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The Effect of Combination Aerobic and Resistance Home Exercise Training on Endothelial Function in Chronic Kidney Disease Stages 3-4: a Randomised Controlled Pilot Study

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Award date:
2023

Awarding institution:
Bangor University

School of Human and Behavioural Sciences, Bangor University

Masters by Research

The Effect of Combination Aerobic and Resistance Home Exercise Training on Endothelial Function in Chronic Kidney Disease Stages 3-4: a Randomised Controlled Pilot Study

By Jenna Crosbie, MBBS iBSc

Thesis submitted to Bangor University in fulfilment of the requirements for the Degree of Masters by Research, September 2023.

Declaration

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy.

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

Acknowledgements

I'm grateful to many people for helping me to complete this work. There are those who encouraged me to start this journey, those who supervised and taught practical skills, and those who gave time and thought-provoking guidance during my write-up. I also want to thank those who kept me grounded, motivated and sea swimming through it all. In particular: Jamie, Aamer, Mark, Jonathon, Bing, Jules, Tess, Zane, Sioned, and Jack. Thank you!

Contents

Abstract

Endothelial dysfunction is a major cause of cardiovascular and renal morbidity and mortality in chronic kidney disease. Exercise poses an attractive intervention for improving endothelial function in this cohort. This study aimed to evaluate whether a six-month exercise intervention could improve physical activity and endothelial function in people living with stage 3 – 4 chronic kidney disease. A randomised controlled pilot study was conducted with 10 CKD patients (60% male; mean age 59 ± 14 years). Five exercisers received a personalised home aerobic and resistance exercise schedule led by exercise instructors with weekly virtual meetings. Five controls received usual care. Data were analysed by repeated measures analysis of variance with a between factor of group and a within factor of time (baseline and six months), with $p<0.1$ considered statistically significant. Questionnaire and accelerometer results showed that the intervention failed to significantly increase physical activity levels from baseline, but significantly decreased daily sedentary time by 30 ± 50 minutes as compared to an increase of 50 ± 36 minutes in the controls ($p=0.062$). There were no significant changes in large vessel or microvascular endothelial function between study groups. This study highlights the challenging nature of delivering and evaluating exercise interventions in CKD patients during the COVID-19 pandemic. It also highlights the importance of collecting and reporting physical activity measures alongside primary outcomes in exercise intervention trials. Future studies in this field should elucidate the optimal intensity, modality, and volume of exercise training to best improve endothelial function in patients living with CKD.

Keywords: CKD; exercise; endothelial dysfunction; flow-mediated dilation; laser doppler imaging; physical activity

Structure/approach of thesis

This study developed as part of a masters degree during an academic component of a medical foundation doctor single-year post, with up to two days per week allocated to academic work, as allowed alongside hospital rotations and shift work. The research presented forms a sub-study looking at specific vessel health and physical activity outcomes from a larger randomised controlled pilot study investigating the feasibility of a 12-month chronic kidney disease exercise scheme. This sub-study was unable to be large or long enough to assess hard cardiovascular outcomes, but was able to fill a current research gap by assessing a surrogate cardiovascular outcome (endothelial dysfunction) over a six-month mixed resistance and aerobic home exercise intervention in pre-dialysis chronic kidney disease patients. The author was personally involved in recruitment calls, data collection at participant study visits, and blinded data analysis of exercise logs. The author analysed all ultrasound scans involved in this study (and the wider pilot study) to generate flow-mediated dilation readings. Finally, the author worked on literature searching, data cleaning, statistical analysis of results, creation of figures and producing this thesis.

The literary chapter of the thesis will form the overall introduction; explaining the global impact of CKD, the need for more research into low-resource earlier-stage interventions, and the key concepts of endothelial dysfunction in the context of CKD and CVD. It will then highlight current knowledge surrounding physical activity, exercise interventions and endothelial dysfunction particularly in CKD, concluding by outlining the aims and hypotheses of the study.

The experimental chapter will include the study methods and results. It will follow the most recent CONSORT guidelines for reporting randomised trials, including specific guidance for social and psychological interventions and reporting exercise interventions in clinical trials (1-3). The author drew from supervisor advice, EQUATOR guidance and further articles to decide how to present data in the results, for example using flow charts, repeated measures plots and displaying statistical uncertainty (4, 5). The formatting will follow the style of a popular nephrology journal, Kidney International.

To conclude, there will be an overall discussion summarising and exploring the study findings and the limitations of the study, followed by an overall conclusion.

Introduction

Chronic kidney disease as a global problem

Chronic kidney disease (CKD) is a state of impaired kidney function, evidenced clinically either by elevated renal protein excretion or by decreased estimated glomerular filtration rate, for at least three months (6, 7). Unlike in acute kidney injury, the damage to the kidney in CKD is irreversible. The damage is also progressive, often insidiously; the kidneys are able to compensate through early stages of impaired renal function, and it commonly takes many years for CKD to become symptomatic. By the end stages of disease progression patients become highly comorbid and require renal replacement therapy (either dialysis or transplant) to survive (6).

CKD now represents a leading global health crisis (7, 8). The disease affects approximately 11-13% of people worldwide and can be caused by a wide range of underlying processes resulting in kidney damage (7, 8). The most common causes of CKD are type two diabetes mellitus (30 to 50% of cases) and hypertension (27%); as such, prevalence has increased rapidly in the context of the global obesity crisis and ageing population (6, 7, 9).

Global Burden of Disease data found that in 2017 the number of individuals with CKD was greater than the number of individuals with chronic obstructive pulmonary disease (COPD), asthma, depressive disorders, osteoarthritis or diabetes ((8). The same data found CKD to be the 12th leading cause of death worldwide in 2017, with the global mortality rate attributed to CKD having risen by 41.5% since 1990 (8). Furthermore, CKD was found to be a leading cause of years of life lost (calculated from the number of deaths attributable to CKD and the life expectancy of individuals in various age groups at the time of their death from CKD).

As shown in Figure 1, CKD is forecasted to rise from the 16th to the 5th highest cause of years of life lost globally between 2016 and 2040; surpassed only by ischaemic heart disease, stroke, lower respiratory infections and COPD (10).

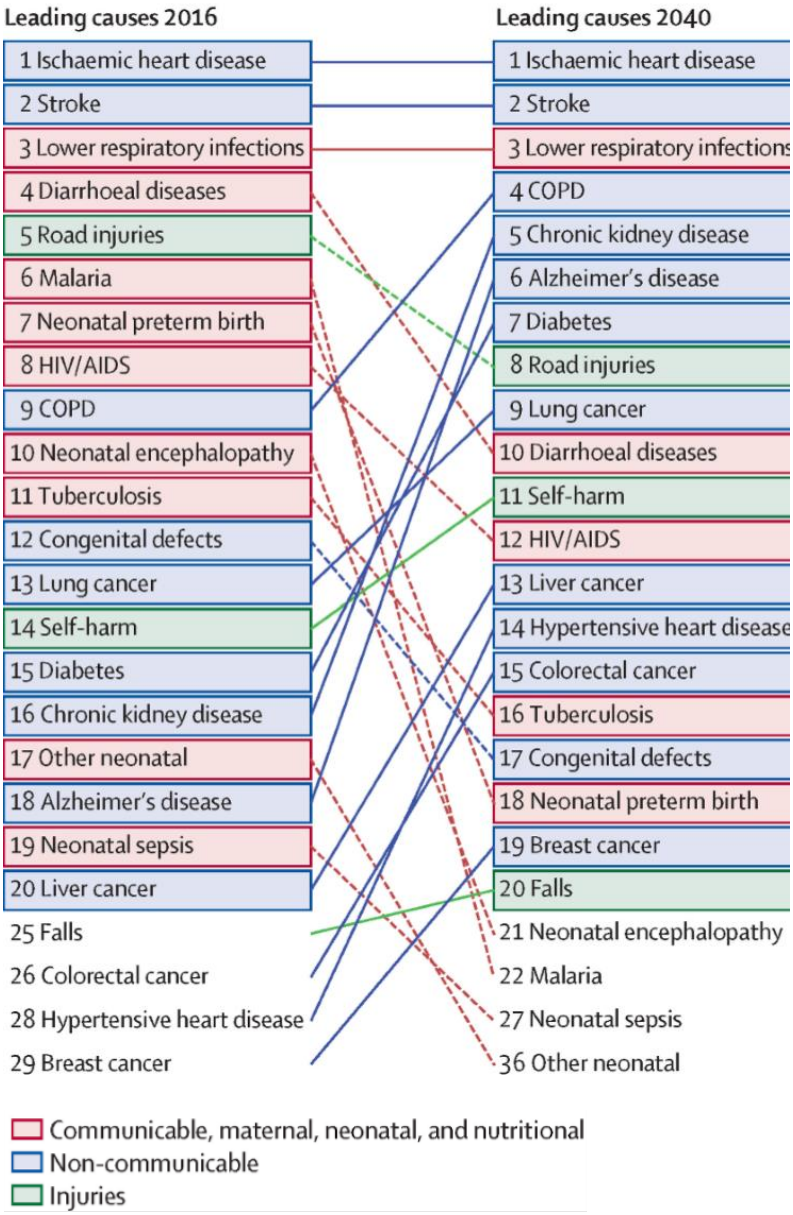

Figure 1 Leading 20 causes of years of life lost globally in 2016 and 2040 by rank order, adapted from Foreman et al (10). COPD = chronic obstructive pulmonary disease. HIV = human immunodeficiency virus. AIDS = acquired immunodeficiency syndrome.

Compared to other leading causes of death, CKD receives relatively little public or healthcare attention worldwide. It can be diagnosed with basic serum and/or urine tests, but estimates suggest that less than 10% of patients with CKD are aware of their disease, both in developing and developed countries (7). The lack of obvious symptoms in the early stages of the condition results in many diagnoses occurring late (11). Resources for CKD globally tend to be directed at providing treatments for end-stage renal failure, most commonly dialysis, which is a high-cost treatment with intensive requirements for staff, consumables, equipment, utilities, transport, and interventional procedures (12). In the UK, the most common regime of three-times-per-week dialysis costs the NHS approximately £30,000 per patient per year (13). Globally, there is a significant shortage of accessible renal replacement therapy, and estimates suggest at least 2.28 million people have already died prematurely as a result (14).

Aside from the morbidity, mortality and expense to healthcare systems for individuals who reach end-stage renal failure, CKD augments an additional large-scale health burden. It is a powerful risk factor for cardiovascular disease (CVD) independent of other conventional cardiovascular risk factors. This risk increases with albuminuria and declining GFR, but still affects those who are asymptomatic with earlier stage CKD (15-17). The most common cause of death in all-stage CKD is not renal failure, but CVD (16). Moreover, death by a cardiovascular event is a more likely outcome in CKD than progressing to end-stage renal failure (6).

Compared to individuals with normal kidney function, cardiovascular mortality is twofold higher in those with stage 3 CKD, and threefold higher in those with stage 4 CKD (9). This corresponds with data demonstrating markedly decreased life expectancies for patients with pre-dialysis CKD. Compared to people with normal kidney function, stage 3B and 4 CKD has been associated with a 17-year and 25-year reduced survival, respectively, with cardiovascular disease the predominant cause of morbidity and mortality (9). In fact, approximately 7% of the total global cardiovascular disease burden is attributable to impaired kidney function according to a Global Burden of Disease analysis (8).

It follows that improvement in the publicity, prevention, diagnosis, and management of CKD is urgently needed worldwide. Research should investigate treatment options for those with pre-dialysis CKD which aim to slow kidney function decline, reduce cardiovascular disease, and delay end stage renal failure; particularly focusing on treatments which could be used in resource-poor settings.

CKD, CVD and endothelial dysfunction

The intimate link between CKD and CVD is complex with many proposed explanatory mechanisms (9, 12, 18). The traditional core risk factors for CVD overlap with those for CKD - for example increasing age, hypertension, diabetes mellitus, smoking, dyslipidaemia and sedentary lifestyle (19). But the CVD burden in CKD is greater than can be explained by these traditional risk factors (20). Growing evidence shows that there are further non-traditional risk factors for CVD specifically in CKD patients; for example, the reduced ability to metabolise elements such as calcium and phosphorous, and accumulation of toxic substances cleared by the kidney (12, 21). CKD also causes a reduction in cardiorespiratory fitness and an increase in sympathetic nervous system activity, both of which heighten the risk of CVD (22, 23). The development of CVD has in turn been shown to exacerbate the progression of CKD, creating a vicious cycle (18)

Mechanistically, both CKD and CVD involve dysregulation of intrinsic systems which maintain the homeostasis of the vascular endothelium (24-26). This leads to a prothrombotic, proinflammatory and less compliant vessel wall via a process called endothelial dysfunction, which ultimately paves the way to atherosclerosis (20, 27). This process can affect the heart, the vasculature and the kidneys (28, 29).

The causes of endothelial dysfunction in CKD are extremely complex (18). Persistent systemic hypertension, for example, can transmit into glomerular capillary beds causing glomerular hypertension (6). This can cause irreversible glomerular damage, with inflammatory processes causing increased vessel permeability, allowing excessive proteins to filter out of the blood and exert toxic effects on the kidney tubules (28, 30). Hyperglycaemic states trigger increased formation of advanced glycation end products, which bind to receptors and induce oxidative stress, pro-inflammatory cytokines, and migration of inflammatory cells into the vessel wall (20, 31). Through a complex sequence involving inflammation, intrinsic renal cell death and extracellular matrix proliferation, the small blood vessels supplying the nephron can fibrose, causing ischaemic nephron loss (6). In a positive feedback loop, nephron loss generates an extra strain on remaining functional nephrons in the kidney, leading to more glomerular capillary hypertension, and hence more endothelial dysfunction, in what is thought to be an adaptive response to maintain filtration rate (30).

There are many causes of CKD, with pathophysiological processes which differ from each other greatly, but endothelial dysfunction is a unifying occurrence in most of them. Endothelial dysfunction in the kidneys gives rise to the glomerulosclerosis, tubulointerstitial fibrosis, and vascular sclerosis seen in most CKD cases (28). Ultimately, these fibrotic changes leave patients with scarred kidneys which are devoid of normal kidney tissue architecture and unable to perform their essential functions such as filtering the blood. In addition, over time this vascular damage and inflammation can have systemic effects, leading to the cardiovascular complications of CKD like arterial stiffness, atherosclerosis, cardiomyopathy, calcification, ischaemic heart disease, heart failure, and cerebrovascular and cardiovascular death (18).

Since the 1990s, the vascular endothelium has increasingly been recognised as playing a crucial role in the pathological processes contributing to a host of conditions, from CVD to CKD, dementia and cancer (28). The endothelium is located between flowing blood and the vascular wall (32). It is lined internally by specialised endothelial cells, and controls vascular tone, haemostasis, vessel permeability, and immune responses to various types of vessel injury (27). The most critical molecule governing these processes is the soluble gas nitric oxide (NO), which can be synthesised by an enzyme in endothelial cells and the brainstem. NO has strong anti-inflammatory, antioxidant, vasodilatory and anti-platelet effects (27). NO also has a central nervous system effect, decreasing sympathetic outflow from the brainstem (33). Endothelial dysfunction is highly dependent on NO; it can be defined by a decreased amount of bioavailable NO in the vasculature. Impaired NO bioavailability is also a hallmark of CKD, evident even in the early stages (20, 27, 28). On average, endothelial dysfunction worsens across the CKD stages in parallel with an increasingly higher risk of cardiovascular death (19, 20).

The pathophysiology giving rise to reduced NO bioavailability is not fully understood. The endothelium constantly modifies NO production in response to endogenous and exogenous stressors. Reactive oxygen species are upregulated during acute and chronic pathological states. They react with NO and decrease NO bioavailability by consumption; the role of NO shifts from being a vasodilator to a radical scavenger (27, 34). This same oxidative stress further increases the availability of asymmetric dimethyl-L-arginine (ADMA), an endogenous inhibitor of the enzyme which produces NO in endothelial cells (27). CKD patients have been shown to have greater oxidative stress and ADMA levels compared to healthy controls (35). Inflammatory cells can express enzymes called arginases, which break down a key substrate required for endothelial NO production (27). Oxidised LDL cholesterol disrupts NO production, as does oestrogen depletion (20). Premature cell aging and bacterial endotoxins may also contribute (18) In vitro studies suggest that uraemia can induce a state of premature aging in endothelial cells, dysregulating micro-RNA expression and blocking endothelial enzymatic NO synthesis (18) Dietary intake of nitrate provides a physiological substrate for NO production, and research is underway investigating the effect of nitrate (NO3) or nitrite (NO2) consumption, GI absorption and microbiome processing on NO bioavailability in the blood (27). Preclinical trials show that pharmacological treatments to improve NO bioavailability can reduce endothelial dysfunction and have beneficial effects on various kidney pathologies (28).

Shear stress is another factor regulating NO bioavailability. Endothelial cells experience constant mechanical loading from blood pressure (compressive stress) and the frictional force of blood flow (shear stress) (32). Shear stress is detected by mechanosensors which span the gaps between endothelial cells and project from the cells out into the vessel lumen. As a result, these sensors trigger a pathway which increases expression of the endothelial NO producing enzyme (20, 36). NO can then diffuse into neighbouring vascular smooth muscle cells, where it stimulates active eviction of intracellular calcium, causing muscle relaxation and hence vasodilation (20). This process enables vessels to dilate in response to periods of high blood flow. It also acts to prevent atherosclerosis, which preferentially occurs in areas of turbulent flow, probably because shear stress helps to inhibit thrombosis, decrease endothelial cell apoptosis, limit endothelial cell permeability, prevent inflammatory cell binding, and increase bioavailability of NO (37).

Complex processes involving shear stress are thought to be the predominant mechanism by which exercise may help to reverse endothelial dysfunction (38). The ability of exercise to stimulate increased NO production is well-established. Evidence suggests that regular bouts of muscle contractions during exercise can induce acute oscillatory shear stress, promoting important beneficial adaptations to the endothelium including increased NO bioavailability by the mechanism described above (34, 39). By inducing shear stress, exercise may also help modify protein transporters which in turn can increase the transport of important substrates for NO production into endothelial cells (38). Exercise is therefore an attractive, inexpensive, nonpharmacological and low-carbon treatment target for improving endothelial function in CKD and subsequently reducing cardiovascular events and progression towards end-stage kidney disease worldwide.

Measurement of endothelial dysfunction

Endothelial dysfunction is a useful phenomenon to measure. It is recognised as the earliest clinically detectable indication of subclinical atherosclerosis, preceding gross clinical symptoms of the inflammatory process and CVD (34, 40). Evidence suggests that endothelial dysfunction acts as a reliable surrogate outcome predicting future CVD events. It can either be measured by functional methods (measuring the response of the endothelium to stimuli that stimulate the generation of NO) or by quantifying circulating levels of biomarkers molecules linked to endothelial dysfunction (such as NO and ADMA) (20).

The “gold standard” measure of endothelial dysfunction, and the most common method used in research to date (in use since the early 1990s) is a non-invasive ultrasound-based measure called flow-mediated dilation (FMD) (25). FMD quantifies the capacity of an artery to dilate in response to shear stress induced by increasing blood flow during post-ischaemic hyperaemia (41). This method was initially criticised for being highly user-dependent with poor reproducibility, however thanks to adoption of standardised protocols and analysis software, FMD is now considered a reliable indicator of endothelial dysfunction in multiple clinical settings including CKD (20, 42-44).

FMD in the forearm has been found to reflect endothelial function in the coronary arteries, the severity of coronary artery disease and the likelihood of future cardiovascular events (20, 41, 45) The American Heart Association considers FMD to be a measure of subclinical atherosclerosis (15). FMD has also been shown to improve with modifications of CVD risk factors and with the use of drugs known to reduce cardiovascular risk (15, 43). Intervention strategies that improve the lipidaemic profile (e.g. healthy diets, weight loss, physical activity, and certain anti-lipidaemic agents) can successfully improve FMD, it is thought via increasing exposure to shear stress and suppressing oxidative stress and oxidised LDL levels (20, 34, 46). Compared to some other vascular markers/cardiovascular outcomes, FMD changes occur quickly, over only a few months (43). As such, FMD is considered a useful surrogate endpoint for CVD outcomes in clinical trials (43).

FMD, by nature, needs to be performed using a vessel with a diameter large enough to be assessed visually using ultrasound – usually the brachial artery. However, the majority of the arterial vasculature is made up of far smaller vessels (arterioles and capillaries) which are more directly connected to tissues and subject to the effects of shear stress (47). In parallel to large vessel endothelial dysfunction, measures of microvascular reactivity have been shown in a recent systematic review to decrease across a continuum throughout the pathogenesis of CVD, from healthy populations to those with impaired glucose tolerance, overweight, metabolic syndrome and type two diabetes with vascular complications (48) Importantly, current evidence suggests that coronary microvascular disease may appear before large vessel disease in CVD (47, 48). In 253 subjects followed up for median of 9.3 years, microvascular dysfunction in patients with CVD risk factors predicted CKD onset and progression (24).

Microvascular endothelial function can be measured non-invasively using the cutaneous circulation. Evidence suggests that assessing microvascular function in this way can act as a model to echo the systemic microvasculature by mirroring the endothelial function of other vascular beds including coronary microvascular function, conduit artery endothelial function, and renal blood flow (49). Skin vasodilation can be triggered by sympathetic nerve terminals releasing acetylcholine, amongst other neurotransmitters, which activate NO-mediated vasodilatory mechanisms involving endothelial cells (50). To measure the extent of this cutaneous vasodilation, stimuli such as heat or chemicals can be applied to the skin and the response detected using laser Doppler technology, in which lasers measure the Doppler shift induced by the movement of red blood cells through the skin vasculature (50). These techniques - including laser Doppler flowmetry, laser Doppler imaging (LDI), and laser speckle perfusion imaging - are relatively uncommonly used compared to FMD and lack standardised protocols. However, they are still considered reliable indicators of cutaneous microvasculature function and are recommended for use in conjunction with FMD to measure global endothelial function in vivo (20, 51).

Laser Doppler microvascular function measures correlate with angiographically demonstrated CVD and have been used in CKD patients to help predict CVD mortality (20, 52). In a pilot longitudinal study of 70 end-stage CKD patients and 33 controls, laser Doppler microvascular function measures were able to add enhanced prognostic information, particularly for the group of patients with no known coronary artery disease or diabetes. In this group, patients who had microvascular function changes similar to those seen in coronary disease or diabetes were found to have a significantly increased CVD mortality. This increase, correctly predicted by laser Doppler findings, was not predicted by other cardiovascular risk assessment methods including the Framingham score (52).

However, data on microvascular endothelial dysfunction in pre-dialysis CKD patients are scarce (20, 52). Most of the available studies in CKD patients focus on laser Doppler flowmetry, which measures skin perfusion over a smaller area than LDI and is considered to be the less reproducible of the two techniques (53). Methods to evaluate microvascular function should be increasingly used to develop understanding of the role of endothelial dysfunction in cardiovascular and renal disease progression in early-stage CKD (25).

Physical activity, exercise interventions, and CKD

Large-scale evidence (a meta-analysis totalling 36,383 participants) shows that higher levels of physical activity and less time spent sedentary are associated with reduced risk of all-cause mortality in adults (54). The beneficial effects of regular aerobic exercise on reducing CVD risk have historically been attributed to favourable modification of risk factors like blood glucose control, blood pressure, plasma lipids, and BMI. However, epidemiological data suggest that only about 50% of the CVD risk-lowering effects of regular aerobic exercise are due to such risk factor modification; other factors must be involved (55). One additional factor is likely to be endothelial function, as exercise increases NO bioavailability (56). Furthermore, regular aerobic exercise seems to preserve endothelial dysfunction during adult aging. Observational studies have investigated FMD in young (15-30 years) and older (50-79 years) age subjects with raised circulating stressors such as low-density lipoprotein cholesterol and impaired fasting glucose. Older sedentary adults had a greater impairment of FMD than the young age subjects, suggesting that young age infers some kind of protective resistance against these harmful stressors. Older adults who did regular aerobic exercise demonstrated this same ”resistance” to harmful stressors as the young age participants (Figure 2) (55, 57, 58).

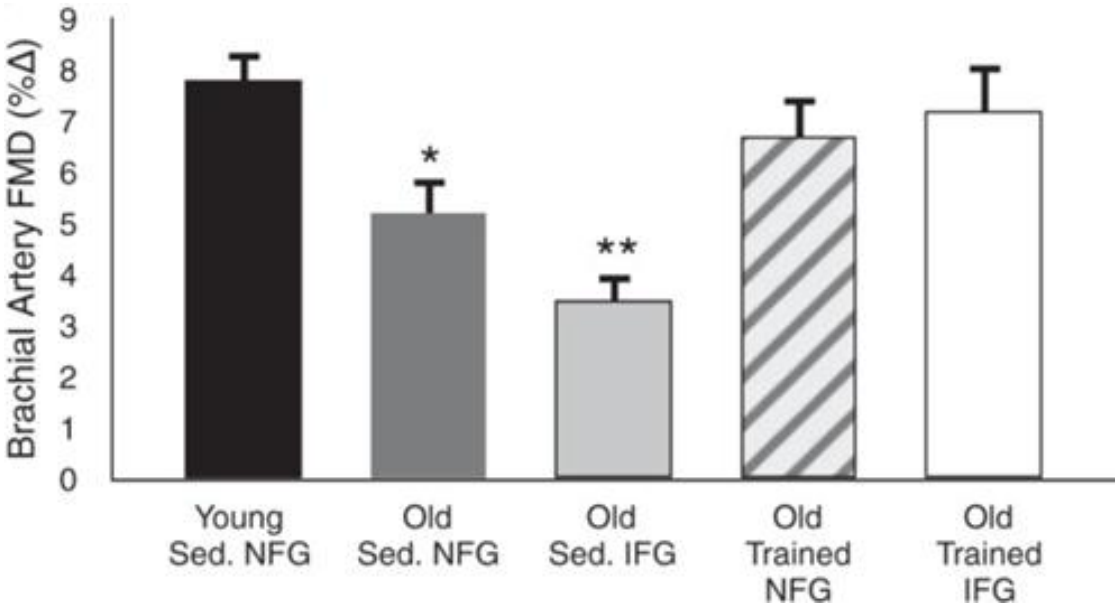

Figure 2 Aging, aerobic exercise, and "resistance" to cardiovascular risk factors, from Seals DR (55). FMD = flow-mediated dilation. NFG = normal fasting blood glucose (<100 mg/dl). IFG = impaired fasting blood glucose (100-126 mg/dl). Sed = sedentary. * = P < 0.05 vs. young. ** = P < 0.05 vs. old NFG. Data are from DeVan et al (57).

Evidence shows that physical activity levels are extremely low across all stages of CKD (59). CKD incidence is reduced in people with higher physical activity levels and physical activity has been identified as predictor of patient survival in this condition (11, 60). Reduced cardiorespiratory fitness in CKD patients was independently associated with increased left ventricular afterload, aortic stiffness, and burden of cardiovascular risk (22). Acute exercise has been shown to improve endothelial function in CKD. A study involving 20 individuals with pre-dialysis CKD tested FMD and serological markers of endothelial dysfunction at baseline, 1 hour and 24 hours after an acute bout of aerobic exercise. FMD improved in both time measurements after exercise. Markers of oxidative stress were shown to be decreased or unchanged, and all antioxidant markers were positively increased following the acute bout of exercise (19).

However, it is challenging to increase physical activity in clinical populations. Education is just one aspect of behaviour change, as shown by data from a RCT assessing a group education programme centred primarily on increased physical activity in multimorbid patients (n=353) across several GP practices in the UK. 90% of participants attending one of the trial sessions reported that they agreed moving more would benefit them, 86% reported they were considering a change in their physical activity habits, but only 66% reported feeling able to do so. After 12 months, the results showed that the intervention failed to increase physical activity and in fact elicited a significant reduction in participants' end-point physical activity compared to the control group (61). As per the behaviour change wheel framework (shown in Figure 3), capability, opportunity and motivation interact to generate behaviour; in turn, enacting a behaviour can alter capability, motivation and opportunity (62).

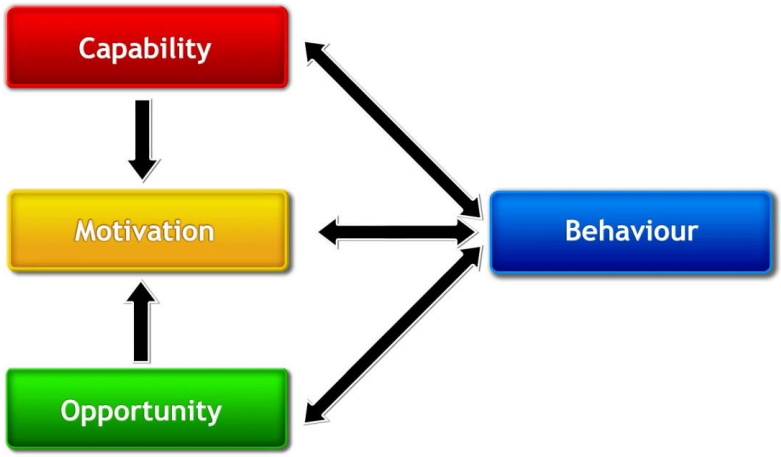

Figure 3 The COM-B system - a framework for understanding behaviour, from Michie et al (62). Capability = the individual's psychological and physical capacity to engage in the activity concerned, including have the necessary knowledge and skills. Motivation = brain processes which energise and direct behaviour, including habitual processes emotional responding, and analytical decision-making. Opportunity = All factors which lie outside the individual which make the behaviour possible or prompt it (62).

For high-risk populations where exercise may be particularly beneficial, rather than simply advising people to exercise, perhaps more success could be had by providing the opportunity to exercise, in a way that participants are capable of, with motivational support. Cardiac and pulmonary exercise rehabilitation programmes are routinely offered to patients with disease-specific long-term conditions in the UK, and engagement with these programmes has been shown to reduce all-cause mortality for those populations (60). At present, exercise-based renal rehabilitation is not routinely offered to CKD patients.

Exercise training may be delivered in numerous ways, from supervised in-centre training to unsupervised home-based training. Home-based exercise may be more physically and financially accessible to participants but can lack the level of accountability and coaching of in-centre training. Limited access to in-centre training during the COVID-19 pandemic has led to emerging digital health approaches to increase engagement in home-based exercise training (63). Many factors can influence the level of uptake and adherence by individuals offered to partake in an exercise scheme, including age, gender, location (rural or urban), level of deprivation, type of health problem (mental health, physical health), level of pre-existing physical fitness, the setting of referral (GP, nurse, specialist, other healthcare provider), duration between referral and initial session, characteristics of exercise leaders, convenience, and incentivisation (64). There is limited evidence concerning the improvement of physical activity with interventions in CKD, and the optimal choices for design, delivery and evaluation of exercise interventions in this cohort are uncertain (65)

The evidence base surrounding exercise training in CKD is small, often with a focus on patients in end-stage renal failure (66). As kidney function declines, physical function (exercise capacity, walking distance, strength and balance) also declines, so patients are usually severely deconditioned by this stage (67). Even in earlier stages of CKD, individuals may be unable to manage more than brief, low-intensity exercise initially, so it is important to adjust the level and dose of activities accordingly. As such, there are many challenges to designing a successful scheme in this cohort (67).

However, in the last decade, interest has been growing in providing exercise interventions to slow the progression of CKD, and there have been several large randomised clinical trials and systematic reviews on the topic (67). A recent systematic review of exercise interventions in pre-dialysis CKD including 11 studies (410 participants) found that exercise training significantly alleviated kidney function decline (11). There is further promising evidence for exercise training decreasing proteinuria, improving physical fitness, boosting quality of life, and diminishing oxidative stress in pre-dialysis CKD patients - albeit in a small trial with a duration of just 3 months (68). Across all stages of CKD, most long-term exercise training studies were found to have relatively good adherence rates, with >60% attendance to possible training sessions, and high rates of improvement in quality of life and functional capacity measures (69). Only one study has been able to evaluate the effects of exercise training on morbidity and mortality in CKD; a retrospective longitudinal study of all-stage CKD patients (n=757) who were assessed for a renal rehabilitation scheme at a single NHS trust in London. Results of this non-randomised retrospective study must be interpreted with caution due to the many possible confounding variables involved. However, participants who completed the scheme were found to have a significantly longer event-free survival period than those who didn’t (60).

Exercise interventions and endothelial dysfunction

Two of the main aims of exercise interventions in CKD are to reduce CKD progression and CVD mortality and morbidity; of which endothelial dysfunction can act as a surrogate outcome. In studies not specific to CKD patients, exercise training has been shown to improve FMD. A systematic review assessing the effect of exercise training on brachial artery FMD in 66 studies (including adults, children, subjects with cardiovascular/metabolic/thyroid disease and asymptomatic/healthy subjects) demonstrated statistically significant improvements in FMD compared to controls, with higher intensities, longer durations, diseased patients and aerobic training showing a greater effect size than lower intensities, shorter durations, healthy subjects and resistance training (34). Exercise training has also been shown to improve endothelial function in elderly subjects and cardiac patients, COPD, and peripheral vascular disease (34, 70, 71). There are limited data, however, for CKD patients.

It is still unclear whether aerobic, resistance or combination exercise training are most effective at improving FMD with variable results between studies. Both aerobic and resistance exercises produce the shear stress needed to benefit the endothelium (56). A trial designed specifically to compare aerobic, resistance and combination training effects on FMD for patients with hypertension found that all modes had similar effects, and combined training was the most effective (Figure 4). Combined training has the additional benefit of enhancing both cardiorespiratory fitness and muscle strength, which is important for daily functioning e.g. reducing risk of falls, musculoskeletal injuries, and osteoporosis (56). Muscle mass and physical function limitations have been identified as predictors of disease progression and survival for patients across all stages of CKD (60).

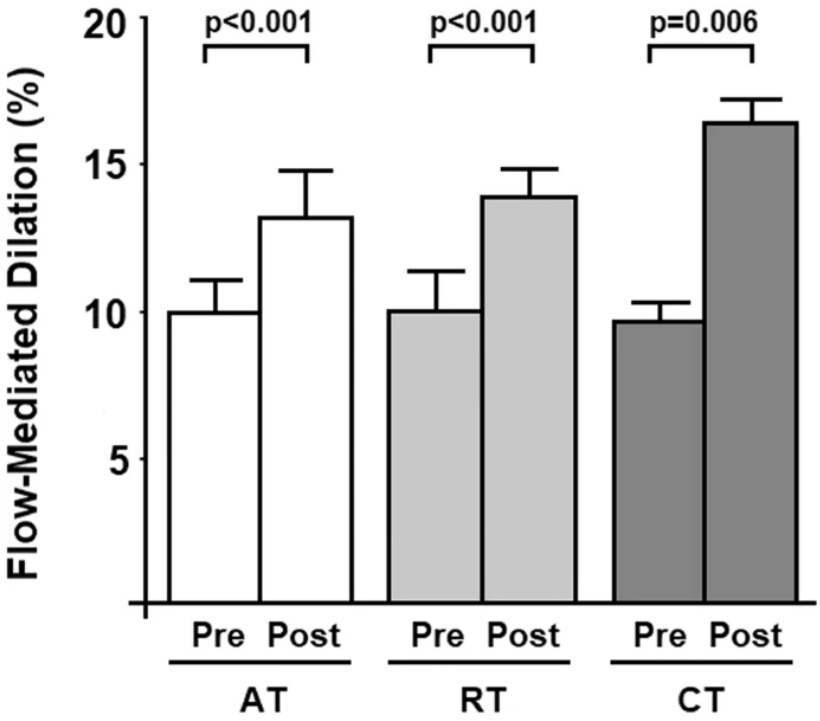

Figure 4 Flow-mediated dilation before (pre) and after (post) different modalities of exercise training in individuals with prehypertension or hypertension from Pedralli et al (56). AT = aerobic exercise training. RT = resistance training. CT = combined aerobic and resistance training. Results are expressed as mean ± SD.

Furthermore, a systematic review and meta-analysis reported that although exercise training was able to maintain healthy vascular function, there was not enough evidence to suggest that short term training programmes could improve FMD in previously sedentary individuals. They reported several limitations – one being a lack of randomised controlled trials in this field, and two, that many studies used assessment of the brachial artery despite the main exercise being cycling – perhaps leading to missed effects within the most active vascular beds of the lower limbs (72). Further randomised controlled research is needed in this area, potentially with inclusion of upper limb exercises where brachial FMD is used (72).

To the author's knowledge, only two trials have investigated the effect of exercise interventions on endothelial function for pre-dialysis CKD patients. The first was a RCT with 40 participants and a 3-month home exercise programme of four 10-minute cycle sessions per week, which found no statistically significant effect of exercise training on FMD, although exercise capacity was significantly improved in the intervention group (73). The second was a RCT of 36 participants, in which a 12-week aerobic exercise intervention of three supervised 45-minute sessions per week was shown to improve microvascular endothelial dysfunction and maintainn large vessel endothelial dysfunction (measured by laser-Doppler flowmetry and FMD respectively). The authors suggested investigating for further effects with a longer exercise training duration (74).

Measurement of physical acitivity

In exercise intervention trials, measurement of physical activity is important. The level of activity performed by each group acts as a manipulation check; it helps determine the effectiveness of the manipulation (exercise intervention) in the experimental design, and as such draw more accurate conclusions regarding the relationship between partaking in exercise interventions, performing physical activity, and the primary outcomes (75). When interpreting primary outcome data, physical activity measurements enable researchers to gage whether the exercise intervention has had the expected positive effect on physical activity levels.

However, physical activity is challenging to accurately measure. It is usually measured broken down into the components of intensity, frequency and duration. These components may be measured subjectively through questionnaires, diaries, or direct observation (76). They may also be measured objectively using pedometers (which measure step count, not activity intensity), accelerometers (which measure body acceleration as counts per unit time, and can calculate activity intensity from this), heart rate monitors, or multi-sensors (which measure multiple factors e.g. heat, heart rate and sweat level) (76, 77). There are two further objective methods, widely considered as the golden standard methods of measuring physical activity, called indirect calorimetry and the doubly labelled water method (76) However, these methods are highly specialised and energy-intensive for staff, equipment, and participants, as they involve measurements of oxygen consumption, carbon dioxide production, and elimination rates of different isotopes from the body; usually in a laboratory. Devices such as waist-worn accelerometers that participants can wear during their daily lives provide a more feasible and acceptable objective measurement option for most clinical research studies (65).

Different intensities of physical activity may be considered separately, and much physical activity research today specifically focuses on moderate to vigorous physical activity (MVPA) and sedentary time. MVPA is the key measure used in the UK and WHO physical activity guidelines (78, 79). It can be calculated from subjective data, by summing up activities reported in questionnaires using validated metabolic equivalent of task (MET) values, or from objective data, by recording activity using accelerometers and categorising this based on number of counts per unit time. Evidence suggests that partaking in more MVPA reduces all-cause mortality (54, 79). This effect has been shown both in individuals without comorbidities (where the beneficial effect of MVPA plateaued at high MPVA volumes) and in people with CVD (where there was a linear relationship between increasing MVPA and reducing mortality) (54).

Sedentary time is also mentioned in the UK and WHO physical activity guidelines (78, 79). It can be defined as time spent in any waking behaviour with an energy expenditure of ≤1.5 METs whilst in a sitting, reclining or lying-down posture (80). High levels of sedentary time are linked to increased risks of mortality, and evidence suggests that replacing time spent in sedentary behaviour with any intensity of activity (including light intensity physical activity) is beneficial to health (65, 79).

Compared to objective measures, subjective measures of physical activity like questionnaires or logs require the individual to report or recall activities performed and are likely to have lower reliability and validity than objective measures; for example over-reporting of activity levels and under-reporting of sedentary behaviour (81). However, questionnaires or logs can provide a simple, cheap, overview of activity including measurement of resistance or muscle-strengthening type exercise, swimming, upper body movements, and the different contexts within which exercise is performed (65). For example, the International Physical Activity Questionnaire (IPAQ) is the most widely used tool to collect self-reported physical activity (82). The IPAQ long-form questionnaire (recommended for research requiring more detailed assessment) includes 27 questions divided into five domains based on the context in which physical activity was performed over the last seven days (83). These domains are job-related, transport-related, housework/gardening-related, and recreational physical activity, as well as the final section which asks about time spent sitting down on weekends and weekdays. Given the lack of any single method to comprehensively measure physical activity, consensus suggests that a combination of simultaneous methods - including subjective and objective measures - is helpful to amalgamate results most accurately into overarching physical activity conclusions. Specifically, combined use of both accelerometers and questionnaires has been recommended to yield the most complete set of data for assessing physical activity in clinical trials (81).

Study aims and hypotheses

This study aimed to investigate endothelial function in both small and large vascular territories in pre-dialysis CKD patients before and after a 6-month home aerobic and resistance training exercise scheme, as compared to a control group randomised to usual care. Alongside measures of endothelial dysfunction, exercise data including the IPAQ questionnaire and accelerometer data were compared across both groups as a manipulation check. We hypothesised that participating in a 6-month home exercise scheme would significantly improve or maintain macrovascular and microvascular endothelial dysfunction and significantly increase physical activity levels in this population. Evidence of a protective effect against worsening endothelial dysfunction could support proposals for larger trials, exercise-based advice and interventions for those with pre-dialysis CKD – a growing global public health problem.

Methods

Trial design

This was a prospective parallel randomised controlled pilot study. Between March 2021 and February 2022, 14 pre-dialysis CKD patients were randomised and received either their usual CKD care or a 12-month prescribed exercise regime plus usual care. Measures of vessel health and physical activity were collected at 0 and 6 months (amongst other data as part of a larger feasibility trial).

All testing and data collection were carried out at Bangor University, North Wales. The study was approved by Wales Research Ethics committee 5, with REC reference 20/WA/0213. The IRAS ID is 270575. The study is registered at https://doi.org/10.1186/ISRCTN12609324.

Participants and Recruitment

Patients were recruited from the general nephrology clinic in Ysbyty Gwynedd district general hospital, North Wales. Records of patients attending the clinic were screened by a renal clinic doctor using the eligibility criteria listed below.

Inclusion criteria

- Adult patients (18 years +)
- Male or female
- Written informed consent
- Stages 3-4 CKD patients (GFR >15-60 mL/min/1.73m2) from local lab CKD Epidemiology Collaboration equation determined eGFR
- Declining CKD with no likelihood of reversibility: minimum decline = 5ml/min/1.73m^2 per year over previous 12 to 24 months, as measured by linear regression analysis (minimum of 3 measurements of eGFR over 12 months)

Exclusion criteria

- Age < 18 years
- Pregnancy
- A history of untoward reactions to iodinated contrast media
- A recent (last 6 months) episode of acute kidney injury
- Sickle cell disease
- Require support for ambulation less than 20m
- Psychosis, bi-polar, and eating disorders, and any other cognitive/neurological related issues
- Infection or course of antibiotics within the last month
- Enrolled in supervised exercise training in previous 3 months

Those deemed eligible were contacted by members of the research team who discussed partaking in the study. If interested, patients were provided with an information sheet (the current Research Ethics Committee and Health Research Authority approved version) and a cover letter explaining the trial and inviting them to participate. Patients were given approximately 2 weeks to consider the information before being recontacted by the research team, who then discussed any questions, collected informed consent for study participation, and booked participants for their baseline study visit. With consent, participants' General Practitioners were also informed of their patients' participation in the study. Language line was used if preferred by non-English speakers. It was not necessary to determine a minimum sample size for this feasibility study.

Data collection

Data was collected at two study visits - one baseline visit and one at six months (+/- two weeks) later. As part of a larger pilot study, the exercise group continued the exercise programme for a further six months (12-month data not reported in this sub-study).

Study visits took approximately two hours each and followed the same data collection routine. Participants always reported to the laboratory at the same time of day (early morning), following an overnight fast and continuation of normal medications. Multiple outcome measures were collected as part of the larger study. Testing started with BMI and body fat percentage measurements, blood pressure recording and venepuncture, and 10 minutes of questionnaires including the IPAQ. In total this equated to at least 30 minutes of mostly sitting still for the participant.

Participants then lay supine on a couch and underwent non-invasive vascular assessments, including laser Doppler imaging and flow-mediated dilation. If suitable, participants then undertook a graded exercise test to exhaustion on a cycle ergometer for assessment of aerobic capacity. All visits ended with the provision of an ActiGraph GT3x accelerometer, which participants were asked to wear for seven days following the visit (to be removed only whilst sleeping, washing, or swimming).

Randomisation

After their first study visit, participants were randomised to receive either usual clinical care or usual clinical care plus prescribed exercise intervention. A study schematic is shown in Figure 5. Randomisation was performed using a web-based service “Sealed Envelope” (https://www.sealedenvelope.com) with a 1 to 1 ratio. The allocation sequence was generated by the web service. This sequence was concealed from the research team by the web service. No blocking or restriction was used. The participants were enrolled and assigned to their allocations by a senior medical doctor on the research team.

Study schematic diagram

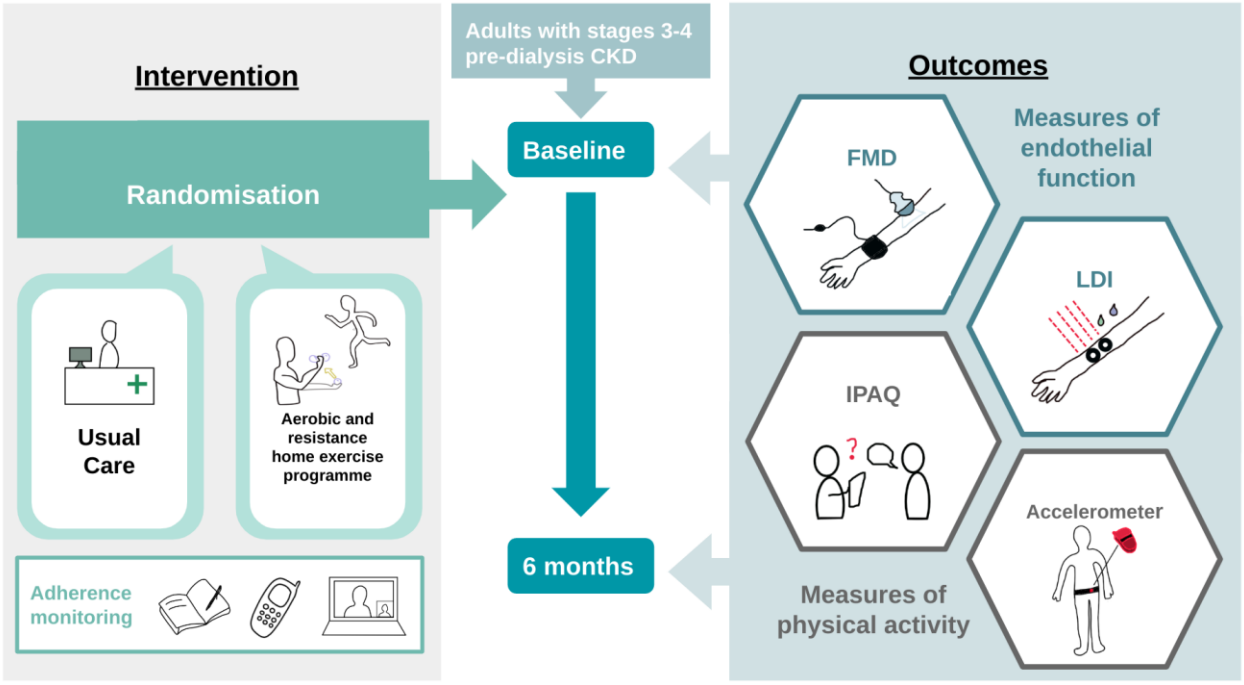

Figure 5 Study schematic diagram. CKD = chronic kidney disease, FMD = flow-mediated dilation, LDI = laser Doppler imaging, IPAQ = international physical activity questionnaire.

Intervention

Participants randomised to the exercise intervention arm of the study received a prescribed individualised exercise plan, designed to be performed either at home or in local gym facilities. In line with recommendations from the most recent Cochrane systematic review on exercise training in CKD, the plan included a combination of aerobic and resistance training (84). Exercise sessions were designed and prescribed by a trained level two gym instructor / level one weightlifting coach who also held a PhD in Sports and Exercise Physiology. Participants started the programme asynchronously from each other. They completed sessions in their own time and had weekly communication with the research team via video call, telephone call or email. There were no group sessions.

Each participant had a face-to-face induction session with the instructor to outline the programme, demonstrate resistance exercises, and discuss individual needs, aims and equipment. Sessions were subsequently designed according to participants' previous experience, ability, goals and preferences. Personalised exercise programmes were then sent to patients in the form of an electronic spreadsheet detailing specific exercises to be completed each week. The spreadsheets were interactive to allow participants to log their progress for each exercise. Some participants were provided with static bikes, dumbbells, resistance bands and/or ankle weights, whilst others used their own exercise equipment or local gym equipment.

Most participants were prescribed four aerobic and four resistance sessions per week. Aerobic sessions involved walking, jogging or cycling on an exercise bike for a prescribed time period. Resistance sessions involved a prescribed number of consecutive repetitions of six to eight exercises targeting large multi-joint muscle groups; examples included squats, bicep curls and knee extensions. Resistance training exercises were chosen with an aim to improve core strength, balance and function. For some participants, weights were used during resistance exercises. If used, the initial level of external load prescribed was the maximum weight that still allowed patients to perform 10 to 15 repetitions with correct technique.

Duration of sessions varied. The aim for participants was to accumulate 40 minutes of moderate intensity exercise four times a week (160min per week) by six months of training. Resistance training sessions aimed to last between 10 to 20 minutes each and were designed around completing a prescribed number of exercise repetitions. Weekly communication with participants and examination of data from their exercise logs enabled monitoring of participants' progress, adherence, and any adverse events.

Usual care

Usual care for patients with stages 3-4 CKD included thrice-yearly appointments at a hospital general nephrology clinic, typically receiving several interventions including blood pressure control, anaemia treatment, phosphate control, dietary advice, and cardiovascular risk mitigation strategies. Many of the patients take renin angiotensin blockers, aspirin and/or lipid lowering agents. Patients did not receive formal physical activity or exercise recommendations as part of usual care.

Primary outcomes

The primary outcomes examined in this sub-study were brachial artery FMD and forearm LDI with iontophoresis. These two outcomes are evidenced-based non-invasive measures widely considered as conduit measures of endothelial function for large vessel and microvascular vessel health, respectively.

FMD and LDI can be measured in a variety of ways; in our laboratory they were measured following described techniques published in a methodological protocol by Sandoo and Kitas, with the exception that our laboratory was not temperature controlled (85).

Flow-mediated dilation

FMD was measured using a Doppler ultrasound machine with a high-frequency (8.9 – 11.4 MhZ) linear array transducer. Scan depth was set to either 35 or 40 mm for all recordings, and dynamic range was reduced to 40 to ensure contrasting black and white images. The gain settings were optimized to display the vascular borders clearly.

Participants were asked to relax on a semi-recumbent couch and to rest their arm still on a pillow out to their side, level with the heart. A sonographer then identified the brachial artery in the longitudinal scanning plane and took a two-minute digital ultrasound video recording of the brachial artery at rest.

Next, a blood pressure cuff placed around the forearm was inflated to suprasystolic pressures for five minutes to occlude blood flow to the hand. This was then deflated to induce post-ischaemic reactive hyperaemia in the brachial artery. In a healthy vessel, this hyperaemia will stimulate NO-mediated vasodilation. A second brachial artery ultrasound video was acquired with recording starting 30 seconds before cuff deflation and ending 3 minutes after cuff deflation. Software analysis of the second video was used to identify the maximum (peak) arterial diameter in the cuff-deflation period. The sonographer who performed all the FMD scans in this study had an intra-observer coefficient of variability of 10.7%.

All FMD scans were analysed using the program “Brachial Analyzer for Research, Medical Imaging Application, USA” version 6, which uses an automated method for near and far vessel wall tracking in ultrasound image sequences. Analysis was carried out by a single research team member who inputted the ultrasound scan depth calibration and manually selected an optimal region of each ultrasound video recording for analysis, based on showing a clear distinction between the arterial walls and lumen, and a consistent image quality. The software confidence threshold for identifying the vascular borders was set to 75%. The software algorithm then ran frame-by-frame through the video clip, calculating the vessel diameter for each frame. These data were used to calculate the minimum, average, and maximum vessel diameter for each ultrasound scan video.

To increase the accuracy of the measurement of the vessel diameters, all images were analysed at the same point of the cardiac cycle. A simple ECG trace was recorded alongside the images, and the software was programmed to automatically detect the R-R peak, which enabled diameter readings to be preferentially collected during the diastolic phase of the cardiac cycle, when the artery is relaxed. The frame-by-frame analysis was also manually screened to clean the data before the average and peak diameter values were recorded. Occasionally the software misidentified edges of surrounding soft tissue as vessel wall, clearly distinct from the true vessel wall identified in most frames of the clip. If this had surpassed the software confidence threshold of 75% and hence was being included in the results (at risk of overstating the vessel diameter), this frame was either removed or the measurement corrected using a specific correction tool in the software.

The overall brachial artery FMD value was calculated as a percentage using the following formula:

[(Peak deflation diameter − baseline diameter) ÷ baseline diameter] ×100

Laser Doppler imaging with Iontophoresis

Iontophoresis is a process of transdermal drug delivery using a voltage gradient on the skin. LDI with iontophoresis of acetylcholine (ACh) and sodium nitroprusside involves measuring microvascular endothelial function by topically applying positively and negatively charged vasoactive agents to the forearm, passing an electric current through the skin into the microvasculature and assessing the perfusion with a laser Doppler scan of the area. Detection of changes in frequency of light scattered by moving red blood cells enables the laser Doppler signal to provide a perfusion value, which represents the concentration and average velocity of red blood cells in that area of tissue. As the laser beam moves across a section of the forearm and detects perfusion values, a “perfusion map” can be created.

The LDI protocol used to collect data in this study has been described in the literature in full detail (85). Participants were asked to relax in a semi-recumbent chair with their forearm rested still on a firm black mat out to their side, directly underneath a Laser Doppler Imager machine’s scanner head. Two circular Perspex chambers were attached to the volar aspect of the participants right forearm using double sided adhesive pads. The chambers were connected via wired plugs into an iontophoresis controller on the Laser Doppler Imager machine (moorLDI2-IR, Axminster, Devon). 2.5 ml of 1% acetylcholine (a classical vasodilator which stimulates endothelium-dependent generation of NO) and 2.5ml of 1% sodium nitroprusside (used to directly activate receptors on smooth muscle cell to cause endothelium-independent vasodilation) were diluted with 0.5% saline solution and added to the anodal and cathodal connected chambers, respectively. The chambers were then covered with coverslip lids, taking care to avoid air bubbles which may interfere with laser imaging.

Next, the Laser Doppler Imager machine performed ‘Auto distance’ measurement to determine the distance from the scanner head to the participant’s arm. A trained researcher manually programmed the skin region to be scanned, ensuring that the diameters of both chambers were within the region. The iontophoresis was started and the Laser Doppler Imager scanned the defined region over several minutes. Ambient lighting was restricted so that all scans were performed in natural lighting conditions. The subsequent perfusion maps were then analysed using moor LDI review v6.1 software to determine a median baseline perfusion value and a median peak perfusion value in response to the iontophoresis.

Due to the limited scope of this study, data for endothelial-dependent vasodilation (a microvascular-equivalent of the response measured during reactive hyperaemia in FMD) were prioritised. The sodium nitroprusside-mediated endothelium-independent vasodilation results are not reported here.

Percentage change in perfusion in response to ACh was then calculated using the following formula:

[(Peak perfusion value − baseline perfusion value) ÷ baseline perfusion value] ×100

All units were in arbitrary units (AU).

Ancillary outcomes

To aid interpretation of the relationship between exercise interventions, physical activity and vascular function, three further outcome measures were examined in this study: adherence to the exercise programme (exercise group only), and IPAQ scores and accelerometer data (all participants).

Adherence

In accordance with a large BMJ review, uptake was defined as either attendance at the initial consultation with the exercise professional or attendance at ≥1 exercise session, and adherence was measured using the number of sessions attended divided by number prescribed (64). Absolute numbers of sessions attended will also be presented in the results.

Adherence to the exercise intervention was calculated from participants' self-reported exercise logs, using a session-based approach. The number of weekly sessions completed was divided by the number of weekly sessions prescribed to give a score for each week. For example, if the instructor had prescribed four resistance training sessions for a certain week, and the participant completed three of these, this gave a weekly score of 0.75. The sum of these scores over 26 weeks (the total number of weeks) was then divided by 26 to give a percentage adherence score for the whole six-month scheme.

As part of the personalised nature of the exercise scheme, if a participant was noted to be struggling with adherence, this was discussed during their weekly contact with the research team and strategies/modifications to their weekly plans were used to help boost adherence.

Accelerometer data and IPAQ scores

ActiGraph GT3x accelerometers include an acceleration sensor (functioning in vertical, horizontal, and perpendicular axes) and an ambient light sensor. The 3-axis (triaxial) data collected is automatically interpreted by an inbuilt inclinometer feature which estimates the orientation of the device. The device is worn on the hip and captures activity in terms of 'counts', which are used to categorise activity by intensity level, depending on number of counts per minute (29). Cut points for these classifications used were set by the ActiLife software Freedson1998 (86). The cut points were sedentary (0-99 counts per minute), light (100-1951), moderate (1952-5724), vigorous (5725-9498), and very vigorous (9499 and above).

Accelerometer analysis for this study focused on two parameters: minutes per week of MVPA and minutes per day of sedentary activity. These parameters were chosen as they feature in the UK's national physical activity guidelines (78). These guidelines recommend performing at least 150 minutes per week of moderate physical activity (or 75 minutes per week of vigorous physical activity, or an equivalent time combination of the two) and minimising sedentary time (78). As such, MVPA was calculated from the data by giving minutes of vigorous exercise twice the credit of minutes of moderate activity; a MVPA score of 150 minutes per week or more was then considered as meeting the guideline targets.

The International Physical Activity Questionnaire (IPAQ) was used as an additional measure of physical activity (87). For this study a member of the research team acted as the interviewer asking and recording the questionnaire results. As opposed to the shortened version, the longer 'last 7 days' format of the IPAQ was used. This comprises 27 questions about time over the past 7 days spent active at work, during transport, during household/garden chores, or for leisure – as well as time spent in a car or sitting. The results are entered into an IPAQ-specific calculator and a score expressed in metabolic equivalents (METs) is produced. METs are multiples of the resting metabolic rate.

Harms

Harms data were collected for all participants. Researchers asked participants about harms at study visits and exercise intervention check-ins. Participants' hospital records were checked after the study for admissions or deaths.

Blinding

Due to the nature of the intervention, participants were unable to be blinded to their allocation. Due to small staffing levels, some research staff were unable to be fully blinded because they were jointly involved in the provision of the exercise programme and collection of outcome measures. However, the sonographer was blinded to allocation. During ultrasound scan analysis, numbered IDs were used to anonymise patient data, and the researchers involved were blinded to allocation.

Changes to methods after trial commencement

Due to the COVID-19 pandemic, the initial planned exercise intervention for this study (which involved supervised in-person gym sessions) was changed to one of prescribed self-directed home exercises, with weekly contact from the research team. This change was implemented before any participant exercise sessions had started. The delivered exercise protocol is the one described here in the methods.

Statistical methods

Statistical analysis was carried out in IBM SPSS Statistics version 27 (IBM, United States of America, New York) comparing baseline and follow-up data for control and intervention groups using a repeated measures general linear analysis, including calculation of interaction terms, and both within-group and between-group comparisons. Data were expressed as mean ± standard deviation. Due to the small sample size, a p-value <0.1 was considered statistically significant.

A minimal clinically important difference (MCID) was decided for each outcome to support interpretation of the results of the analysis. This value was pre-determined by the authors before the study results were identified.

A meta-analysis involving 14 cohort studies and 5,547 patients (mean follow-up of 3 years) examining prediction of CVD events by FMD found that a 1% absolute decrease in brachial artery FMD was associated with an 8% increase in risk of future CVD events, and this association was consistent across a broad range of populations (41) As such, a minimal clinically important difference of 5% absolute decrease in FMD was chosen – implying a 40% increased risk of future CVD events – which the author felt would be deemed an important change for patients. Particularly given the large intra-observer coefficient of variability (10.7%) when collecting ultrasounds for FMD, this minimally clinically important difference was used with caution, and only applied to mean changes in the data. It was not applied to individual data changes.

As LDI is a less commonly used technique, the predictive value of LDI outcomes on future clinical events is less understood. Research has not yet established whether LDI can be used to predict cardiovascular outcomes. In the absence of prospective data, a cross-sectional study comparing laser Doppler flowmetry measures in patients with metabolic syndrome (n=78) to healthy controls (n=40) was used to determine an estimated MCID for LDI-measured percentage change in perfusion response to ACh (47). The difference between the mean percentage change in healthy controls (612%) compared to patients with metabolic syndrome (324%) was chosen to give an MCID of 288% increase in percentage change in perfusion response to ACh.

This MCID was again used with caution. Our trial used laser Doppler imaging, which is a newer technique than laser Doppler flowmetry. Compared to laser Doppler flowmetry, LDI is slower and enables a larger area of skin to be imaged, with the aim of reducing movement artefact and site-to-site heterogeneity (50). However, the two methods work on the same principles and the same iontophoresis method was applied to the forearm in both studies, so the percentage change results can be comparable in the absence of more relevant evidence. Again, this MCID was applied only to mean data and not individual data changes.

Results

Participant flow

As shown in Figure 6, 925 patients from the renal clinic were screened according to the eligibility criteria by a senior nephrologist. 857 were excluded due to not meeting inclusion criteria. A clinical decision was made by the nephrologist to exclude 28 patients due to medical conditions which made them unsuitable, despite meeting formal inclusion criteria. A further six patients were excluded due to time constraints for recruitment. 34 patients were approached. Due to changes in renal function and physical mobility since the screening process, two patients who had been successfully recruited to join the study became ineligible before entering the randomisation process. During the study, two participants in the intervention group and one in the control group withdrew from the study, either due to moving away from the area or in one case due to a family member becoming unwell. One participant in the intervention group had to be excluded from study analysis due to an ultrasound machine fault during one of their study visits.

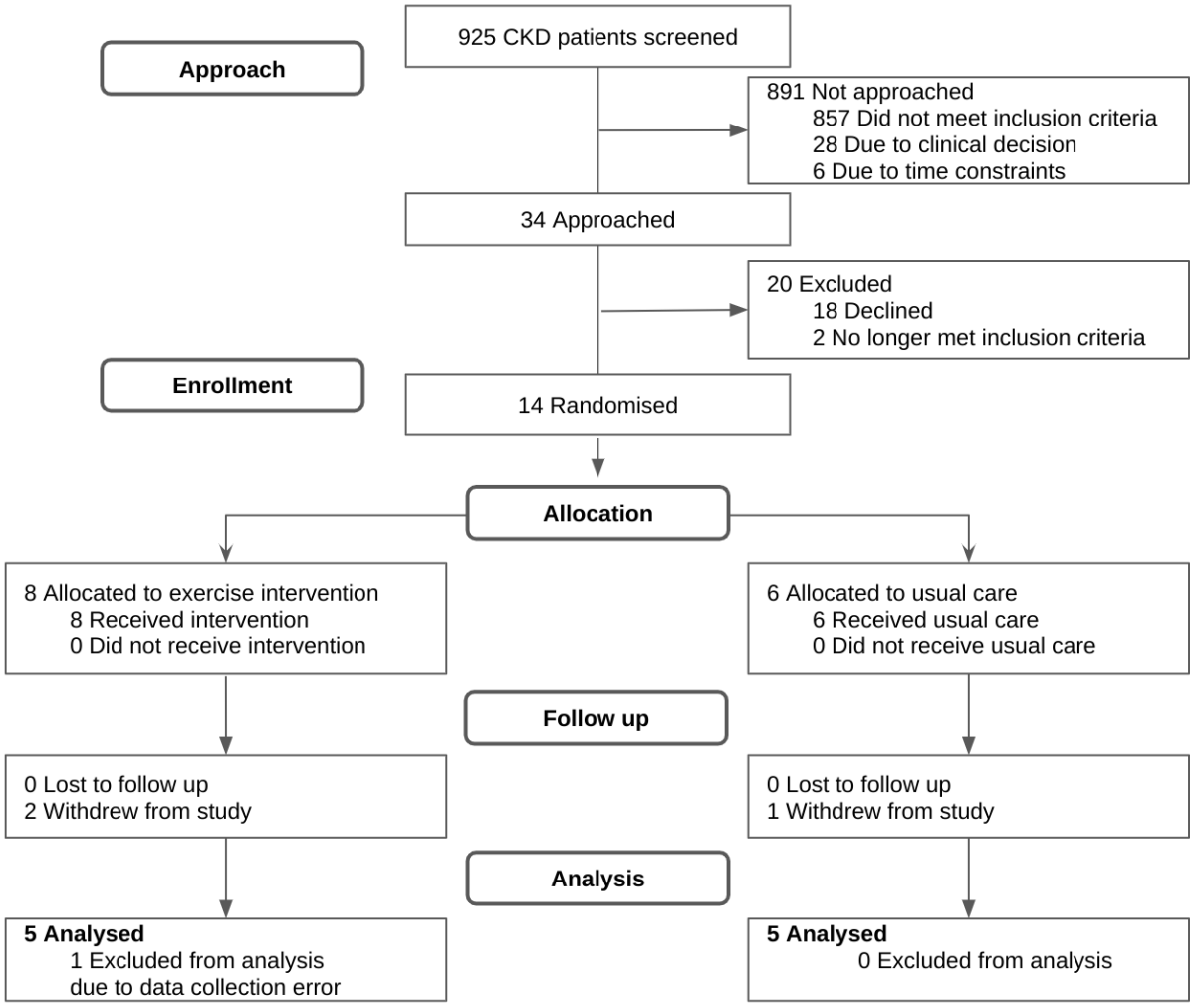

Figure 6 Participant flow diagram. CKD = chronic kidney disease.(56)

Baseline data

The study population characteristics are outlined in Table 1. Each group had a similar average age, BMI, and sex ratio. All participants identified their ethnicity as White.

Table 1 Baseline population characteristics.

Characteristics	Exercise Training (n=5)	Usual Care (n=5)
Age (years)	58.9 ± 9.4	58.2 ± 18.4
Male sex (%)	60%	60%
BMI (kg/m²)	29.7 ± 3.2	26.9 ± 4.1
Systolic BP (mmHg)	141.9 ± 20.6	133.6 ± 13.6
Diastolic BP (mmHg)	78.8 ± 4.2	76.6 ± 5.9
Kidney disease cause (n)		
IgA nephropathy	3	1
Diabetic nephropathy	1	1
Renovascular disease	1	0
Hypertension	0	1
Collapsing glomerulopathy	0	1
Polycystic kidney disease	0	1
Never smoked (%)	40%	60%
EGFR (ml/min/1.73m²)	32.6 ± 13.4	34.0 ± 10.2
Creatinine (umol/L)	189.6 ± 66.9	167.2 ± 25.7
Triglyceride (mmol/L)	1.46 ± 0.63	2.58 ± 1.82
HDL cholesterol (mmol/L)	1.44 ± 0.50	1.38 ± 0.28
LDL cholesterol (mmol/L)	2.74 ± 0.97	2.72 ± 1.64
Total cholesterol (mmol/L)	4.86 ± 1.18	6.22 ± 1.65
CRP (mg/L)	4.2 ± 0.5	4.0 ± 0.0
HbA1c (mmol/mol)	43.2 ± 16.3	37.4 ± 4.93

Mean ± standard deviation shown. BMI = body mass index, BP = blood pressure, eGFR = estimated glomerular filtration rate, HDL = high density lipoprotein, LDL = low density lipoprotein, CRP = c-reactive protein, HbA1c = glycated haemoglobin.

Primary outcomes

Flow-mediated dilation

As shown in Figure 7, the usual care group started with a higher mean FMD value compared to the exercise group. This trend was maintained in the six month data. Mean FMD tended to increase in both groups. However, the mean changes in these data are highly influenced by individual participant results due to the small sample size, and the change data between individual participants were highly variable.

Specifically, in the usual care group, FMD increased in two participants and fell in three participants. In the exercise group, FMD increased in three participants and fell in two participants. Overall, there was no statistically significant change in FMD between groups from baseline to six month data ($p=0.90$). The mean FMD change was +3.6% in the usual care group, and +4.3% in the exercise group, which did not surpass the MCID of a 5% increase in FMD. The overall increase in FMD for the cohort (regardless of group) over time was not found to be statistically significant ($p=0.183$).

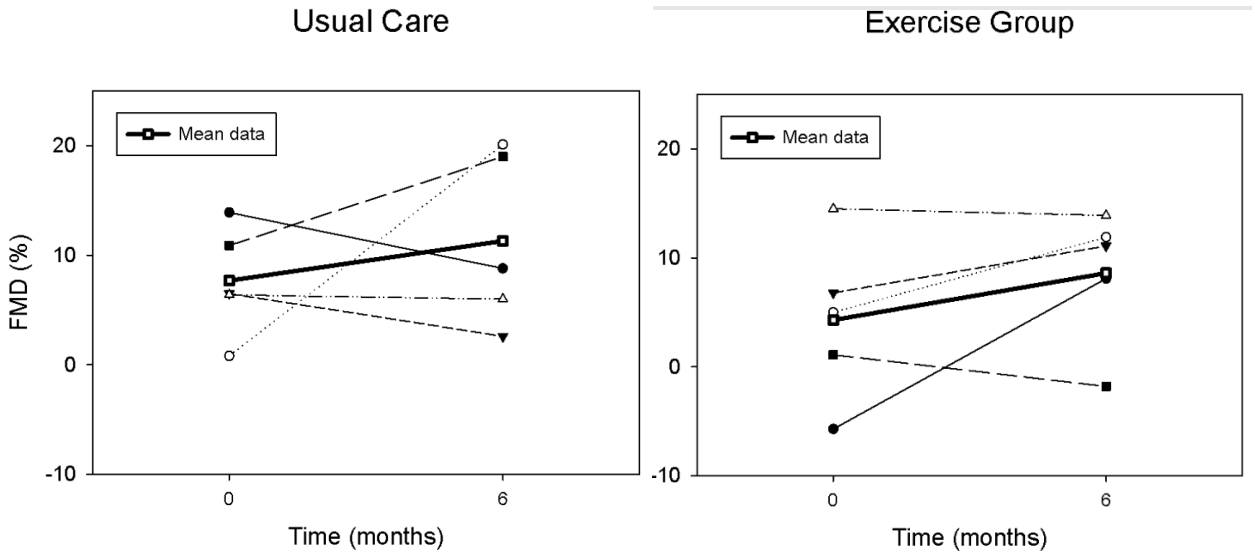

Figure 7 Flow-mediated dilation in the brachial artery at 0 and 6 months by study group (56). FMD = flow-mediated dilation.

Laser Doppler imaging with iontophoresis

One participant in the exercise group had incomplete LDI data due to a machine fault during one of their study visits, so was excluded from the LDI analysis. Overall, there was no statistically significant change between study groups across baseline and six month data in percentage change in perfusion in response to ACh ($p = 1.00$). The individual change data was highly variable. As shown in Figure 8, the usual care group on average started with an increased perfusion reaction following ACh iontophoresis as compared to the exercise group. This trend was maintained in the six month data. In the usual care group, perfusion change increased in three participants and fell in two participants. In the exercise group, perfusion change increased in two participants and fell in two participants. The mean data changes were small; 7% and 6% for the control and exercise groups respectively. They did not surpass the MCID of a 288% increase in percentage change in perfusion. The overall increase in perfusion response for the cohort (regardless of group) over time was not found to be statistically significant ($p=0.909$).

Comparing data from both measures of endothelial function (large vessel and microvascular), similar trends were observed. The usual care group on average started and ended with better endothelial function than the exercise group. Neither large vessel or microvascular endothelial function statistically or meaningfully increased in the intervention group over time as compared to the control group.

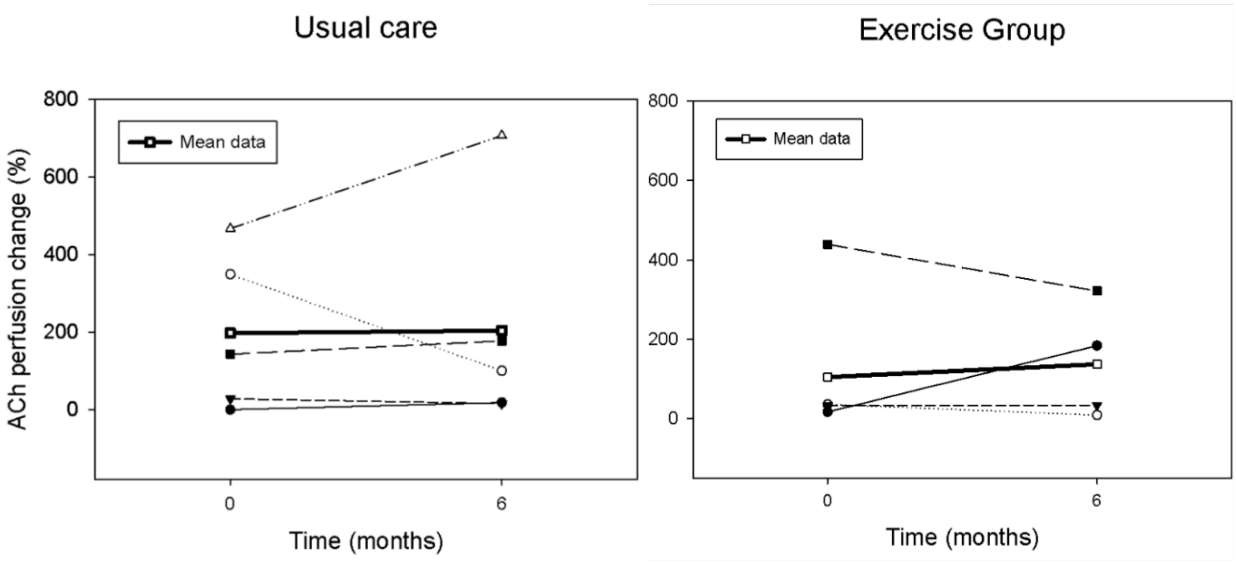

Figure 8 Percentage change in perfusion to forearm skin in response to acetylcholine (measured by Laser Doppler imaging with iontophoresis) at 0 and 6 months by study group(56). ACh = acetylcholine.(56)

Ancillary outcomes

Adherence

The uptake for the exercise intervention was 100%. The mean adherence (± standard deviation) to exercise training sessions was 61.7% ± 11.7 for aerobic exercise and 57.7% ± 12.2 for resistance exercise. The mean absolute number of sessions attended (± standard deviation) was 109.4 ± 26.0.

Figure 9 shows the total number of exercise sessions completed on average per participant per week. Aside from a dip in week 19, in which just four sessions were completed across all five participants, session numbers remained relatively stable across the programme.

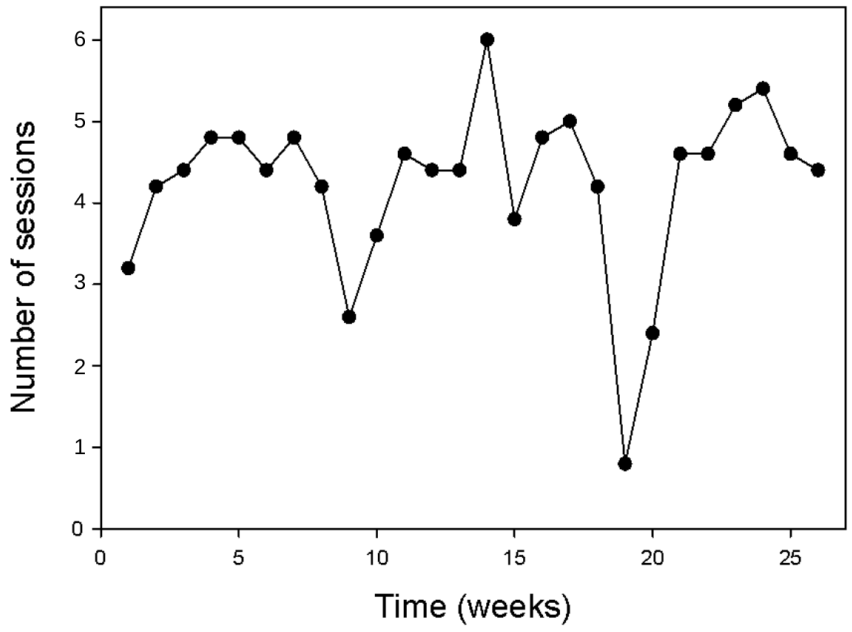

Figure 9 Mean total number of exercise sessions (aerobic and resistance) completed per exercise participant per week.(56)

Accelerometer data and IPAQ scores

IPAQ scores represent the number of METs expended in the seven days prior to the date of completing the questionnaire on the study visit. Accelerometer data represent the MVPA and time spent sedentary for the seven days following the study visit. One participant from the usual care group and two from the exercise group were excluded from accelerometer analysis due to incomplete data collection. The remaining participants completed an average of 7.5 days wearing an accelerometer post each study visit – with the minimum length of time being 5 days, and the maximum 13 days.

The two measures of physical activity (IPAQ scores and accelerometer-derived MVPA) showed closely aligned trends (Figure 10). Compared with control data, there was no statistically significant increase or decrease in IPAQ scores (p=0.33) or accelerometer MVPA (p=0.35) following the intervention. However, there was a change in the number of participants meeting the 150 minutes per week MVPA target. At baseline, two of the four participants in the usual care group and two of the three in the exercise group were meeting the target. By the end of the study, the same two participants were meeting the target in the usual care group, but none of the exercise group participants were meeting the target. As such, there is evidence of a meaningful decrease in physical activity in the exercise group compared to the usual care group, although this was not found to be statistically significant. The overall IPAQ and MVPA changes for the cohort (regardless of group) over time were not found to be statistically significant (p=0.355 and p=0.847 respectively).

At baseline, the exercise group had a mean sedentary time of 739 minutes per day and the usual care group a mean of 623 minutes per day. Overall, as shown in Figure 10, the exercise group remained the more sedentary group. However, accelerometer results showed a statistically significant difference between the daily sedentary time of the two groups following the intervention (p=0.062). The exercise group decreased their mean daily sedentary time by 30 minutes (standard error 50 minutes), and the usual care group increased theirs by 50 minutes (standard error 36 minutes). The overall change in minutes spent sedentary per day for the cohort (regardless of group) over time was not found to be statistically significant (p=0.579).

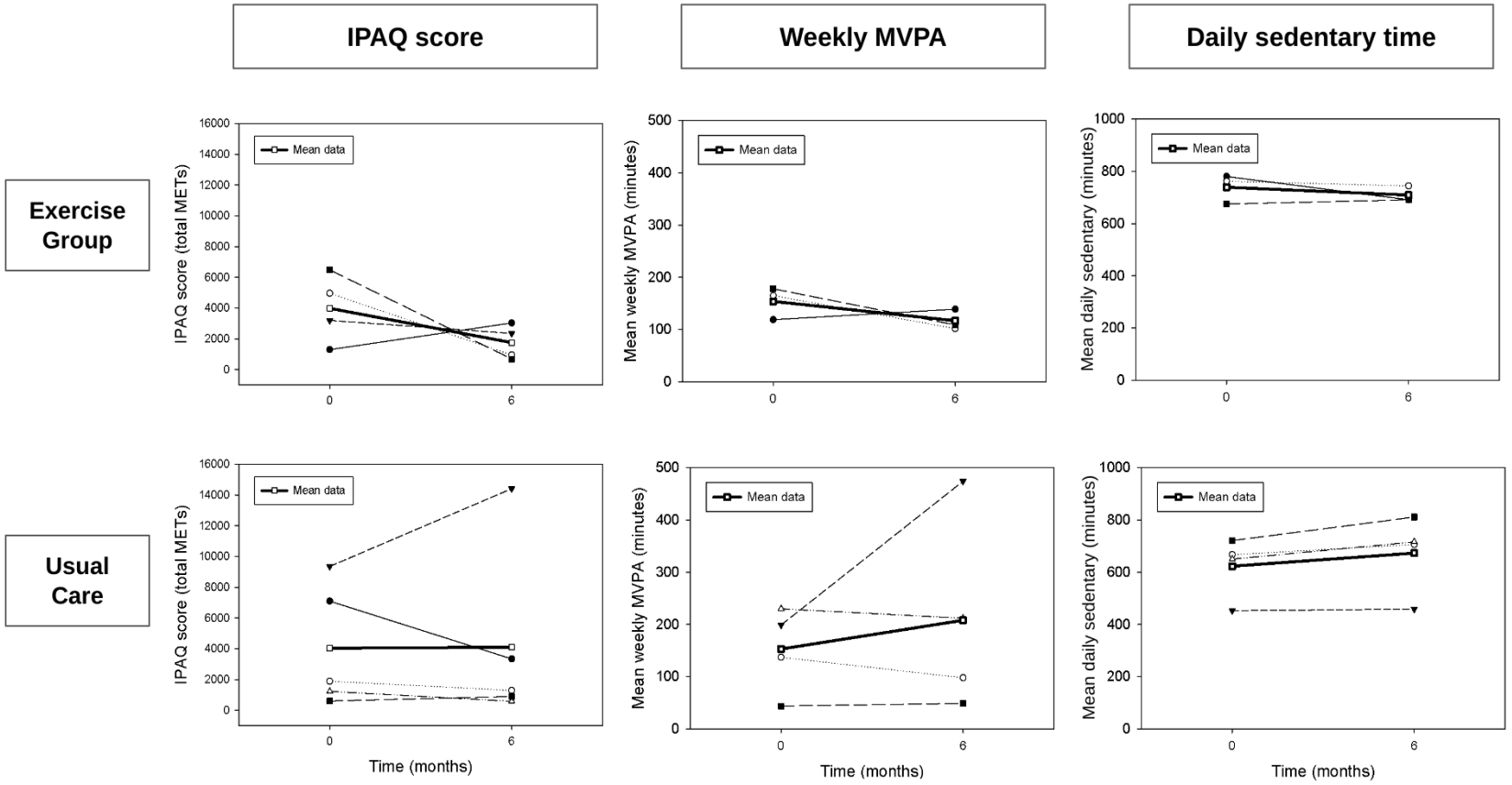

Figure 10 International physical activity questionnaire score, weekly minutes of moderate to vigorous physical activity, and daily minutes of sedentary time at 0 and 6 months by study group. IPAQ = international physical activity questionnaire, MPVA = moderate to vigorous physical activity(52).

Harms

There were no confirmed study-related harms in the exercise group. One exercise participant reported ankle pain at the site of a previous ankle injury. This could have been possibly related to following their exercise regime - although the participant also felt it could have been from having driven 300 miles shortly before the pain onset. Following this, the team worked with the participant to reduce their exercise plan to a manageable intensity for their ankle. One participant had a fall in the shower and sustained a possible vertebral fracture. This was not thought to be related to this study. They experienced a delay in starting their exercises due to pain. Another participant had an anaphylactic/angioedema reaction with no temporal relationship to any trial procedure or intervention. This was managed in the emergency department. There were no hospital admissions or deaths in the exercise group.

There were no confirmed study-related harms in the control group. One patient had a day case surgery for insertion of a Tenckhoff catheter, which was later used for commencement of peritoneal dialysis. This was not trial related. There were no deaths and no other hospital admissions in the control group.

Discussion

Summary of results

Previous evidence would suggest that exercise interventions can improve endothelial function in several chronic disease groups, but whether this is true for pre-dialysis CKD patients is unclear. In this study, the six-month home exercise intervention failed to significantly increase or maintain endothelial dysfunction in either small or large vessels when compared to a control group. The intervention also failed to significantly increase physical activity levels. As such, the study hypothesis cannot be accepted.

There were multiple unexpected findings in our results. Our manipulation check measures show no evidence of an effect of the intervention on physical activity compared to the control group. Remarkably, the number of people achieving the 150 minutes per week physical activity target decreased (two to none) in the exercise group whilst remaining stable in the control group (two participants). Considering the relatively high reported adherence to the programme throughout the 6 months, it is difficult to explain why this finding was reached. One possibility is that, as seen in other exercise intervention trials, structured exercise can incur individual compensatory behaviours, for example reduced non-exercise physical activity, which negate any activity-boosting effect of the programmes (88, 89).

Another possibility is that the measures used in the study failed to accurately detect the kinds of physical activity which may have increased during this intervention, for example, the accelerometers failing to detect an increase in upper limb exercises like bicep curls. This possibility is further discussed in the limitations section below. However, the levels of activity suggested from IPAQ scores aligned closely with accelerometer MVPA readings in this study (Figure 10), which adds internal validity to these results and makes this explanation less likely. This close alignment contrasts with data from previous research examining estimated physical activity from the long-form IPAQ compared to accelerometer data in older adults in Portugal, which found large differences and low correlation between the two methods (82).

It is also possible that the intervention used in this study was insufficiently designed or monitored to increase the amount of physical activity completed by the exercise group. For example, perhaps a regime with more frequent sessions, more intense exercises, or more robust adherence monitoring could have resulted in successful improvements in physical activity measures. Although weekly communication with participants aimed to provide opportunity for discussion and support around adherence, participants were not observed during their home exercises so may have been able to overstate their adherence in their exercise logs.

In those seven patients for whom accelerometer data was available, there was a statistically significant change in the daily amount of sedentary time between the groups, with the exercise group decreasing and the control group increasing their sedentary time. Although not predicted in our study hypothesis, this reduction in sedentary time likely represents a positive health benefit for the intervention group. Sedentary behaviour is associated with muscle weakness and poor mobility in older adults and carries increased risk for poor health and mortality (82). Both groups on average spent most of their daily activity (between 10 and 13 hours) in sedentary behaviour. The intervention may have caused this significant change in sedentary time in several ways. It may have been that the intervention directly influenced participants to spend less time sedentary and more time lightly active in a way which was not detected by our measures of physical activity. If replaced by activity, this comparative reduction in sedentary time could be expected to contribute to endothelial dysfunction improvements by increasing shear stress and NO bioavailability. However, sedentary time may instead have been replaced by sleep. This could be plausible if partaking in resistance/aerobic exercises had led to increased fatigue and/or prolonged sleep. Sleep would not increase shear stress in the vasculature, though it may play a role in reducing endothelial dysfunction as sleep deprivation has been linked to impaired FMD and increased CVD (90).

In the context of the manipulation check results, the intervention appears to have had conflicting effects on physical activity levels in this cohort. This makes it difficult to draw conclusions regarding the impact of physical activity on the primary outcomes of the study. However, important discussion can still be had regarding the effect of the overall exercise intervention on measures of endothelial dysfunction.

The FMD and LDI data align in many aspects, despite the small sample. This is consistent with previous research demonstrating a statistically significant association between the values of FMD and the magnitude of ACh-induced skin perfusion (91). In both FMD and LDI data the control group had healthier mean endothelial function at baseline and 6 months. Neither group saw a decrease in endothelial function in either measure; an unexpected finding in this cohort of CKD patients. Neither group had a statistically significant increase in endothelial dysfunction. Given that physical activity levels were not significantly increased by the intervention, this does not challenge the theory that increasing physical activity levels reduces endothelial dysfunction. The FMD values themselves also seem to correlate with previous data on average FMD values from a meta-analysis of FMD values comprising 16680 subjects across healthy participants and participants with CHD and diabetes mellitus. The meta-analysis reported average FMD values between 0.2-19.2% in healthy participants, -1.3-14% in CHD patients, and 0.75%-12% in diabetes mellitus patients. Given that CKD is closely linked to both CVD and diabetes, mean FMD across our whole study cohort was 6.02% (± 6.22% standard deviation) at baseline and 9.97% (± 6.82% standard deviation) at six months. Our results included several negative FMD values, implying that the maximum vessel diameter was reduced following shear stress exposure compared to baseline. Negative values are unusual but were also reported in this large-scale meta-analysis, and so are consistent with previous findings (92).

Limitations

There are many limitations to this study and as such our results must be interpreted cautiously. These will be discussed under the following five subheadings to consider limiting factors related to the wider setting of the study, the sample population, the intervention, the outcomes chosen, and specifics regarding how the outcomes were measured.

The wider setting of the study

Starting this study intervention within a year of the onset of the international COVID-19 pandemic poses a limitation. The external influences of the pandemic will have affected both groups equally, so are less likely to have affected between-group comparisons, but are worth mentioning and may have added additional layers of individual data variation to our already small sample. It remains unknown whether these results would be replicated during pre- or post-pandemic circumstances. The pandemic created challenges recruiting and testing chronic disease patients despite social distancing fears and isolation episodes during coryzal symptoms. The team had to undertake a design change for the intervention away from the initially planned in-person exercise sessions, and develop an alternative mode of delivery in uncertain conditions. Society was still growing accustomed to virtual communication at the time; since the intervention was carried out, there have been societal advances in virtual interventions and communication methods which may have made a similar home-based intervention more effective and acceptable for participants.

Physical activity is a complex behaviour affected by demographic, biological, cognitive, emotional, sociocultural and environmental factors; and evidence suggests that national COVID-19 restrictions reduced physical activity overall in the UK adult population, although this may be returning to pre-pandemic levels as restrictions have eased (93-96). There were multiple national COVID-19 restrictions in place during the exercise intervention timeframe from March 2021 to February 2022 in Wales. The period started during a lockdown in Wales with a ”stay at home” order, with restrictions easing generally throughout the period but occasionally heightening by appearance of the Indian, Delta and Omicron variants of COVID-19. These events may have affected patterns of physical activity, as well as physical and mental health, within the cohort. The fact that the participants did not start and finish the intervention concurrently may have reduced the impact of these external events on the results.

The sample population

Sample size is an important limitation of this study. The small sample limits the ability for randomisation to create groups with equal possibility of changing their activity levels and endothelial function in response to different stimuli. It limits the power of the study to draw statistically significant conclusions, even with a less stringent P value of 0.1. To display the heightened effect of individual data on mean data, individual data was presented graphically alongside the results for each outcome. To help gage whether results may have been meaningfully different, if not statistically significant, an MCID was pre-determined for each primary outcome.

The baseline characteristics of the study population align relatively well in terms of age, sex, BMI and blood pressure between the study groups. Groups had similar blood results including kidney function, lipid profiles, inflammatory markers and glycated haemoglobin. Notably, the causes of CKD varied greatly between groups. This is perhaps inevitable in a generalised CKD study of this size given the broad range of causes of CKD; however, it is possible that this affected the way that the intervention acted on participants' endothelial function. There was a lack of ethnic diversity within this population, and socioeconomic status was not recorded, limiting the generalisability of this data. The study population live in a predominantly rural area, and as such the results may not be representative of the urban CKD population, who may have easier access to health facilities, active commuting and public transport, for example.

The generalisability of this study to the wider CKD population is additionally limited by the fact that 857 (93%) of the 925 screened CKD patients were not eligible due to the specific exclusion and inclusion criteria. The most prevalent stage of CKD is stage 3; this stage represents over half of all-stage CKD cases in both national and international data (17, 97). As such, the low eligibility rate is unlikely to be explained by including only those with stage 3 and 4 CKD. It is more likely to be explained by other exclusion criteria or the requirement to have had a minimum decline in eGFR of 5ml/min/1.73m2 per year over previous 12 to 24 months (including a minimum of 3 measurements of eGFR over 12 months).

Furthermore, participation bias is common in exercise research, wherefore those agreeable to participate in studies of this field have been found to be more positive about their health, physically fitter, on fewer medications, and less likely to suffer from chronic pain (59, 98, 99). Of the 34 eligible patients invited to join this study, 18 (53%) refused. 28 patients who formally met the inclusion criteria for the study were not approached by the research team due to a clinical decision by the doctor that they had medical conditions which made them unsuitable for the study. Six patients were also discounted from the study having not been contacted due to time constraints. This recruitment process could certainly have allowed for participation bias as well as further selection bias to affect the study cohort, further limiting the generalisability of the data. Any atypical attributes of the participants, such as activity level or motivation, could affect both groups equally thanks to the randomisation process. However, all these factors put the study at risk of misrepresenting the target population.

The intervention

The exercise intervention was carefully targeted with multiple components aimed at maximising impact in this chronic disease population despite their physiological limitations and the effects of the COVID-19 pandemic. However, some aspects of the design and delivery of the intervention may limit the study. The exercise programme and self-reported adherence measures both required technical ability from participants to access and use an electronic spreadsheet. The lack of observation during exercise sessions may have permitted for incorrect exercise techniques and left adherence reporting open to response bias. The limited scope of the study meant that some researchers were involved in the delivery of the intervention as well as the collection of data at study visits, which limited the blinding of the study and increased the risk of biased data.

The intervention duration of six months, although longer than those seen many studies investigating endothelial dysfunction outcomes, limits the conclusions that can be drawn from the data. A longer intervention could have assessed for potential longer-term effects on endothelial dysfunction. Alternatively, an additional data collection visit at a later date could have assessed the ability for the intervention to have long-lasting effects in this cohort, even after the end of the intervention. For example, a randomised controlled trial of 422 underactive adults with at least one chronic condition followed participants up 15 months after the start of a three month exercise programme, and found that the exercise intervention group had maintained significant higher physical activity levels than the usual care group, indicating that the scheme had produced sustainable physical activity effects (93).

Another possible limitation of the intervention was that it neglected other aspects of behaviour change which could have been targeted, for example formal motivational interviewing. Evidence in Chronic Obstructive Pulmonary Disease patients suggests that interventions improved physical activity levels significantly more when combined with an element of counselling - predominantly based on the principle of goal-setting and implementation of that goal (100). Finally, this study did not incorporate any feedback from participants or researchers regarding the design and/or delivery of the intervention. Feedback may have helped interpretation of the results, particularly given the lack of evidence of any effect on physical activity following the intervention; without it only limited speculations can be made as to why this may have occurred.

The chosen outcomes

Due to the limited scope of this study, it was not possible to measure hard endpoints such as morbidity and mortality. Only surrogate outcomes of CVD (measures of endothelial dysfunction) could be measured. Short-term trials measuring surrogate outcomes are commonly used to improve trial efficiency. They have also been shown to overestimate treatment effects and miss significant harms, likely due to effects which are not mediated through the surrogate-to-final-outcome causal pathway (101). As such, any effects from this study must be interpreted as surrogate effects only.

A recent review has highlighted the diverse heterogeneity in outcomes used (and how those outcomes are measured and reported) in exercise research in CKD, raising concerns about the significant impact this may have on the inferences which can be derived and the potential for data-pooling into meta-analysis on this topic (59). Although our study employed some commonly used and validated measures such as the IPAQ, FMD, and accelerometery, the use of LDI in this context is relatively novel. Similar cutaneous methods such as laser Doppler flowmetry have shown promising results, but current research is insufficient to establish LDI as a confirmed marker of cardiovascular risk. As such, it is difficult to establish scientifically what would form a meaningful set of LDI results, and only limited conclusions could be drawn from these data. Additionally, exercise studies in CKD populations often report a quality-of-life measure, which could have been an important additional outcome in our study.

There are important limitations for the IPAQ. As a self-reported measure of physical activity over the preceding seven days, it is subject to recall bias and participants giving socially desirable answers (65). It was also developed for population-level surveillance, as opposed to intervention studies, and hence was not designed to be responsive to physical activity change (83, 102, 103). It was initially designed and tested in adults 18 to 69 years old, however has since been extrapolated across a far wider age range, including in the present study, in which there is limited evidence for its use (83, 102, 103).

IPAQ results are converted into METs based on a standardised list of activities with estimates of their corresponding energy use. However, given that people may carry out the same tasks (such as gardening) with varying degrees of intensity, and that resting metabolic rate is different between different individuals, this score system risks oversimplifying physical activity data. Older, frailer or less fit patients may find that a task considered by IPAQ cutoffs to be "light exercise" may actually require over three times their resting oxygen consumption per minute (>3 METs) and as such should be considered as "moderate exercise" by the METs classification (65). However, these limitations are difficult to avoid as other questionnaires carry similar risks of bias and inaccuracy, and METs provide a useful, widely used comparable measure amongst researchers.

Including accelerometer data alongside IPAQ scores helps to negate some of the limitations of including the questionnaire alone. However, the chosen accelerometer outcomes present limitations in themselves. Weekly MVPA and daily sedentary time are important measures, and are commonly used in studies and guidelines. But these do not account for light intensity exercise, which is also included in accelerometer results and may also have a mortality benefit(104). One of the advantages of accelerometers over questionnaires is that they can capture ubiquitous light-intensity activity throughout the day (29). Particularly in CKD patients who are highly sedentary, the absence of light exercise data limits interpretation of the physical

activity changes which occurred during this study and gives potential for missing an important change in this cohort.

Finally, this study did not report on endothelium independent vasodilation. Endothelium dependent vasodilation measures such as FMD and ACh infusion into forearm vessels are commonly referred to alone in research as measures of endothelial function (105). However, there are mechanisms which cause vasodilation which are not mediated by the endothelium. These processes are known as endothelium independent vasodilation. An example would be providing unconfined NO directly into the circulation. This can act on smooth muscle cells and cause vasodilation without requiring the endothelium to secrete any vasoactive agents (106). The vascular response to administering nitroglycerine, nitroprusside or other NO donors exogenously has therefore been used as part of endothelial function assessment in studies to measure endothelium independent vasodilation. There is some consensus that endothelium dependent and independent vasodilation should both be tested before defining endothelial function in studies. If both measures were impaired, for example, it could imply that the vascular smooth muscle response to NO is impaired, as opposed to endothelial function alone (107). As such, our study can only make limited conclusions about endothelial function.

The measurement of the outcomes

Although certain factors known to affect vascular testing were controlled, e.g. time of day, fasting, caffeine, body position, duration of cuff inflation - other factors such as temperature control in the room during study visits and stage of menstrual cycle were not actively controlled ((92, 108). Blood pressure can affect vascular perfusion but was not recorded during FMD or LDI readings. Dietary intake of nitrate is known to affect NO production and may subsequently affect measures of vascular function, but dietary habits were not reported or controlled in this study. Furthermore, although analyses of FMD and LDI were largely automated, there were elements of manual input for these processes and manual interpretation for FMD - as described in the methods - which could have introduced error. The shear rate area under the curve was not recorded during FMD assessment; a measure which could have helped compare stimulus for brachial artery dilation between groups and across timepoints. These limitations impacted both the control group and intervention group, so should not have impacted between-group comparisons, however with this small sample size the chances of invalid results may have been increased.

There are a wide array of possible limitations arising from using accelerometers to measure physical activity in this study. Accelerometers measure activity in terms of “counts” of acceleration in different axes. Research suggests that they may have a limited sensitivity for low-intensity and sedentary behaviour, and may include sitting, lying and standing within their metric of sedentary time – despite the accepted definition of sedentary behaviour requiring a seated or reclining posture (80, 82). Furthermore, it is important to consider the calibration process which converts the number of counts into metrics such as “moderate activity” or " sedentary activity”. Studies using direct observation and indirect calorimetry to assess accelerometer calibration softwares have reported that MVPA derived from monitors calibrated only to ambulatory activities is substantially underestimated compared to true MVPA values (104). Our study used the Actilife software Freedson1998 calibration, which was found to be one of the methods calibrated only to ambulatory activities in a large study of older adults using ActiGraph GT3X accelerometers. Broader calibration measures, aimed to capture lifestyle as well as ambulatory activity, were shown to give higher estimates of MVPA in this population, and align better with previous-day recall (104).

There is also controversy surrounding the optimal placement for accelerometers; with options including hip, wrist, thigh, lower back, chest and ankle placements. When examined in a small trial of eight participants wearing six triaxial accelerometers simultaneously whilst walking, jogging, sitting, lying, climbing stairs and standing, however all six aforementioned locations were found to be similar in their level of accuracy, however the hip was shown to be the optimal single location for recording data (109). The study tested a good range of normal daily activities, but lacked inclusion of resistance training work, such as upper body exercises, which may be neglected by hip placement (65).

The Actigraph accelerometers specifically used in this study have been shown in a large-scale trial to underestimate physical activity energy expenditure compared to doubly labelled water, and the authors proposed this could be linked to waist-worn accelerometery failing to register activities like arm movements, carrying weights, or walking uphill vs walking on the flat (29). It is possible that combining two locations such as wrist and hip concurrently may provide increased accuracy, although this could risk reducing the acceptability of the measure for participants (82).

Finally, participants were also asked to remove accelerometers whilst swimming and washing, which risked underestimating physical activity. On two accounts, participants wore accelerometers for less than seven days (both following their baseline visit; one participant for five and one for six days instead). Data was extrapolated from this time to give an estimated weekly MVPA. Guidelines recommend at least seven days of accelerometer monitoring in CKD patients, particularly to capture differences in activity over weekdays and weekends (65). Further technical issues limiting our results included accelerometers which went missing in the post whilst being delivered back to the lab, and an ultrasound scan machine fault during a study visit, which reduced our already limited data from a small sample.

Adherence data was also limited. Data was reported in a session-based form and did not include information regarding the intensity of exercise achieved during the exercise intervention (e.g. from heart rate monitors or a Rate of Perceived Exertion scale), posing an obvious limitation. Session-based adherence reporting is very common but may not adequately reflect the total volume of exercise performed (110). Resultingly, we were unable to monitor the intervention stated aim for participants to increase their exercise intensity over time to accumulate 40 mins of moderate intensity exercise four times a week by six months. Additionally, there was no specific goal defined for “acceptable adherence”. A decision was made not to set this goal, in part because goals set for adherence to non-pharmacological therapies are rarely set based on any sound theoretical framework, and in part because in this cohort of CKD patients, any improvement in activity is likely to represent a health benefit (110).

Due to their weekly contact with exercise group participants for exercise log discussions, the research team had more frequent communication with the intervention group than with the control group, and hence risked comparatively overreporting adverse events in the exercise group. Similarly, a final limitation to mention is that the control group did not receive any kind of sham or placebo form of the intervention, such as sham weekly virtual meetings. They still experienced monitoring of parameters including blood pressure, body mass index, and physical activity questionnaires and accelerometers at study visits. These interventions, and simply being part of the clinical trial, may have motivated the control group to increase or maintain their physical activity despite not participating in the exercise intervention. As such it is unclear whether the same results would have been noted in an exercise scheme being run outside of a clinical trial environment.

Conclusion

To our knowledge, the present study is the first evaluation of the effect of a six-month home-based mixed aerobic and resistance exercise programme on endothelial function in patients with CKD stages 3-4. Interpretation and generalisability of this study findings are limited by multiple factors and a small sample. However, in summary, the exercise programme did not improve physical activity or endothelial function despite a significant relative decrease in sedentary behaviour. These results contradict with our study hypothesis and with the limited previous evidence in this area, in which exercise training has successfully improved physical fitness and intermittently improved endothelial function in pre-dialysis CKD patients. Self-reported adherence was relatively high and did not wane over six months. There were correlations between both measures of endothelial function (microvascular and large vessel), but no evidence of a statistically significant effect on endothelial function as compared to the control group. This is plausibly due to failure of the exercise intervention to increase physical activity levels in the intervention group, as supported by aligning results from both questionnaires and accelerometers. This study highlights the challenging nature of designing, delivering and evaluating exercise interventions. It also highlights the importance of collecting and reporting physical activity measures alongside primary outcomes in exercise intervention trials.

Future studies in this field should consider less specific inclusion and exclusion criteria, inclusion of light-intensity or total activity counts from accelerometers, laboratory temperature control, reporting endothelium independent vasodilation data, and collecting feedback from participants and the research team about the exercise intervention to help elucidate the optimal intensity, modality, and volume of exercise training in this population.

Disclosure statement

This study was funded by the UK government via the NIHR Trainees Co-ordinating Centre (TCC); Grant Codes: ICA-CL-2017-03-020.

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