a global measure of HRV. These findings are clinically significant, as by identifying oxidative stress as an associate with autonomic dysfunction, appropriate therapeutic targets may be able to be investigated with the view of restoring normal autonomic function.

Table 1 Time and frequency HRV parameter descriptions

Frequency domain HRV measures		Unit	Description
LF	Low frequency power (0.04 - 0.15Hz)	ms^2	Correlated to both parasympathetic and sympathetic modulations.
HF	High frequency power (0.15 - 0.4Hz)	ms^2	Indicator of parasympathetic modulations.
LF/HF	Ratio of LF/HF	No units	Indicator of sympathovagal balance.
TP	Total Power	ms^2	A global measure of HRV. Calculated from the addition of VLF, LF and HF
Time domain measures of HRV		Unit	Description
pNN50	Ratio between the number of pairs of adjacent NN intervals differing by more than 50ms and the total number of NN intervals	%	Reflects high frequency heart rate variations due to parasympathetic modulation.
SDNN	Standard deviation of all NN intervals	ms	Indicator of overall HRV
RMSSD	Square root of the mean of the squares of successive R-R interval differences	ms	Reflects high frequency heart rate variations due to parasympathetic modulation. Indicates short-term HRV components.

Table 2 Characteristics, medications, heart rate variability parameters and biochemical measures of CKD patients.

Variable	**Normal plasma F2-isoprostanes (n=62)**	**Elevated plasma F2-isoprostanes (n=16)**	**p value**
Demographics			
Age (years) (M-W U)	59.8 ± 10.2	56.6 ± 11.8	0.364
BMI (kg/m^2)	33.0 ± 6.5	34.0 ± 6.9	0.604
Diabetes Status (%)	26 (41.9%)	5 (31.3%)	0.569
eGFR (mL/min/1.73m^2)	41.1 ± 9.7	40.9 ± 8.1	0.952
eGFR Rate of Change	0.05 ± 0.5	-0.1 ± 0.1	0.415
Medications			
β-Blockers (%)	23 (29.9%)	2 (2.6%)	0.055
Angiotensin Converting Enzyme Inhibitor (%)	31 (40.3%)	5 (6.5%)	0.163
Statins (%)	61 (79.2%)	16 (20.8%)	0.535
Blood biochemistry			
Total Cholesterol (mmol/L) (M-W U)	4.2 ± 1.0	5.3 ± 1.2	**0.014**
Triglyceride (mmol/L)	1.7 ± 0.8	2.8 ± 3.1	0.03
HDL cholesterol (mmol/L) (M-W U)	1.1 ± 0.4	1.6 ± 0.9	**0.007**
LDL cholesterol (mmol/L)	2.6 ± 0.9	2.6 ± 0.9	0.943
Glutathione Peroxidase (GPx) (U/L)	24.6 ± 4.0	23.4 ± 4.8	0.216
Total antioxidant capacity (mmol/L)	1.7 ± 0.2	1.8 ± 0.2	0.635
Albumin (g/L) (M-W U)	38.0 ± 2.6	37.4 ± 4.2	0.845

Haemoglobin (g/L)	132.5 ± 14.5	130.5 ± 15.5	0.628
Phosphate **(**mmol/L)	1.1 ± 0.2	1.1 ± 0.2	0.733
Magnesium (mmol/L) (M-W U)	0.8 ± 0.1	0.9 ± 0.1	0.500
HOMA (M-W U)	8.7 ± 21.1	7.7 ± 11.3	0.537
HaemoglobinA1c (%) (M-W U)	6.5 ± 1.4	6.2 ± 0.5	0.590
Interferon-γ (ng/mL)	1.3 ± 2.8	0.7 ± 0.5	0.528
Tumour Necrosis Factor α (M-W U) (ng/mL)	6.7 ± 3.4	7.2 ± 3.8	0.520
C-Reactive Protein (mg/L)	3.8 ± 3.6	5.2 ± 3.8	0.481
Heart Rate Variability			
Mean RR (ms)	895.0 ± 167.9	837.5 ± 114.8	0.2
Mean HR (1/min)	69.5 ± 13.4	73.0 ± 10.4	0.334
STD HR (1/min)	2.2 ± 0.8	1.8 ± 0.7	0.102
SDNN (ms^2)	29.3 ± 13.2	20.9 ± 8.8	**0.018**
RMSSD (ms^2)	18.9 ± 10.7	15.1 ± 8.3	0.190
Very Low Frequency (VLF) (ms^2)	530.8 ± 656.7	212.4 ± 159.3	**0.039**
Low Frequency (LF) (ms^2)	318.7 ± 580.9	106.4 ± 128.3	**0.037**
High Frequency (HF) (ms^2)	405.4 ± 1556.1	108.4 ± 105.3	0.400
Total Power (TP) (ms^2)	1254.8 ± 2588.7	427.2 ± 289.6	0.085
LF:HF (ms^2)	2.5 ± 2.5	1.8 ± 1.5	0.099
NN50 (count) (M-W U)	10.8 ± 20.7	4.2 ± 7.0	0.138
pNN50 (count) (M-W U)	3.5 ± 7.3	1.2 ± 2.0	0.125

(M-W-U) = Mann-Whitney-U test; CCB= calcium channel blocker. Values are reported as mean ± SD for normally distributed variables, or median (IQR) for not normally distributed or log transformed variables.

Table 3 Bivariate relationships between plasma F2-isoprostanes and HRV parameters.

	F2-isoprostanes	
HRV Parameter	r Value	*p* Value
Mean RR (ms)	-0.200	0.079
Mean HR (1/min)	0.178	0.119
STD HR (1/min)	**-0.248**	**0.029**
SDNN (ms^2)	**-0.337**	**0.003**
RMSSD (ms^2)	-0.198	0.083
Very Low Frequency (ms^2)	**-0.281**	**0.013**
Low Frequency (ms^2)	**-0.315**	**0.005**
High Frequency (ms^2)	-0.190	0.095
Total Power (ms^2)	**-0.288**	**0.01**
LF/HF (ms^2)	-0.156	0.173

(S) = Spearmans Test

Table 4 Heart rate variability parameters of CKD patients *not* on β-blockers.

Heart rate variability variables	**Normal F2-isoprostanes (n=39)**	**Elevated F2-isoprostanes (n=14)**	***p* value**
Mean RR (ms)	876.6 ± 148.6	846.4 ± 100.9	0.487
Mean HR (1/min)	69.4 (14.9)	74.2 (15.2)	0.611
STD HR (1/min)	2.2 ± 0.9	1.7 ± 0.7	**0.042**
SDNN (ms^2)	29.5 ± 14.4	19.8 ± 6.9	**0.019**
RMSSD(ms^2)	17.9 (14.3)	13.8 (15.3)	0. 399
Very Low Frequency (VLF) (ms^2) (M-W-U)	266.9 (657.9)	152.0 (202.2)	0.083
Low Frequency (LF) (ms^2) (M-W-U)	179.2 (277.8)	47.9 (82.2)	**0.009**
High Frequency (HF) (ms^2)	91.7 (189.9)	70.1 (163.2)	0.408
Total Power (TP) (ms^2)	759.7 (1179.4)	387.0 (431.6)	0.096
LF:HF(ms^2)	1.7 (2.4)	1.5 (2.2)	**0.05**
NN50 (count)	4.0 (16.3)	0.0 (8.0)	0.607
pNN50 (count)	1.2 (4.8)	0.0 (2.2)	0.503

(M-W-U) = Mann-Whitney-U test. Values are reported as mean ± SD for normally distributed variables, or median (IQR) for not normally distributed or log transformed variables.

Table 5 Bivariate relationships between plasmaF2-isoprostanes and HRV parameters on CKD patients *not* on β-blockers.

	F2-isoprostanes	
HRV Parameter	r Value	*p* Value
Mean RR (ms)	-0.203	0.149
Mean HR (1/min)	0.195	0.166
STD HR (1/min)	**-0.363**	**0.008**
SDNN (ms^2)	**-0.416**	**0.002**
RMSSD (ms^2)	-0.254	0.07
Very Low Frequency (ms^2) $_{(S)}$	**-0.282**	**0.043**
Low Frequency (ms^2) $_{(S)}$	**-0.351**	**0.011**
High Frequency (ms^2)	-0.261	0.062
Total Power (ms^2)	**-0.333**	**0.016**
LF/HF (ms^2)	-0.141	0.32

(S)= Spearman's Test

Table 6 Bivariate and multivariate associations with SDNN

	SDNN (ms^2)			
Variable	r Value	*p* Value	β	*p* Value
Age (years) (S)	-0.205	0.076		
Sex (S)	**-0.366**	**0.001**	**-0.263**	**0.014**
BMI (kg/m^2)	0.044	0.710		
Diabetes Status (S)	**-0.344**	**0.002**	-0.228	0.064
eGFR ($mL/min/1.73m^2$)	0.131	0.257		
Blood Biochemistry				
Glutathione Peroxidase (U/L)	0.050	0.666		
Total Antioxidant Capacity (mmol/L)	0.112	0.332		
Plasma F2-isoprostanes (pg/mL)	**-0.337**	**0.003**	**-0.272**	**0.010**
Albumin (g/L) (S)	0.165	0.151		
Phosphate (mmol/L)	0.145	0.210		
HOMA-IR (S)	0.226	0.053		
HbA1c (%) (S)	**-0.417**	**0.000**	-0.145	0.239
C Reactive protein (mg/L)	0.104	0.372		
Medications				
β-Blockers (%) (S)	0.074	0.525		
Angiotensin Converting Enzyme Inhibitor (%) (S)	-0.027	0.816		
Statins (%) (S)	0.200	0.081		

(S)= Spearman's Test

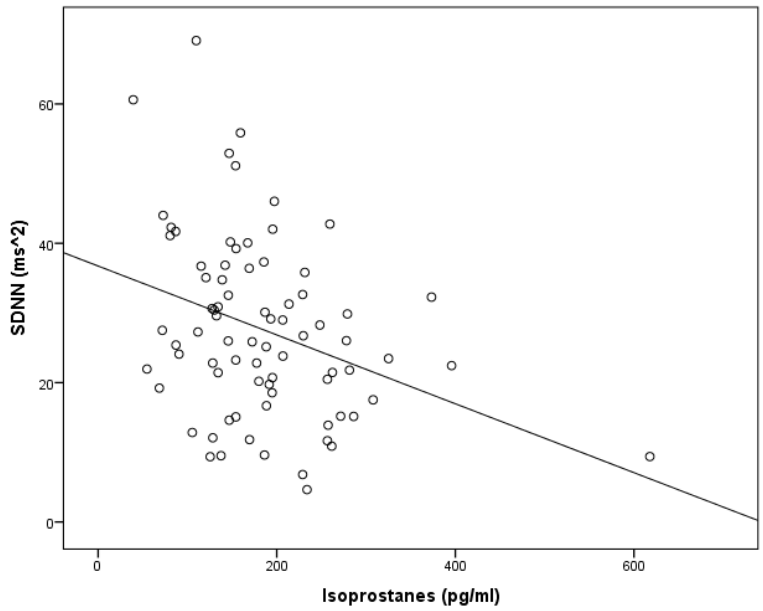

Figure 1. Relationship between SDNN and plasma F2-isoprostanes in patients with CKD (r=0.337, *p*= 0.003).

11.9 Changes in cardiorespiratory fitness are associated with changes in autonomic function in patients with chronic kidney disease

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ABSTRACT

Purpose- To investigate the effects of a 12-month lifestyle intervention (LI) on autonomic nervous system function, as indicated by heart rate variability, heart rate recovery, and heart rate reserve.

Methods- 120 patients (54 females) with stage 3 or 4 chronic kidney disease participated in the study. Exercise training consisted of an 8-week individualized training program supervised by a clinical exercise physiologist for 2-3x/week. Patients were then provided with a swiss ball, a theraband, and given a home program to complete for the remaining 8 months. Patients received regular telephone follow-up calls and gym refresher sessions. Cardiorespiratory fitness (VO_2peak) was determined from a graded exercise stress test and indirect breath-by-breath calorimetry. Heart rate variability (standard deviation of NN intervals [SDNN], root mean square of successive differences [RMSSD], very low frequency [VLF], low frequency [LF], high frequency [HF], and total power [TP]) were measured for 5 minutes in the supine position in a quiet temperature controlled environment. Heart rate at one, two, and three minutes following peak exercise was subtracted from peak heart rate, measured in a supine position, to determine the heart rate recovery. Chronotropic competence was derived using a percent heart rate reserve formula from the Framingham Heart Study.

Results- There were significant ($p<0.05$) improvements in the LI group from baseline to 12 months compared to changes in the control (CON) group with absolute VO_{2peak} (LI +0.1±0.3, CON -0.1±0.3 L/min), relative VO_{2peak} (LI +1.8±3.8, CON -1.0±3.9 ml/kg/min), resting DBP (LI -6.0±15.5, CON 0.5±14.1 mmHg), and LF power (LI 214.6±723.9, CON -108.2±506.9). There were no significant improvements in any other autonomic function variables in both the LI and CON group. However, when both groups were combined, the change in relative VO_{2peak} was significantly associated with the change in heart rate recovery at one minute ($r=0.287$, $p=0.01$) and two minutes ($r=0.218$, $p=0.05$), and high frequency heart rate variability ($r=0.288$, $p=0.05$).

Conclusion- Changes in cardiorespiratory fitness are associated with an improvement in autonomic function.

Chapter 11 Appendices

Key words: autonomic dysfunction, heart rate variability, heart rate recovery, heart rate reserve, exercise, chronic kidney disease

INTRODUCTION

Compared to a healthy adult population, the risk of cardiovascular disease (CVD) is remarkably greater in patients with chronic kidney disease (CKD), where the likelihood of experiencing cardiovascular mortality is much higher than reaching end-stage kidney failure (Tonelli et al, 2006; Gansevoort et al, 2012). Since CVD is the primary cause of death at every stage of kidney disease, it is important that the CKD population is regarded as one of the highest risk groups for the disease (Tonelli et al, 2006).

This study focuses on autonomic nervous system (ANS) dysfunction, a significant contributor to the increased risk of CVD (Bronas, 2009). ANS dysfunction can be evaluated by a variety of measures including resting heart rate variability (HRV), heart rate reserve (HR reserve) during exercise, and heart rate recovery (HRR) after exercise. HRV is the interaction of the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) to regulate heartbeat. HRR is measured as the number of decreased heart beats per each minute following peak exercise. HR reserve is a measure of chronotropic competence with patients deemed as having a chronotropic incompetent response if unable to utilize >80% of their HR reserve (Lauer, 2004). Patients with chronotropic incompetence are incapable of sufficiently increasing their HR to correspond with the increase in exercise demands (Brubaker et al, 2011). The aforementioned autonomic function parameters are predictors for all-cause and cardiovascular mortality with low levels reflecting an increased risk of mortality in a variety of clinical populations (Lauer et al, 1999; Chacko et al, 2008; Georgoulias et al, 2009).

Despite no studies investigating the impact of lifestyle interventions on autonomic function in patients with CKD, patients undergoing dialysis have shown improvements in HRV parameters as a result of two hours of intra-dialysis exercise training on three days a week in a one year intervention program (Kouidi et al, 2010). HRR also improved in response to a one-year lifestyle intervention that included regular exercise in patients with type 2 diabetes mellitus (T2DM) (Ribisl et al, 2012). Home-based exercise of a moderate intensity over a 6-month period has also shown improvements in HRR in patients with coronary artery disease (Neves et al, 2011), while partial reversal of chronotropic incompetence was identified in patients with stable heart failure after a 30-minute exercise intervention that occurred three times a week for 24 weeks (Keteyian et al, 1999).

Though there is ample evidence illustrating improvements in autonomic function as a result of lifestyle changes and exercise training in other clinical and diseased populations, there are no indications to suggest whether the same benefits can occur in patients with stage 1 to 4 CKD. The aim of this study is therefore to investigate the impact of a 12 month LI on parameters of autonomic function, specifically HRV, HRR, HR reserve and chronotropic response in patients with stage 3 or 4 CKD. It is hypothesized that HRV, HRR, HR reserve and chronotropic response will improve as a result of the LI. Furthermore, it is hypothesized that changes in cardiorespiratory fitness (VO_2peak) will be associated with changes in autonomic function parameters.

METHODS

Setting & Participants

The data from this study originates from the prospective randomized controlled trial 'LANDMARK 3' (Longitudinal Assessment of Multiple Discrete Atherosclerotic Risk Factors in Kidney Disease). Patients were recruited from the chronic kidney disease population attending the Department of Nephrology, Princess Alexandra Hospital in Brisbane, Australia. Patients aged 18 to 75 with CKD stages 3 and 4 (eGFR: 25-60 ml/min/1.73m^2) were invited to participate in the study, with the exclusion criteria containing patients that i) were pregnant or breast feeding, ii) had a life expectancy of 6 months or less, iii) a current involvement in other research studies, or iv) was or expecting to be a recipient of an organ transplant.

The study protocol was approved by the Princess Alexandra Human Research Ethics Committee (HREC 2007/190), and the University of Queensland Medical Research Ethics Committee (MREC 2008000184). The study was registered with the Australian and New Zealand Clinical Trials Registry (ANZCTR; www.anzctr.org.au), Registration Number ACTRN12608000337370.

Study Design

Subsequent to obtaining written informed consent, patients completed a baseline visit that included resting brachial blood pressure measured by mercury sphygmomanometry (BP), anthropometry (weight and height), resting and exercise electrocardiograph (ECG) recordings, cardiorespiratory fitness and exercise capacity (VO_{2peak} and METS) measures. Patients were randomized to the lifestyle intervention group or standard care control group. The standard care control group received treatment as per normal according to their nephrologist. After 12 months of receiving either the lifestyle intervention or standard care, patients completed a full series of tests identical to that of the baseline visit.

Lifestyle Intervention

Patients in the LI group received 8 weeks of individualized exercise training under supervision. In the initial 8 weeks, patients were required to attend an hour-long exercise session 2 to 3 times per week. The sessions were individualized based on each patient's needs and comorbidities. However, the majority of sessions consisted of 20-30 minutes of aerobic exercise, 20 minutes of resistance training, and a 10 minute cool-down period. Throughout the 8 weeks of supervised training sessions, the aim was to develop confidence in performing exercises and teach the patient an array of exercises that could be safely performed at home. Exercise progression was tailored accordingly. Subsequent to the 8 weeks, patients were provided with a swiss-ball, therabands, and an exercise handbook to encourage independent home exercises. The aim was to complete at least 150 minutes of moderate to high intensity aerobic exercise and two resistance training sessions per week, as appropriate. Patients were then regularly contacted via telephone; weekly in the first month, then monthly afterwards. Patients were also provided with the opportunity to attend refresher supervised exercise sessions as needed. In the initial 4 weeks of the LI, patients also attended weekly dietician and psychologist group sessions. Regular contact, as well as quarterly appointments, with the nurse practitioner and dietician occurred for the remainder of the intervention.

Outcome Measures

Cardiorespiratory Fitness and Exercise Capacity

Participants completed a standardized maximal treadmill exercise stress test (protocol: Bruce, Naughton, Balke or modified Balke). Before, during, and for five minutes after the exercise stress test, a 12-lead ECG was recorded (CASE, GE Medical Systems, Milwaukee, WI, USA). Cardiorespiratory fitness, measured as peak oxygen uptake (VO_{2peak}) was determined by breath-by-breath indirect calorimetry (Vmax29c, Sensor Medics, CA, USA) during the exercise stress test. Exercise capacity was calculated as metabolic equivalents (METs) based on the grade and speed of the treadmill at the termination of the test.

Heart Rate Variability (HRV)

Participants completed a supine 5 minute resting ECG recording before the maximal exercise stress test. Three Ag/AgCl electrodes were used in a modified lead II configuration connected to an analysis program (SpyhmoCor 8.1, AtCor Medical, Sydney, Australia). The R-R interval tachogram was analyzed in Kubios (2.1, Kuopio, 2012) by which ectopic beats were removed using linear interpolations of the previous and subsequent beats. HRV was assessed by time and frequency domain parameters. For the time domain, consecutive R-R intervals were measured against the

number of total beats. The standard deviation of N-N intervals (SDNN) is used as the global measure of HRV. The root-mean square of the successive differences (RMSSD) of the R-R intervals is also calculated. For the frequency domain, HRV was divided by spectral power analysis into high frequency (0.15-0.40 Hz) (HF), low frequency (0.04-0.15 Hz) (LF), and very low frequency (0.0033–0.04 Hz) (VLF) powers. Total power (TP) was also calculated as a sum of all frequency powers.

Heart Rate Recovery (HRR)

HR was measured immediately after the termination of the exercise stress test at one (HRR-1), two (HRR-2), and three (HRR-3) minutes into the cool-down period. HR at each of these time-frames was subtracted from peak HR of the exercise stress test. HRR-1, HRR-2, and HRR-3 are used to describe the overall HRR at those respective time points.

Heart Rate Reserve (HR reserve)

Chronotropic competence was determined by calculating the percent HR reserve (%HR reserve). The following equation used in this study was taken from the Framingham Heart Study (Lauer et al, 1996) with the recommendation that patients are deemed chronotropic incompetent with a %HR reserve of <80:

$$\%\,\mathrm{HR} - \mathrm{Reserve} = \frac{(\mathrm{HR\ stage} - \mathrm{resting\ HR})}{(\mathrm{APMHR} - \mathrm{resting\ HR})} x\ 100$$

'HR stage' refers to the HR corresponding to the end of the exercise stress test stage. In this study, stage 2 is used to calculate %HR reserve, meaning that patients must have at least completed two stages on the exercise stress test. APMHR was calculated as 208 - (0.7 x age).

Statistical Analyses

The Kolmogorov-Smirnov test was used to determine normality of distribution for all variables. Non normally distributed variables [control: METs, HR reserve, HF(%), VLF(ms^2), LF(ms^2), HF(ms^2), TP(ms^2), HF(n.u.), LF/HF ratio; intervention: VO_2(L/min), VO_2(ml/kg/min), peak HR 60s, peak HR 180s, SDNN, HF(%), VLF(ms^2), LF(ms^2), TP(ms^2)] were transformed using the natural logarithm [X=log(X)]. Paired t-tests were used to determine within group differences between baseline and 12 month values. Non-parametric Wilcoxon signed-rank test was used for not normally distributed variables that were not able to be transformed. Independent samples t-test was used to identify significant differences between groups at baselines as well as group differences of the change from baseline to 12 months. Spearmans correlation test was used to determine univariate correlations between autonomic function and exercise fitness. Pearsons chi square test was used to

determine the difference between groups for categorical variables at baseline. Mean ± standard deviation (SD) was used to describe baseline characteristics and measured outcome variables. Median and interquartile range described variables that were not normally distributed. Statistical analysis was performe d using GraphPad Prism 6 (La Jolla, CA, USA). Statistical significance was set at $p<0.05$.

RESULTS

120 patients that met the inclusion criteria were included in the analysis. The clinical characteristics at baseline are summarized in Table 1. Patients had a mean age of 62 years and were obese (median[interquartile range]) (31.6[7.6]). There were no statistical differences between the control and intervention group in any of the baseline variables.

Baseline vs. 12 Months

Baseline and the change scores to 12 months for the control and intervention group on all measured variables are summarized in Table 2. The control group showed significant decreases for absolute VO_2 ($p=0.04$), peak HR ($p=0.01$), peak SBP ($p=0.002$) and DBP ($p=0.003$) at the end of the 12 months. The intervention group showed significant improvements in a number of exercise parameters, specifically increasing absolute VO_2 ($p=0.03$), relative VO_2 ($p=0.003$), METs ($p<0.001$), and decreasing resting HR ($p=0.04$). Resting SBP ($p=0.05$), resting DBP ($p=0.01$), and peak DBP ($p=0.03$) additionally decreased in the intervention group. HR reserve decreased by a mean of 12.2% in the intervention group ($p=0.02$). No other significant differences were seen for any other autonomic function parameters in both the control and intervention groups from baseline to 12 months.

Control vs. Intervention

The change scores from baseline to 12 months between the control and intervention group are summarized in Table 2. The findings illustrate that the control and intervention group differed in delta absolute VO_2 ($p=0.003$), relative VO_2 ($p=<0.001$), METs ($p<0.001$), resting DBP ($p=0.03$), and LF (ms^2) power ($p=0.05$), with the intervention group improving as a result of the LI. Additionally, no changes in other autonomic function parameters were statistically different between the two groups.

Autonomic Function and Exercise Parameters

The association between the change from baseline to 12 month values in HR variability, HR recovery, and HR-reserve variables to the change in relative VO_2 for all patients are represented in Table 4. Specifically, HF (ms^2) power showed a significant association with cardiorespiratory fitness (r=0. 0.288, p=0.05). In addition, both HR recovery at 1 and 2 minutes showed a significant association as well (r=0.287, p=<0.01) (r=0.218, p=0.05). No associations were seen between change in HR reserve and change in relative VO_2.

DISCUSSION

This is the first study to investigate the impact of a 12 month LI on autonomic function through measurement of HRV, HRR, HR-reserve and chronotropic response in patients with stage 3-4 CKD. The findings illustrate that despite the increase in cardiorespiratory fitness and exercise capacity, the LI produced no beneficial changes in autonomic function measures outside of LF power. Nonetheless, there was a marked association between the change from baseline to 12 months in HF power, HRR at 1 minute, HRR at 2 minutes and the change in cardiorespiratory fitness for all patients.

The literature on the effect of exercise on autonomic function in the CKD population is scarce, yet other studies have demonstrated similar findings to the current study in other healthy and clinical populations. The following studies support the present findings in which no or little changes were seen in any of the time or frequency HRV domains. Tuomainen et al. (2005) studied the effects of a six-year exercise intervention in a population of healthy males. The results suggest no changes in HRV, despite the 16% increase in ventilatory aerobic threshold. Change in certain HRV parameters, specifically SDNN, TP, VLF, and HF, were nevertheless significantly correlated to the change in VO_2max in all participants. Oya et al. (1999) found no significant difference in HRV parameters in post myocardial infarction (MI) patients after 3 months of exercise training. Despite little improvements seen in the early phases of acute MI, these changes were not significant at the end of the intervention. On the other hand, Figueroa et al. (2007) showed significant increases in LF and HF power after a 16 week exercise intervention in obese women with and without T2DM. Kouidi et al. (2010) further consolidated these results in patients undergoing hemodialysis showing marked improvements in SDNN and LF/HF ratio after one year of regular cycling and strength exercise training. In the present study the mean age of patients is 62 years, a factor that could attenuate the impact of exercise on HRV parameters. This is supported by previous literature that highlights

additional decreases in short-term supine HRV measurements and overall autonomic function in elderly patients, suggesting a reduction in parasympathetic tone (Stein et al, 1999; Fukusaki et al, 2000; Richard et al, 2011). Although a low HRV has been linked with an increased risk of all-cause mortality, there are no accepted normative values for comparison. There is consequently little insight into the varying degrees of HRV measures and its clinical impact in the CKD population.

To adjust to the demands of exercise, the ANS activates the SNS and withdraws the PNS to cause an increase in HR throughout the exercise duration (Pierpont, 2013; Esco, 2010). Contrariwise, a decrease in HR following exercise is characterized by the reactivation of the PNS independent of withdrawal of the SNS (Carter et al, 2003; Imai et al, 1994). The intervention group showed an increase in HRR at 3 minutes of 2.9 bpm compared to the control group that showed no improvement, although these findings are not statistically significant. This trend is supported by previous findings that also portray increases in HRR at 3 minutes following exercise (Neves, 2011; MacMillan, 2006). Neves (2011) illustrated that a home-based exercise program of moderate intensity improved HRR in diabetic patients with CAD over a duration of 6 months. MacMillan (2006) also showed an increase in HRR by 13.5% in males and 14.3% in females after exercising three times a week for a period of 12 weeks. Patients in this study in both the control and intervention group showed normal responses to HRR at baseline as indicated by a decrease of more than 18 bpm at one minute following peak exercise (Watanabe et al, 2001). Therefore, the expectations for already established normal values to further improve during an intervention may not be of any significance. The control group showed a slight, although statistically insignificant, decline. It is thus perhaps more important to note that no significant decreases in HRR was seen in the intervention group.

The impact of exercise on HR reserve and chronotropic response is a topic rarely explored in the clinical population. The findings of this study demonstrate a marked and significant reduction of 12.2% in HR reserve in the intervention group. Although the controls also showed a similar decrease in HR reserve by 10.1%, this was not statistically significant. Keteyian (1999) studied the impact of 100 minutes of exercise per week for 24 weeks on HR reserve in patients with stable heart failure. The results show an increase in both peak HR and HR reserve, signifying a reversal of chronotropic incompetence, with a significant association between the change in peak HR and change in HR reserve. The correlation suggests that an increase in HR reserve parallels an increase in peak HR. The present study demonstrated no significant increases in peak HR in the intervention group, which may explain the lack of improvement in HR reserve. It is also important to note that both groups had a chronotropic incompetent response at baseline.

Compared to the control group, the intervention group had higher absolute VO_2peak, relative VO_2peak, METs, a lower resting HR, resting SBP, resting DBP, and peak DBP. This is consistent with previous research assessing the impact of exercise training in patients with renal dysfunction (Clyne, 2004; Deligiannis, 1999; Suh et al, 2002, Violan et al, 2002; Kouidi et al, 2010; Koufaki et al, 2002). Unlike the intervention group, the control group showed significant reductions in peak HR and peak SBP. It is likely that the significant decrease in cardiorespiratory fitness over the 12 months in the control group attenuated the exercise capacity and therefore affected the intensity reached during the exercise stress test from baseline to 12 months, thereby explaining the lower values.

Even though no advances were seen in the autonomic function variables measured outside of LF power, the change in HRR at one minute, HRR at two minutes, and HF power from baseline to 12 months showed a significant association with the change in relative VO_2. These relationships suggest that a long-term exercise intervention has the potential to improve autonomic function. Nevertheless, the impact of exercise on autonomic function may vary with different exercise intensities. Martinmaki (2008) demonstrated that high intensity endurance training was more effective in improving cardiac vagal tone compared to low intensity endurance training. Trapp (2007) similarly reported a greater impact on the ANS in high intensity training compared to steady state aerobic exercise. More specifically, high intensity or interval exercise interventions ranging from 12 to 24 weeks have been shown to improve HRV, HRR, and HR reserve in the elderly patients, patients on beta-blockers, patients with T2DM, and patients undergoing coronary intervention (Malfatto et al, 1998; Pichot et al, 2005; Munk et al, 2010; Heydari et al, 2012). The aforementioned studies reported increases in maximal oxygen uptake ranging from 16% to 46%. The intervention group in this study only showed an increase of 8.0%. The large discrepancy of maximal oxygen uptake between the present study and the discussed literature alludes to a probable reason for the non-significant or little changes found in autonomic function parameters. For autonomic function to markedly improve, it is possible that a large change in cardiorespiratory fitness, especially for elderly individuals, needs to be seen. Future research should investigate the effects of high intensity exercise training on cardiorespiratory fitness and autonomic function in patients with CKD.

It is possible that for improvements in autonomic function to occur, an intensive lifestyle program that puts a primary and stronger focus on weight loss in addition to improving cardiorespiratory fitness is needed. Ribisl (2012) studied the combined effects of diet and exercise in patients with

T2DM, finding that weight loss and fitness gains resulted in a greater improvement in HRR than a diabetes support education program after 12 months. The findings also suggest that greater weight loss is associated with greater increases in HRR. Brinkworth (2006), on the other hand, showed an increase in HRR in overweight males with metabolic syndrome in response to a weight loss program alone. Exercise was kept within baseline levels, evident by the lack of significant changes in peak HR, physical activity levels, and cardiorespiratory fitness. This attributes the beneficial change in HRR purely to weight loss. The intervention group in the present study exhibited no signs of significant weight loss, as measured by body mass, from baseline to 12 months. Since the average patient is classified as obese, significant weight loss may augment the effect of exercise on autonomic function.

Limitations

Despite measuring a number of time and domain frequencies to infer HRV, additional measures taken during orthostatic posture and following peak exercise may have provided further insight into the impact of exercise on autonomic function in different situational demands. Furthermore, the lack of a reference of normative values for the assessment of autonomic dysfunction represents an important limitation.

Conclusion

The 12 month LI produced no significant changes in HRV, HRR, HR reserve, and chronotropic response in patients with stages 3 or 4 CKD, outside of the improvement in LF power. The change in cardiorespiratory fitness is, however, associated with HF power and HRR at one and two minutes. Future research is needed to explore the clinical implications and the impact of different forms of exercise as a therapeutic intervention on autonomic dysfunction in pre-dialysis CKD patients in hopes of reducing the risk of cardiovascular mortality.

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CONFLICT OF INTEREST

There are no conflicts of interests.

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Table 1. Clinical characteristics of 120 stage 3-4 CKD patients

Variable	**All** (n=120)	**Control** (n=59)	**Intervention** (n=61)
Age (years)	62[13]	63.5[9]	60[15.2]
Female sex (%)	54(45)	26(44)	28(46)
Diabetes (%)	46(38)	24(41)	22(36)
eGFR (ml/min/1.73m^2)	39[13]	40[16]	39[12]
Weight (kg)	89.9±22.0	89.8±23.2	90.7±21.0
Body mass index (kg/m^2)	31.5[7.5]	30.9[5.4]	32.8[8.4]
Fat (%)	35.6±7.7	35.4±6.8	35.8±8.4
Appendicular lean mass (%)	60.6±9.4	60.2±8.9	61±10
LDL (mg/dL)	2.5[1.0]	2.3[1.3]	2.6[1]
HDL (mg/dL)	1.1[0.4]	1.1[0.4]	1.1[0.5]
Total cholesterol (mg/dL)	4.4[1.2]	4.3[1.5]	4.5[1.2]
Triglycerides (mg/dL)	1.5[1.1]	1.5[1.6]	1.4[1.1]
ACE inhibitors (n, %)	56, 46.7	31, 52.5	25, 41
α-blockers (n, %)	9, 7.5	4, 6.8	5, 8.2
α2-blockers (n, %)	57, 47.5	25, 42.4	32, 52.5
B-blockers (n, %)	43, 35.8	28, 47.5	15, 25.0
Calcium channel blockers (n, %)	50, 41.7	28, 47,5	22, 36.1
Centrally acting anti-adrenergics (n, %)	2, 1.7	1, 1.7	1, 1.6
Thiazide (n, %)	23, 19.2	14, 23.7	9, 14.8
Spironolactone (n, %)	2, 1.7	2, 3.4	0, 0
Loop diuretics (n, %)	21, 17.5	12, 20.3	9, 14.8
Nitrovasodilators (n, %)	10, 8.3	7, 11.9	3, 4.9
Statins (n, %)	70, 58.3	34, 57.6	36, 59.0

Mean ± standard deviation given for normally distributed variables, median [interquartile range] given for not normally distributed variables. Number (%) given for categorical variables. eGFR = estimate glomerular filtration rate. LDL = low density lipoproteins. HDL = high density lipoproteins. ACE = angiotensin converting enzyme.

Table 2. Differences between baseline, 12 month, control and intervention values

Variable	**Control**		**Intervention**		
Characteristics	**Pre**	**Change**	**Pre**	**Change**	**P-value**
Weight (kg)	91.5±15.0	-3.6±15.4	91.8±16.9	-3.7±20.2	0.1603
Body mass index (kg/m^2)	32.0±4.4	0.6±5.1	32.6±5.6	0.1±7.6	0.4956
Exercise Parameters					
VO_2peak (L/min)	2.1±0.6	-1.0±0.3*	2.0±0.5	0.1±0.3*	**0.0028***
VO_2peak (ml/kg/min)	22.7±5.2	-1.0±3.9	22.5±5.6	1.8±3.8*	**0.0007***
RQ	1.0[0.13]	0.01±0.3	1.0±0.1	0.07±0.3	0.2926
METs	7.3±2.6	-0.1±1.9	7.5±3.6	1.9±1.8*	**0.0001***
Resting heart rate (bpm)	78.3±12.1	-3.0±16.4	82.6±13.8	-4.2±13.9*	0.6887
Peak heart rate (bpm)	144±17.1	-5.3±14.9*	150±25.6	1.2±21.9	0.0701
Haemodynamics					
Rest systolic blood pressure (mm/Hg)	130[26]	-0.2±25.3	130[20]	-7.4±23.5*	0.1525
Rest diastolic blood pressure (mm/Hg)	80[12]	0.5±14.1	80[10]	-6.0±15.5*	**0.0322***
Peak systolic blood pressure (mm/Hg)	180±26	-9.8±24.0*	173[21]	-3.6±24.3	0.1834
Peak diastolic blood pressure (mmHg)	80[20]	-4.1±12.0*	82[15]	-4.2±13.3*	0.9759
Heart rate variability					
SDNN (ms)	25.3±12.4	1.3±11.0	29.7±14.3	5.0±26.1	0.4702
RMSSD (ms^2)	13.4[14.6]	0.8±10.0	19.1[13.3]	5.3±22.5	0.3293
TP (ms^2)	976±2607	-447.2±2553	880±979	625±2713	0.1336
VLF (ms^2)	416±548	57.5±573.3	475±578	219.2±1111	0.4725
LF (ms^2)	240±567	-108.2±506.9	250±295	214.6±723.9	**0.0452***
HF (ms^2)	374±1615	-295.2±1676	124[138.6]	242.9±845.9	0.1111
VLF (%)	53.8±21.5	5.3±28.3	52.7±21	-2.2±22.5	0.2750
LF (%)	24.7[20]	-2.0±19.2	24.8±12.5	1.7±15.0	0.4213
HF (%)	20.5± 17.5	-3.3±21.3	22.5±18.0	0.5±24.5	0.5376
VLF (Hz)	0.004[0.02]	-0.002±0.01	0.008[0.01]	0.001±0.009	0.3126
LF (Hz)	0.06[0.05]	-0.002±0.03	0.07[0.05]	-0.001±0.03	0.8493
HF (Hz)	0.24±0.06	-0.007±0.08	0.24±0.06	0.004±0.09	0.6120
LF (n.u.)	67.1[42.2]	-0.2±26.1	55.9±18.4	1.9±26.4	0.7571
HF (n.u.)	40.5±22.5	0.2±26.1	44.1±18.4	-1.9±26.4	0.7571
LF/HF ratio	2.5±2.2	-0.2±2.7	1.3[1.1]	0.4±0.5	0.3441

Heart rate recovery					
HRR-1 (bpm)	22[13]	-0.5±10.7	22.9±11.3	1.9±19.9	0.4529
HRR-2 (bpm)	41.3±13.5	-0.6±13.2	39[17.3]	1.9±21.1	0.4882
HRR-3 (bpm)	48.4±15.7	-1.5±13.5	45.9±13.9	2.9±22.2	0.2664
Heart rate reserve					
Heart rate reserve (%)	59.6±22.8	-6.0±16.3	60.9±23.9	-7.4±18.6*	0.7178

Mean ± standard deviation given for normally distributed variables, median [interquartile range] given for not normally distributed variables. RQ = respiratory quotient. METs = metabolic equivalents. SDNN = standard deviation of N-N intervals. RMSSD = root mean square of successive differences. TP = total power. VLF = very low frequency. LF = low frequency. HF = high frequency. HRR-1: heart rate recovery at 1 minute peak exercise. * in change column refers to significant within group differences. * in p-value column refers to significant between group differences.

Table 3: Autonomic function variables associated with VO_2 (ml/kg/min)

Variable	Univariate (r)	P-value
Heart rate variability		
SDNN (ms)	0.001	0.995
RMSSD (ms^2)	0.078	0.621
TP (ms^2)	0.080	0.610
VLF (ms^2)	0.120	0.422
LF (ms^2)	0.041	0.782
HF (ms^2)	0.288	**0.050***
VLF (%)	-0.034	0.829
LF (%)	-0.167	0.283
HF (%)	0.193	0.215
VLF (Hz)	-0.112	0.454
LF (Hz)	0.210	0.158
HF (Hz)	0.179	0.228
LF (n.u.)	-0.257	0.081
HF (n.u.)	0.257	0.081
LF/HF ratio	-0.261	0.077
Heart rate recovery		
HRR-1 (bpm)	0.287	**0.009***
HRR-2 (bpm)	0.218	**0.047***
HRR-3 (bpm)	0.147	0.204
Heart rate reserve		
Heart rate reserve (%)	-0.048	0.691

SDNN = standard deviation of N-N intervals. RMSSD = root mean square of successive differences. TP = total power. VLF = very low frequency. LF = low frequency. HF = high frequency. HRR-1: heart rate recovery at 1 minute peak exercise.