Exercise in Haemodialysis Patients: Impact on Markers of Inflammation

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Abstract

End-stage renal disease patients have a greatly increased risk of cardiovascular disease partly attributed to the elevated levels of systemic inflammation observed in uraemia. One of the key mechanisms underlying inflammation appears to be the immune dysfunction that afflicts almost every aspect of the uraemic immune system. As a consequence patients experience immunosuppression and reduced responsiveness to antigen as well as a simultaneous over-activation leading to a pro-inflammatory environment. In addition, the haemodialysis (HD) treatment itself induces a pro-inflammatory response but may provide an otherwise opportune time to complete supervised exercise.

Firstly, in characterising HD patients (Chapter 3), patients were found to be highly inactive compared with an age-matched healthy cohort. The patient group had higher circulating levels of all pro-inflammatory cytokines measured, pro-inflammatory monocyte phenotypes were elevated in number and proportion, anti-inflammatory regulatory T cells appeared to be diminished and neutrophils were less responsive to bacterial stimulant.

Exercise at an intensity that the patients could complete during HD was shown to be safe from an immunological perspective (Chapter 4). Exercise did not elicit leucocytosis, plasma IL-6, or subsequent cytokine cascade. Neutrophil degranulation to stimulant appeared temporarily suppressed but this was not statistically significant. Exercising during HD significantly altered blood pressure compared with the resting control trial (Chapter 5). Exercise induced an increase in blood pressure that was expected and the subsequent decrease seen post-exercise mimics that seen in non-chronic kidney disease hypertensive cohorts. Due to the potential negative connotations of intradialytic hypotension markers of cardiac injury were examined but no abnormalities found. The haemodynamic response to intradialytic exercise appears to be normal but superimposed onto the response to HD treatment.

Next, in a 6-month controlled training study (Chapter 6), regular exercise during HD that improved physical function had no clear impact on circulating cytokines but significantly altered the distribution of monocyte phenotypes away from the pro-inflammatory

intermediate subset that has been strongly associated with cardiovascular disease in chronic kidney disease (and other) cohorts; this is the first study in any cohort to report this training adaptation. In addition an increased number of the anti-inflammatory regulatory T cells were found after training.

These studies provide evidence that exercise is safe from an immunological perspective and lay the foundations for further research into the cardiovascular implications of intradialytic exercise. In addition, this work demonstrates regular exercise has significant anti-inflammatory effects that may be beneficial in ameliorating cardiovascular risk in this high-risk cohort.

Key words: Haemodialysis, end-stage renal disease, exercise, inflammation, monocytes, cardiovascular disease, immune dysfunction, regulatory T cells.

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Publications and presentations

To date, the following publications and presentations have been derived from the work presented in this thesis:

Peer-reviewed publications

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Scientific meeting proceedings

Dungey M, Young HML, Watson EL, Hull KL, Clarke AL, Ruttanaporn N, Bishop NC, Burton JO and Smith AC: Blood pressure during dialysis: The acute effect of exercise. *Abstracts from the British Renal Society Annual Conference 2013, Manchester, UK* (May 2013).

Dungey M, Bishop NC, Young HML, Burton JO and Smith AC: Effects of Acute Intradialytic Exercise on Blood Pressure and Circulating Cytokines. *Journal of the American Society of Nephrology, **24**: 457A: Abstracts from the American Society of Nephrology annual conference 2013, Atlanta, Georgia, USA* (November 2013).

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Invited communications

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Abbreviations

All abbreviations are defined within the text on the first instance.

ACE – Angiotensin converting enzyme

ANOVA – Analysis of variance

APC – Antigen-presenting cell

AVF – Arteriovenous fistula

BMI – Body mass index

CCL – C-C chemokine ligand

CCR – C-C chemokine receptor

CD – Cluster of designation

CKD – Chronic kidney disease

CKMB – Creatine kinase MB

COPD – Chronic obstructive pulmonary disease

CRP – C-reactive protein

cTnI – cardiac troponin I

CV – Coefficient of variation

CVD – Cardiovascular disease

CX_3CR – CX_3C chemokine receptor

DASI – Duke activity status index

DBP – Diastolic blood pressure

EDTA – Ethylenediaminetetraacetic acid

ELISA – Enzyme-linked immunosorbent assays

EPO – Erythropoietin

ES – Effect size

ESRD – End-stage renal disease

GFR – Glomerular filtration rate

GHS-R1a – Growth hormone secretagogue receptor 1a

GPPAQ – General practice physical activity questionnaire

HADS – Hospital anxiety and depression scale

HD – Haemodialysis

HDL – High density lipoprotein

h-FABP – heart-type fatty acid-binding protein

Ig – Immunoglobulin

IL – Interleukin

LDL – Low density lipoprotein

LPS – Lipopolysaccharide

LUSS – Leicester uraemic symptom score

MAP – Mean arterial pressure

MDRD – Modification of diet in renal disease study

MET – Metabolic equivalent of task

NHS – National Health Service

NIHR – National Institute of Health Research

PBMC – Peripheral blood mononuclear cell

PD – Peritoneal dialysis

pmp – per million population

RCT – Randomised controlled trial

RM – Repetition maximum

ROS – Reactive oxygen species

RPE – Rating of perceived exertion

RPP – Rate pressure product

RRT – Renal replacement therapy

SBP – Systolic blood pressure

SD – Standard deviation

STS 60 – Sit-to-stand 60 test

TGF – Transforming growth factor

TLR – Toll-like receptor

TNF – Tumour necrosis factor

Treg – Regulatory T cell

UF – Ultrafiltration

URTI – Upper respiratory tract infection

USRDS – United States Renal Data System

$\dot{V}O_{2peak/Max}$ – Peak/Maximum oxygen consumption

Brief glossary of specialist immune terminology

Cluster of designation (CD) – a protocol used for the identification of cell surface molecules that allows identification of cell phenotypes.

CD3 – a T cell co-receptor used as a marker to identify T cells.

CD4 – a co-receptor that assists the T cell receptor in communication with antigen-presenting cells; often used as a marker to identify T helper cells.

CD14 – a pattern recognition receptor for detection of lipopolysaccharide; often used as a marker to identify monocytes.

CD16 – Fc receptor that is present on neutrophils, natural killer cells and monocytes.

CD25 – alpha chain of the IL-2 receptor, this is present on activated T cells.

CD127 – Interleukin-7 receptor subunit and appears in an inverse relationship to FoxP3. Those cells that have a low expression of CD127 are highly suppressive $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T cells.

C-reactive protein (CRP) – an acute-phase protein i.e. the circulating levels rise in response to inflammation.

Cytokines – proteins released by cells as signals and regulate many aspects of the immune system. They can act in an autocrine, paracrine or endocrine-like manner. Cytokines include interleukins (IL) but also tumour necrosis factors (TNF), chemokines and interferons.

Degranulation – The secretion of cytotoxic granules from granulocytes (e.g. neutrophils) with the purpose of destroying a pathogen.

Elastase – A protease enzyme, i.e. it breaks down protein. Elastase breaks down elastin. The neutrophil form breaks down the outer membrane protein A of *E. coli* and other gram-negative bacteria.

Growth hormone secretagogue receptor 1a (GHS-R1a) – a receptor that binds and mediates the effects of ghrelin, recently discovered to be present on leucocytes.

Interleukin-1 beta (IL-1β) – a pro-inflammatory cytokine. IL-1β is involved in a number of functions including leucocyte proliferation, differentiation and apoptosis.

Interleukin-1 receptor antagonist (IL-1ra) – an anti-inflammatory cytokine. IL-1ra binds to IL-1 receptors inhibiting IL-1α and IL-1β signalling.

Interleukin-6 (IL-6) – an inflammation responsive cytokine. IL-6 has a number of roles in stimulating the immune response and inflammation but also stimulates an anti-inflammatory response (see page 14).

Interleukin-10 (IL-10) – an anti-inflammatory cytokine. Down-regulates many aspects of the cellular immune response and promotes a humoral response.

Leucocytes (white blood cells) – heterogeneous cells of the immune system that defend the body from infection and disease through multiple functions.

Lymphocytes – mononuclear leucocytes mainly involved in the adaptive immune system. Lymphocytes are mainly agranular and include T cells, B cells and natural killer cells (although these are granular and part of the innate immune system)

Monocytes – circulating mononuclear leucocytes, the main functions are phagocytosis, antigen-presentation and cytokine production. They differentiate into macrophages upon migration into tissue.

Neutrophils – granulocytes involved in the innate immune system. Neutrophils are a major aspect of the defence against bacterial infection.

Peripheral blood mononuclear cells (PBMCs) – Collectively lymphocytes and monocytes

Reactive oxygen species (ROS) – secreted by some leucocytes as an antimicrobial defence. Accumulation of ROS may lead to oxidative stress which is pathogenic and associated with inflammation.

Regulatory T cells (Tregs) – T cell subset involved in the regulation and suppression of the immune system.

Tumour necrosis factor alpha (TNF-α) – a pro-inflammatory cytokine. Roles include inducing inflammation, fever, cachexia and apoptotic cell death.

Chapter 1

Background

1.1 Chronic kidney disease: an overview

The kidneys have multiple functions that are imperative to life. The kidneys regulate water and electrolyte balance, filter metabolic waste products and potentially harmful foreign substances, control acid-base balance, secrete hormones (e.g. renin, erythropoietin, 1,25-dihydroxyvitamin D_3) and have a role in gluconeogenesis during prolonged fasting. As a result of these numerous essential roles, any disease of the renal system can have various profound and complex pathological consequences.

Chronic kidney disease (CKD) is a collective term used to describe various disorders affecting the function and structure of the kidney, confirmed on two or more occasions at least 3 months apart, leading to deteriorating renal function and abnormal biochemistry. These disorders may vary in severity, causality, pathology and rate of progression (Levey and Coresh, 2012). The severity of CKD is defined by reduced glomerular filtration rate (GFR) and / or the presence of proteinuria. This severity can be classified between stage 1 and stage 5, the higher stages being the most severe (Table 1.1). In the clinical setting an equation is usually used to estimate GFR (eGFR)[1]; the simplified MDRD (modification of diet in renal disease) equation is commonly used in the UK which factors in sex, age, ethnicity and serum creatinine concentrations (Levey *et al.,* 2000).

General nephrology patients (stages 1-3a) are commonly asymptomatic and therefore frequently undiagnosed. The symptoms that are associated with high levels of plasma urea, known as uraemia, often only become apparent when CKD is more advanced (e.g. itching, polyuria, insomnia, fatigue, loss of appetite, anaemia). CKD progression is an irreversible process with escalating complications; patients who progress to CKD stage 5 may require renal replacement therapy (RRT) in order to maintain life (indicated by pulmonary oedema, severe metabolic acidosis, severe biochemical derangement, progressive uraemia or hyperkalaemia). Patients receiving RRT are defined as end-stage renal disease (ESRD) patients.

[1] True measures of GFR can be obtained using the gold-standard inulin, 51Cr-EDTA, 125I-iothalamate or iohexol methods; however, eGFR is more frequently used.

Table 1.1 – Progression of chronic kidney disease

Stage	Description	GFR ($mL \cdot min^{-1} \cdot 1.73m^{-2}$)
1	Normal/increased GFR with other evidence of kidney damage	> 90
2	Mild reduction in GFR with other evidence of kidney damage	60-89
3a	Moderate reduction in GFR with or without other evidence of kidney damage	45-59
3b		30-44
4	Severe reduction in GFR with or without other evidence of kidney damage	15-29
5	Established renal failure	< 15 (or dialysis)

GFR, Glomerular filtration rate.
The suffix (p) is used to denote the presence of proteinuria when staging CKD, and proteinuria is defined as urinary albumin to creatinine ratio ≥ 30 $mg \cdot mmol^{-1}$, or protein to creatinine ratio ≥ 50 $mg \cdot mmol^{-1}$.
(National Collaborating Centre for Chronic Conditions, 2008)

For clarity, in this thesis ESRD is used to define patients who require RRT to maintain life, of which there are haemodialysis (HD) and peritoneal dialysis (PD) patients; pre-dialysis is used to describe patients with recognised CKD who have not progressed to ESRD (usually stages 3b-5) and CKD is used to describe the entire chronic kidney disease population (including ESRD).

Transplantation is often the RRT of choice and is associated with the best survival and quality of life (Thiruchelvam *et al*., 2011). Not all CKD patients are eligible for transplantation; those who are may receive transplant organs from living or cadaver donors. Demand greatly exceeds supply of cadaver organs therefore patients are added to a transplant waiting list. The majority of ESRD patients receive a form of dialysis treatment; HD or PD. In PD fluid is inserted into the abdominal cavity and the peritoneum is used as a membrane across which waste products diffuse. Alternatively, patients may opt for conservative treatment and enter end-of-life care.

Haemodialysis

HD, the more common form of dialysis, is the process of circulating blood through an extracorporeal disposable dialyser. The dialyser contains hollow fibres of a semi-permeable material (impermeable to blood cells and protein but permeable to solutes)

through which highly-treated water flows in the opposite direction to the blood; this counter-current flow maintains the highest possible gradient to maximise dialysis efficiency. The highly purified water (dialysate) contains low concentrations of factors to be removed from the blood and high amounts of bicarbonate to correct acidosis. Fluid is removed through creation of a pressure gradient across the dialysis membrane so the ultrafiltration (UF; removal of fluid) can be controlled. The vascular access of choice is a matured arteriovenous fistula (AVF), created through anastomosis of an artery to a vein (usually in an arm) causing arterialisation of the vein that is then accessible and with good blood flow. If an AVF is unavailable or has failed an indwelling catheter may be used or a graft may be inserted.

Current UK medical guidelines in the UK are for HD to be prescribed "either 3 sessions per week of at least 4 hours duration or more frequent dialysis" (Mactier, Hoenich and Breen, 2009). Treatment is not usually sufficient to completely normalise biochemistry but can provide adequate control of biochemistry and minimise symptoms. Other complications associated with kidney failure are treated through diet modification and medications; some of these are added to the blood during HD (e.g. EPO for anaemia) along with an anticoagulant to prevent clotting during treatment.

Incidence and prevalence

CKD is a worldwide health problem associated with increasing incidence, prevalence and poor outcome and consequently escalating health costs. Incidence of ESRD is as high as 200 per million population (pmp) per year in many countries and nearing 400 pmp in the United States (United States Renal Data System (USRDS), 2013). In the UK the incidence rate of RRT in 2012 was 108 pmp (Gilg, Rao and Fogarty, 2013).

A worldwide systematic review analysing 26 studies reported a median prevalence of 7.2% with CKD stage 3 and greater in individuals aged > 30 y, and between 23.4% and 35.8% in those aged > 64 y (Zhang and Rothenbacher, 2008). The age-standardised prevalence of stage 3-5 CKD in the UK is estimated to be 8.5% (10.6% in females and 5.8% in males; Stevens *et al*., 2007). At the end of 2012 the total RRT prevalence in the UK was 54,824 patients, equating to 861 pmp, considerably lower than the US (1976 pmp), but

similar to that of other northern European countries (Shaw *et al.*, 2013; USRDS, 2013). Despite transplantation rates increasing annually in the UK over the last 5 y HD prevalence rates have also increased from 323 to 367 pmp between 2006 and 2012 (Shaw *et al*., 2013). With continuous strains on the NHS budget the costs of ESRD should also be considered. The average annual cost of HD is around £30,000 per patient, the increasing prevalence of ESRD is therefore coupled with escalating medical costs and already accounts for more than 2% of the total NHS budget (Feehally *et al*., 2008). It is clear that CKD should be considered a national and worldwide health priority.

Increasing incidence can partly be attributed to the ageing population; however, incidence rates are increasing in all age categories (Gilg, Rao and Fogarty, 2013). An increase in a number of modifiable risk factors that are involved in the pathogenesis of CKD may also be to blame (Table 1.2). Physical inactivity, poor diet, smoking and drug abuse contribute to a large number of initiation and progression factors cited, all of which are highly modifiable with lifestyle behaviour changes.

Table 1.2 - Risk factors for chronic kidney disease

Non-modifiable	**Modifiable**
Old age (S)	Systemic hypertension (I, P)
Male sex (P)	Diabetes mellitus (I, P)
Race/ethnicity (S)	Proteinuria (P)
Genetic disposition (S)	Dyslipidaemia (I, P)
Family history (S)	Smoking (I, P)
Low birth weight (S)	Obesity (I, P)
	Alcohol consumption (I, P)
	Infections/infestations (I)
	Drugs and herbs/analgesic abuse (I)
	Autoimmune diseases/obstructive uropathy/stones (I)
	Low socio-economic status (S)

S, Susceptibility factor; I, Initiation factor; P, Progression factor.
Adapted from El Kossi *et al*., 2007.

Causes of chronic kidney disease

The causes of CKD vary depending on the patient demographics (e.g. age, race, geographical area). In the UK the most frequent causes of CKD are diabetes mellitus (25.6% of incident ESRD patients; Gilg, Rao and Fogarty, 2013), essential hypertension and atherosclerotic renal vascular disease (Kumar and Clark, 2012). Of note, the most common causes of renal disease in the UK are secondary to systemic conditions rather than primary renal diseases. A summary of some of the main causes of CKD can be found in Table 1.3.[2] A number of cases remain uncertain because the cause is often insignificant to treatment and kidney biopsies required for diagnosis are of an invasive nature.

Table 1.3 – Common causes of chronic kidney disease

Congenital and inherited diseases
Polycystic kidney disease
Tuberous sclerosis
Congenital obstructive uropathy
Glomerular disease
Primary glomerulonephritis
Secondary glomerulonephritis (e.g. diabetes mellitus)
Tubulointerstitial disease
Drug abuse (e.g. analgesics)
Diabetes mellitus
Toxins
Urinary tract obstruction
Prostatic obstruction (e.g. hypertrophy, tumour)
Gynaecological cancer
Calculi (e.g. renal stones)
Vascular disease
Hypertensive nephrosclerosis
Reno-vascular disease
Small and medium-sized vessel vasculitis

Adapted from Kumar and Clark (2012)

[2] In the UK the primary renal diagnoses are grouped into 8 categories: Uncertain, Glomerulonephritis, Pyelonephritis, Polycystic, Renal vascular disease, Hypertension, Diabetes and Other (UK Renal Registry, 2010).

Complications of end-stage renal disease

Regardless of the underlying cause of CKD the overall outcome is similar; a reduction in the number of functionally active nephrons and consequently a progressive loss of renal function. The aims of treatment for CKD patients are to slow the progression of CKD, reduce the cardiovascular risk and treat the complications. If CKD is diagnosed at an early stage progression may be slowed if the underlying cause can be treated. However, in some individuals, fibrosis of the remaining nephrons and vasculature results in a progressive loss of renal function.

Progressive renal dysfunction is associated with numerous complications (Table 1.4); these symptoms can vary between individuals because of different aetiologies of CKD, different co-morbidities and individual differences. Some symptoms can be ameliorated through careful dietary control, fluid restriction and dialysis treatment, some require daily medication and many have to be managed rather than treated. What is common is that complications are usually exacerbated by progression of CKD and are particularly prevalent in ESRD patients receiving HD.

HD patients must also contend with the complications that are associated with the HD treatment itself. The removal of fluid disturbs haemodynamics and can lead to excessive fatigue, nausea, cramps and acute hypotensive events. Problems with vascular access are common, aneurysms and thrombosis are dangers and the risk of infection is substantially increased (particularly in those without an AVF access). Aside from physiological symptoms the psychosocial burden of HD is great. A large amount of time each week is set aside for treatment and transportation leading to a disrupted schedule. Frequently ESRD patients have self-confidence and body image problems due to fistulae or superficial symptoms and sexual function and fertility is impaired. Depression is as prevalent as 20-30%, which is higher than seen in other chronic diseases (Hedayati and Finkelstein, 2009).

Table 1.4 - Principal clinical features of uraemia

	Symptoms
Central nervous system	Diurnal somnolence, night insomnia, memory and concentration disorders, asthenia, headache, confusion
Peripheral nervous system	Polyneuritis, restless legs, cramps
Gastrointestinal	Anorexia, nausea, gastroparesis, parotiditis, stomatitis
Haematologic	Anaemia, haemostasis disorders
Cardiovascular	Hypertension, atherosclerosis, coronary artery disease
Skin	Itching, skin dryness, calciphylaxis
Endocrinology	Growth impairment, impotence, infertility, sterility
Osteoarticular	Secondary hyperparathyroidism, osteomalacia, β2-microglobin amyloidosis
Nutrition	Malnutrition, weight loss, muscular catabolism
Immunity	Low response rate to vaccination, increased susceptibility to infectious disease
Biochemical	Metabolic acidosis, hyperphosphataemia, hyperkalaemia

Adapted from Almeras and Argiles, 2009.

Prognosis

Not all CKD patients progress to ESRD; rather, in all stages of CKD, death is a far more common event than the initiation of RRT. Keith and colleagues followed 27,998 patients with CKD stages 2, 3 and 4 for a 5-y observation period with 1.1%, 1.3% and 19.9% reaching ESRD, and mortality rates of 19.5%, 24.3% and 45.7% respectively (Keith *et al*., 2004). ESRD patients can be regarded as a select group of patients that have survived many years of the unfavourable pro-atherogenic and pro-inflammatory uraemic milieu.

Patients diagnosed with the need for RRT have an immediately poor prognosis. The 1-y age-adjusted survival rate in 2011 for UK incident HD patients was 89.3%; 5-y survival rate for incident RRT patients (unadjusted for age) was 53.3% and 10-y survival was 28.2% (Pruthi, Steenkamp and Feest, 2013). The median life years remaining for a 25-29 y-old on RRT is under 20 y and the risk of death is 25-fold greater than the general

population (Pruthi, Steenkamp and Feest, 2013). ESRD can be seen as representing extreme premature physiological ageing (Stenvinkel, 2010); as a consequence, the cardiovascular event risk for a dialysis patient aged 40 y is about the same as that of someone aged 80 y without CKD (Foley, Parfrey and Sarnak, 1998).

In addition to the substantially increased mortality rate in ESRD patients many parameters of quality of life are diminished. It is well established that patients requiring HD have a concerning decreased quality of life compared with the general population. Many interventions that have been proposed to improve mortality and morbidity, such as increased HD frequency or dose, have had little or no improvement on quality of life (Kimmel, 2013). A more holistic approach to improving health and health-related quality of life appears desirable (Cukor *et al*., 2007).

1.2 Cardiovascular disease in chronic kidney disease

The most frequent fatal outcome of CKD is cardiovascular disease (CVD) (Levey and Coresh, 2012). Recent data from the US reported 42.3% of CKD deaths were caused by cardiovascular related diseases (acute myocardial infarction, congestive heart failure, cardiac arrest, cerebrovascular accident and other cardiac and vascular deaths; USRDS, 2013). This number was lower in the UK but was still the most common cause of mortality, with 28% of deaths in ESRD patients accounted for by CVD (Pruthi, Steenkamp and Feest, 2013).

Deteriorating kidney failure is associated with a substantially increased risk of CVD at every stage of CKD. Even in the early stages of CKD the risk of a cardiovascular event is elevated and this is worsened by disease progression (Schiffrin, Lipman and Mann, 2007). In a meta-analysis incorporating over 1.2 million individuals the cardiovascular mortality rate was twice as high in stage 3 CKD and thrice as high in stage 4 CKD than those of normal kidney function (Matsushita *et al*., 2010).

Why is chronic kidney disease associated with cardiovascular disease?

The increased risk of CVD in CKD can be partly ascribed to high prevalence of the traditional risk factors for CVD including high systolic blood pressure (SBP), high total and LDL cholesterol, low HDL cholesterol, prevalence of diabetes, smoking and old age (Saran and DuBose, 2008; Wilson *et al*., 1998). CKD itself is considered a well-established risk factor for CVD and the American Heart Association recommend classifying individuals with CKD (particularly advanced CKD) in the highest risk groups for CVD (Sarnak *et al*., 2003).

In HD patients the importance of some of the traditional CVD risk factors have recently been questioned (Kalantar-Zadeh, 2005). Paradoxical results regarding obesity and cholesterol levels have been reported in large observational studies with high body mass index (BMI) and LDL cholesterol concentrations actually appearing protective against all-cause mortality (Kalantar-Zadeh *et al*., 2005). It appears a reverse epidemiology exists in the ESRD population, factors that would normally signal improved survival rates in the general population are associated with a worse prognosis in HD patients. High BMI for example, despite being a risk factor for CKD and associated with faster disease progression in pre-dialysis patients, appears protective against mortality in patients who have progressed to ESRD (Kalantar-Zadeh *et al*., 2005).

One suggestion for why this reverse epidemiology exists is a possible '*time discrepancy*' in the risk factors. Traditional risk factors such as obesity and hypercholesterolaemia, are often deleterious over the long-term, whereas novel risk factors such as inflammation and protein-energy malnutrition appear to rapidly influence short-term survival in ESRD (Kalantar-Zadeh *et al*., 2003). The proposal is that because the adverse effects of novel risk factors develop more rapidly in ESRD than those caused by traditional risk factors, patients die before traditional risk factors can be fatal (Kalantar-Zadeh *et al*., 2003). It appears in the presence of severe uraemia 'novel' risk factors for CVD have emerged that may be of greater importance. Inflammation and immune dysfunction and malnutrition have been found to be strong predictors of CVD and mortality (Kalantar-Zadeh *et al*., 2003; Stenvinkel, 2001).

1.3 Malnutrition-inflammation complex

Patients with advanced CKD have a high prevalence of inflammation and protein-energy malnutrition with epidemiological evidence suggesting a strong association between inflammation and malnutrition and greater mortality risk (Honda *et al*., 2006; Stenvinkel, 2001). Both inflammation and malnutrition are strongly associated with each other; however, the contributions of each aspect to poor outcome in CKD are not well defined "Malnutrition-inflammation complex syndrome" has been suggested to denote the important contribution of both these conditions to ESRD outcome (Kalantar-Zadeh *et al*, 2003). In ESRD the combination of these related conditions is associated with muscle wasting (cachexia), CVD and all-cause mortality; and is more powerfully associated than most traditional risk factors (e.g. hypertension, BMI; Kalantar-Zadeh, 2005).

Inflammation

Under normal conditions inflammation is a local, involuntary, protective response to trauma such as microbial invasion or injury; classically presenting with swelling, redness, pain and fever (tumor, rubor, dolor and calor). The purpose of inflammation is to isolate, inactivate and remove the cause of inflammation and any damaged cells so healing can take place. Inflammation must be fine-tuned and precisely regulated because a deficient or excess inflammatory response may cause morbidity and shorten lifespan (Tracey, 2002).

Inflammation is a complex process involving interaction of a number of leucocytes, cytokines, enzymes and various systems: including the complement, kinin-kallikrein, coagulation and fibrinolysis systems. Inflammation is usually a transient response, when the stimuli persist or when anti-inflammatory systems are dysfunctional the acute inflammatory response becomes chronic. Chronic low-grade systemic inflammation has been introduced as a term for conditions in which a typically two- to three-fold increase in the systemic concentrations of tumour necrosis factor (TNF)-α, interleukin-1 (IL-1), IL-6 and C-reactive protein (CRP) is reflected. Unlike acute inflammation that is necessary to contain, heal and stimulate the immune response; a chronic inflammatory state is often harmful.

Empirical evidence links chronic low-grade inflammation with disorders of several body systems and tissues, including the circulatory (atherosclerosis, heart failure), endocrine (insulin resistance, metabolic syndrome), skeletal (sarcopenia, arthritis, osteoporosis), pulmonary (chronic obstructive pulmonary disease) and neurological (dementia, depression) systems, along with many other adverse health conditions now thought of as inflammatory disorders (Khansari, Shakiba and Mahmoudi, 2009). Even in the absence of disease chronic inflammation is a robust predictor of morbidity and mortality (Harris *et al.,* 1999; Ridker *et al.,* 1997).

Due to the common role of inflammatory diseases in the aetiology of CKD it is difficult to confirm whether renal failure directly causes inflammation during the early stages of CKD. In ESRD numerous potential causes of inflammation have been suggested including those that relate to the uraemic environment as well as the dialysis procedure (Table 1.5) leading to an increased production and decreased excretion of inflammatory factors. It is well acknowledged that HD treatment *per se* has some impact upon systemic inflammation; however, the elevations in systemic inflammation observed in pre-dialysis patients suggest that other mechanisms also have an integral role (Carrero and Stenvinkel, 2010).

Table 1.5 – Underlying causes of inflammation in chronic kidney disease patients

Causes of inflammation in CKD
Decreased glomerular filtration rate
Decreased clearance or increased production of pro-inflammatory cytokines
Volume overload
Oxidative stress
Deteriorating nutritional state and food intake
Alteration in body composition
Uraemic toxins (e.g. urea, advanced glycation end products [AGEs])
Infection
Genetic and epigenetic factors
Additional inflammatory factors related to dialysis treatment in CKD
Intravenous catheter, peritoneal dialysis catheter and its related infections
Dialysis membranes with poor biocompatibility
Impurities in dialysis water and dialysate
Back-filtration or back-diffusion of contaminants
Constant exposure to peritoneal dialysis solution
Peritonitis

CKD, Chronic kidney disease. Adapted from Cheung, Paik and Mak, 2010.

Alterations to circulating cytokines, seen during inflammation and in ESRD, have systemic consequences. The acute-phase reactant CRP is released from hepatocytes in response to inflammation and can increase up to 1,000-fold in response to infection or trauma (Gabay and Kushner, 1999). CRP is widely used as a marker of general inflammation or infection in the clinical setting. Normal circulating CRP levels in western populations are usually between 1-3 $mg \cdot L^{-1}$ or lower, whereas dialysis patients demonstrate average CRP concentrations of 7-8 $mg \cdot L^{-1}$ (median 4-5 $mg \cdot L^{-1}$) (Carrero and Stenvinkel, 2010). Furthermore, as eGFR declines from 60 to 15 $mL \cdot min^{-1} \cdot 1.73m^{-2}$ the probability of having elevated CRP increases from 44% to 81% (Eustace *et al*., 2004). Increases, or persistent elevations in CRP or IL-6 are highly predictive of mortality (Tripepi, Mallamaci and Zoccali, 2005); although IL-6 is reported as a better prognostic marker than CRP in ESRD patients (Barreto *et al*., 2010).

The roles of IL-6 are particularly interesting due to apparent discords depending upon the context and source. Elevations in circulating concentrations of IL-6 at rest can be attributed to adipocytes and macrophages resident to the adipose tissue, immune dysfunction and other uraemic sources of inflammation (Table 1.5). However, an acute infusion of IL-6 (similar to that seen after strenuous exercise) leads to a transient anti-inflammatory cascade of cytokines (IL-10, IL-1 receptor antagonist [IL-1ra] and the hormone cortisol) without the stimulation of pro-inflammatory cytokines (IL-1 and TNF-α) (Steensberg *et al*., 2003). Thus it appears that increases in IL-6 that are well regulated by an effective anti-inflammatory response are not detrimental; conversely, when IL-6 remains chronically elevated and represents an inflammatory environment (and immune dysfunction) these concentrations are associated with poor outcome. The functions of IL-6 include stimulating the differentiation of B cells, neutrophil production in bone marrow, fuel mobilisation, fever, inflammation and the acute-phase response.

TNF-α is a potent pro-inflammatory cytokine primarily responsible for tumour cell killing. In addition, TNF-α induces IL-1 and IL-6 secretion, fever, inflammation, cell apoptosis and catabolism. Consequently, dysregulation of TNF-α, which appears frequently in CKD patients, is deleterious for health (Stenvinkel *et al*., 2005).

Both IL-6 and TNF-α have been implicated in the pathogenesis of endothelial dysfunction, atherogenesis and catabolism (Table 1.6). In addition, CRP may also have pro-atherogenic roles. These findings demonstrate the important contribution of inflammation in two of the greatest causes of morbidity and mortality in ESRD patients.

Table 1.6 – Pro-atherogenic and pro-catabolic roles of IL-6, TNF-α and CRP

	Pro-atherogenic roles	**Pro-catabolic roles**
IL-6	↓ endothelial function via inhibition of nitric oxide ↑ adhesion molecule expression ↑ plaque formation ↓ adiponectin	↓ IGF-1 signalling ↓ appetite ↑ muscle protein breakdown
TNF-α	↑ vascular calcification ↑ endothelial dysfunction ↑ insulin resistance ↓ apolipoprotein E ↑ NADPH oxidative pathways	↓ MyoD = ↓ muscle differentiation and repair ↑ ubiquitin proteasome pathway ↑ muscle protein breakdown ↓ appetite
CRP	↑ monocyte adhesion and migration ↑ binding of oxidised LDL ↓ endothelial function via inhibition of nitric oxide	

CRP, C-reactive protein; IGF, Insulin-like growth factor; IL-6, Interleukin-6; LDL, low-density lipoprotein; NADPH, Nicotinamide adenine dinucleotide phosphate; TNF, Tumour-necrosis factor. Table adapted from Stenvinkel *et al.,* 2005 with data from Yousuf *et al.,* 2013.

It is also noteworthy that a number of anti-inflammatory cytokines are reported to be elevated in CKD. Anti-inflammatory cytokines such as IL-10 and IL-1ra are shown to be elevated in uraemia (Descamps-Latscha *et al*., 1995; Morita *et al*., 1997) possibly as a response to chronic inflammation or due to a reduction in their normal excretion through the kidneys. In the majority of CKD patients the elevations in pro-inflammatory factors far outweigh any anti-inflammatory increases; however, the patients with the highest IL-10 levels have a better immune balance, as shown by improved response to vaccination (Girndt *et al*., 1995).

1.4 Immune dysfunction in chronic kidney disease

Despite chronic inflammation in these patients, immunosuppression is also apparent. It was first noted that immune function must be compromised in CKD patients when improved skin graft survival was observed in this population (Dammin *et al*., 1957). This was substantiated by a lower response to vaccination in CKD patients than in healthy individuals (Rautenberg *et al*., 1988). Infection is one of the leading causes of death in ESRD patients behind CVD. Despite improvements in infection control, infection still accounts for 17% of deaths in ESRD patients in the UK (Pruthi, Steenkamp and Feest 2013). The annual mortality secondary to sepsis is approximately 100- to 300-fold higher in ESRD patients than the general population; these findings remain robust after a wide range of sensitivity analyses (Sarnak and Jaber, 2000).

Immunosuppression and immune over-activation in ESRD patients are complex and inexorably linked; consequently, HD patients find themselves at both ends of Figure 1.1. Increased inflammation and risk of infection can be partly explained by various features of immune dysfunction. It appears that almost no aspect of immune function is left unaffected by the uraemic milieu associated with CKD. The following summarises various aspects of the immune system and their potential role in increased inflammation and immune anergy in the ESRD population (Figure 1.2). For more in-depth information refer to published reviews (Betjes, 2013; Eleftheriadis *et al*., 2007; Girndt *et al*., 1999; Hauser *et al*., 2008; Kato *et al*., 2008; Vaziri *et al*., 2012).

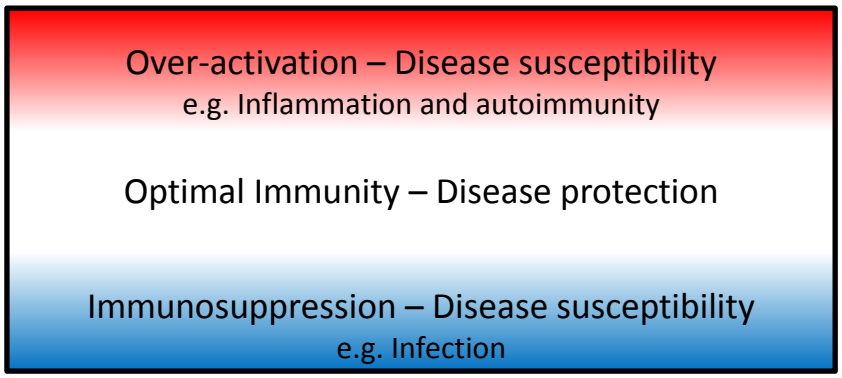

Figure 1.1 - Optimal and sub-optimal immune function. Haemodialysis patients are simultaneously at both ends of the diagram with both immunosuppression and over-activation. Adapted from Walsh *et al.,* 2011.

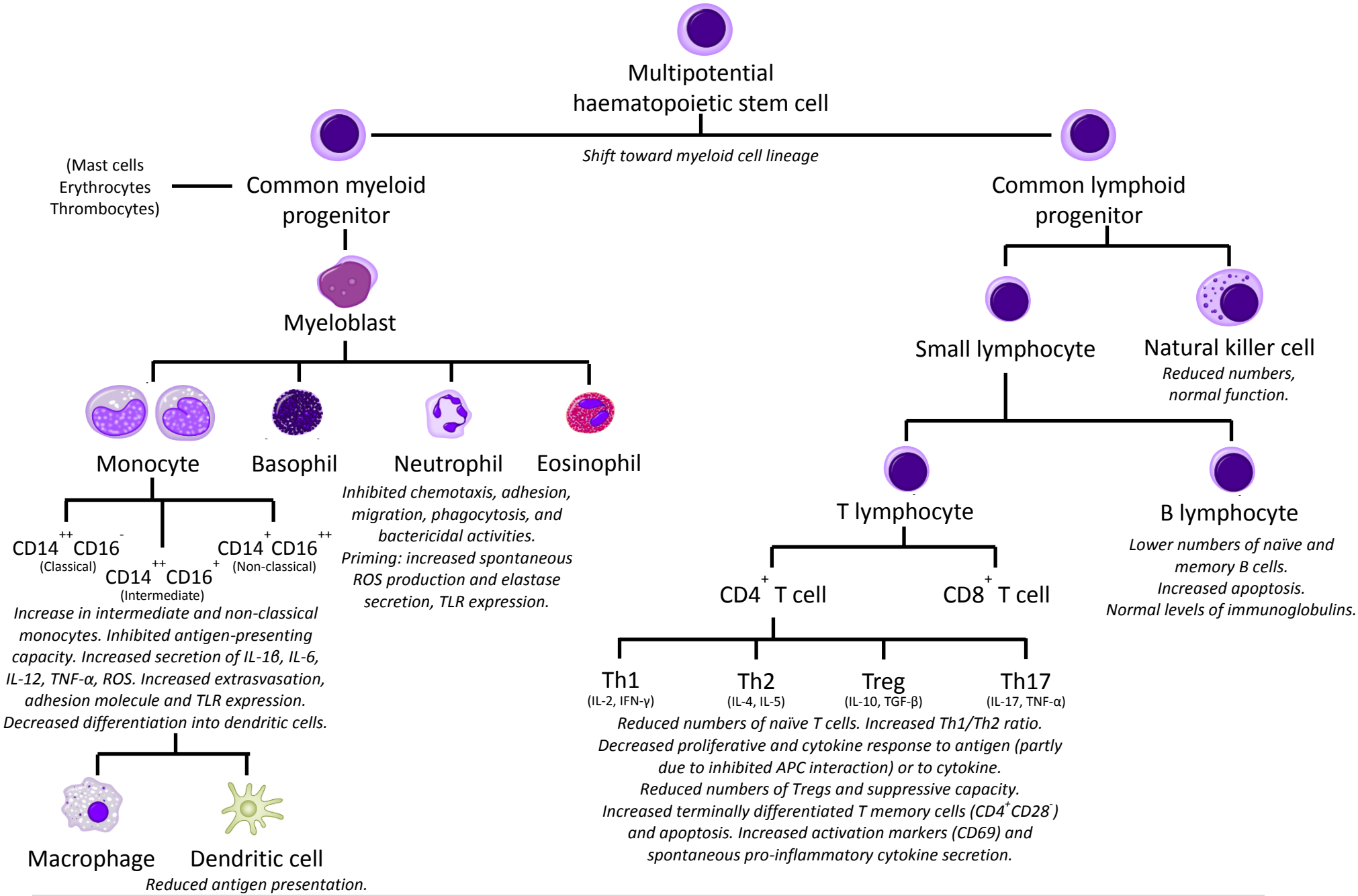

Figure 1.2 - The cellular immune system and end-stage renal disease. Italics denote the marked changes observed in end-stage renal disease. APC, antigen-presenting cell; IFN, interferon; IL, interleukin; ROS, reactive oxygen species; Th, helper T cell; Treg, regulatory T cell; TGF, transforming growth factor; TNF, tumour necrosis factor; TLR, toll-like receptor.

There is an apparent shift toward myeloid cells and away from lymphoid cell lineages in ESRD patients (Betjes, 2013). In addition to the numbers of each leucocyte subset alterations to function are also reported.

Neutrophils

Progression of CKD is associated with an increase in the number of neutrophils (Sela *et al*., 2005); however, overall neutrophil function is impaired. Studies have observed neutrophil chemotaxis, adhesion, migration, phagocytosis and bactericidal activities are all impaired and reactive oxygen species (ROS) production is abnormal (Anding *et al*., 2003; Pesanti, 2001; Yoon, Pahl and Vaziri, 2007).

Neutrophils show clear evidence of spontaneous activation (Pereira *et al*., 2010; Polanska *et al*., 2010; Sela *et al*., 2005); this state of neutrophil priming is reflected by elevated plasma elastase levels and ROS (Costa *et al*., 2008b; Sela *et al*., 2005). In addition, an upregulation of toll-like receptor (TLR)-2, TLR4 and integrin expression is observed in HD patients that may have a role in spontaneous activation resulting in increased spontaneous ROS secretion and degranulation (Gollapudi *et al*., 2010). The increased inflammatory cytokines (e.g. TNF-α) and ROS in circulation in uraemia appear likely to induce neutrophil priming (Mazor *et al*., 2010).

Lymphocytes

ESRD patients may display lymphopaenia; partly explained, by a decrease in the number of B lymphocytes (Deenitchina *et al*., 1995). Absolute cell counts for each T lymphocyte subset are also diminished in the ESRD population, but to a lesser extent than B cells. Consequently, patients have a greater proportion of T cells (cluster of designation [CD]3^+) and T helper cells (Th, $CD4^+$) than healthy controls, whereas the $CD4^+$: $CD8^+$ ratio is unchanged (Costa *et al*., 2008a; Deenitchina *et al*., 1995).

The disturbances in acquired immunity primarily concern the T cells and not the B cells (Degiannis *et al*., 1987). Despite increased apoptosis of B lymphocytes (Fernandez-Fresnedo *et al*., 2000), ESRD patients have been reported to have normal levels of

circulating immunoglobulins (IgG, IgM and IgA) (Bouts *et al*., 2000; Costa *et al*., 2008a; Okasha *et al*., 1997). Further, despite reduced numbers, natural killer cell cytotoxicity, degranulation and interferon secretion in ESRD patients is reported to be normal compared with healthy controls although activation markers CD69 and xNKp44 are increased (Vacher-Coponat *et al*., 2008).

Thymic output of naïve T cells is severely diminished in ESRD patients, there is an increased susceptibility to apoptosis and overall there is a large decline in the number of naïve T cells (Betjes *et al*., 2011). Memory T cells are frequently terminally differentiated, no longer expressing CD28 required for co-stimulation with antigen-presenting cells (APCs) and with decreased telomere length. These $CD4^{+}CD28^{-}$ T cells are typically pro-inflammatory, secreting interferon-γ (IFN-γ) and TNF-α upon activation but also in basal conditions; the presence of these cells in ESRD is associated with CVD (Betjes, 2013).

T cells from ESRD patients have an impaired proliferative response and cytokine production to stimulation (Girndt *et al*., 2001b; Kurz *et al*., 1986; Meier *et al*., 2002). Conversely, T cells also exhibit increased early activation markers (CD69) and this is associated with a lower activation upon stimulation (inhibited IL-2 release, thus reduced proliferative capacity; Meier *et al*., 2005). This is suggestive of a continuously activated immune system unable to adequately react to antigen challenge.

An overproduction of IL-12 by monocytes may cause the shift in differentiation in T cells towards type 1 T helper cell dominance (Sester *et al*., 2000). Furthermore, uraemic T cell activation has been shown to be normal in the presence of APCs from healthy donors; therefore, the disturbances observed in T cells can be somewhat attributed to a defective interaction with APCs (Girndt *et al*., 1993). It appears that at least some of the immune dysfunction observed in T lymphocytes may be accounted for by over-activated monocyte cytokine production and impaired APC function and interaction.

Regulatory T cells

A subpopulation of T lymphocytes described as regulatory T cells (Tregs) suppress the immune response of T lymphocytes and are crucial for the control of autoreactive T cells

in vivo (Sakaguchi *et al*. 1995); therefore they help maintain peripheral tolerance and minimise collateral tissue damage (Wohlfert and Belkaid, 2008). Due to the important regulating capacity of Tregs either a surplus or deficiency in activity is deleterious. Over-activation of Tregs is associated with an increased risk of chronic infections and tumour growth; on the other hand, a deficiency can lead to autoimmunity, inflammation and allergy (Shalev *et al*., 2011).

Tregs produce the anti-inflammatory cytokine IL-10 (Vignali, Collison and Workman, 2008). In ESRD patients low circulating levels of IL-10 are associated with atherosclerotic cardiovascular complications (Seyrek *et al*., 2005), and ESRD patients with a greater response to vaccination show a high secretion of IL-10 upon lipopolysaccharide (LPS)-stimulation (Girndt *et al*., 1995). The presence of a uraemic milieu seen in ESRD is associated with a decreased number of Tregs as well as a diminished suppressive capacity (Hendrikx *et al*., 2009; Meier *et al*., 2009). It is intuitive to suggest a deficiency and dysfunction of Tregs may be a basis for uncontrolled inflammation in ESRD patients.

Monocytes

CKD patients, and particularly patients undergoing regular HD, are associated with a monocytosis (Sester *et al*., 2001) and a shift in the phenotype of monocytes towards the 'pro-inflammatory' $CD16^+$ populations (Heine *et al* 2008; Kim *et al*., 2011; Nockher and Scherberich, 1998).

Recently, the nomenclature of three monocyte phenotypes was formally defined (Ziegler-Heitbrock *et al.*, 2010). The role of each monocyte subpopulation (summarised in Table 1.7) has been an area of contention since the discovery of their apparent trichotomy; disagreements arise about the specific inflammatory properties of intermediate and non-classical phenotypes (Cros *et al*., 2010; Wong *et al*., 2011). Expansion of intermediate and non-classical monocytes (or collectively 'CD16+ monocytes') have been reported in a number of diseases conditions, including sepsis, hepatitis, asthma, tuberculosis, coronary artery disease and stroke (Wong *et al*., 2012). It has so far not been completely clarified whether this expansion is protective or pathogenic, although an increased proportion of intermediate monocytes are associated with a poorer outcome in HD patients (Heine *et*

al., 2008). To add a further complication each monocyte subset may also be altered by the disease condition; for example, angiotensin converting enzyme (ACE; CD143) is expressed highest on intermediate monocytes, increased in ESRD patients and even more so in those with evidence of CVD (Ulrich *et al*., 2006). Not only does the proportion of each subset shift in disease, but each subset may also adapt.

In addition to apparent changes in the distribution of monocyte subpopulations, and partly as a consequence, at a single cell level, monocytes in ESRD patients have an increased basal production of pro-inflammatory cytokines, IL-1β, TNF-α, IL-6 and IL-8 and ROS (Girndt *et al*., 1995; Girndt *et al*., 1998; Higuchi *et al*., 1997; Morita *et al*., 1997). Furthermore, monocytes in HD patients are functionally impaired as shown by reduced antigen-presentation capacity and B7-CD28 interaction (Girndt *et al*., 2001a; Meuer *et al*., 1987).

As with other features of the uraemic immune system, monocytes in CKD patients appear to be a combination of pre-activated (or primed) and unresponsive which leads to chronic overproduction of cytokines and a potential role in chronic inflammation but also susceptibility to infection. The degree of pre-activation is associated with non-responsiveness to hepatitis B vaccination (Girndt *et al*., 1995) and the greater the degree of unstimulated cytokine production the lower the immune response to stimulation (Sardenberg *et al*., 2004). Seemingly, pre-activation of monocytes contributes to impaired immune response as well as chronic inflammation.

Table 1.7 - The profiles of monocyte subpopulations

Subset	Profile	Action	Response to LPS stimulation	Other notes
Classical ($CD14^{++}CD16^{-}$)	Large size $CCR2^{high}$ $CCR5^{low}$ CX_3CR1^{low} HLA-$DR^{low/-}$	Strong phagocytes Unclear migratory behaviour Strong ROS behaviour	CCL2, IL-6, IL-8, IL-10 and CCL3. Limited TNF-α	Traditional monocytes Approximately 85% of monocytes
Intermediate ($CD14^{++}CD16^{+}$)	Large size $CCR2^{+}$ $CCR5^{+}$ CX_3CR1^{+} HLA-DR^{high}	Strong phagocytes Unclear migratory behaviour Spontaneous ROS production	TNF-α, IL-1β and IL-6	Elevations predict CVD events in CKD and HD. Express ACE, high ACE expression predicts mortality. Approximately 5% of monocytes
Non-classical ($CD14^{+}CD16^{++}$)	Small size $CCR2^{low}$ $CCR5^{-}$ CX_3CR1^{high} HLA-DR^{+}	Poor phagocytes Patrolling behaviour Weak ROS production	Lower response except IL-1ra In response to virus: TNF-α, IL-1β and CCL3	Confirmed as monocytes Approximately 10% of monocytes

ACE, Angiotensin converting enzyme; CCL, C-C chemokine ligand; CCR, C-C chemokine receptor; CKD, Chronic kidney disease; CX_3CR1, CX_3C chemokine receptor-1; HD, Haemodialysis; IL, Interleukin; ROS, Reactive oxygen species; TNF-α, Tumour necrosis factor alpha.
Cros *et al*., 2010; Heine *et al*., 2012; Hilgendorf and Swirski 2012; Rogacev *et al*., 2011; Rossol *et al.* 2012; Ulrich *et al*., 2010; Wong *et al*., 2011; Zawada *et al*., 2012.

To conclude, ESRD and the uraemic milieu alter almost all areas of the immune system. Leucocytes are dysfunctional, prematurely aged and unreactive to stimuli contributing to an increased risk of infection. Furthermore, over-active immune cells combined with impaired renal clearance results in chronic increases in circulating pro-inflammatory factors (cytokines and ROS); consequently, increasing the risk of CVD and other complications.

1.5 Ghrelin, malnutrition and inflammation

Given the important but ill-defined relationship between inflammation, malnutrition and prognosis in ESRD, recent findings suggesting an anti-inflammatory role of the orexigenic gut-hormone ghrelin is of potential interest in this population. The interaction between acylated ghrelin and the growth hormone secretagogue receptor 1a (GHS-R1a), recently found to be present on leucocytes, leads to a suppression of pro-inflammatory cytokine secretion (Dixit *et al*., 2004). Further, the infusion of ghrelin may improve energy intake, muscle wasting and reduce inflammation in CKD (Ashby *et al*., 2009; DeBoer *et al*., 2008), therefore having great therapeutic potential. However, so far very little is known about the effect of uraemia on the expression of GHS-R1a by leucocytes.

The contribution of inflammation to the malnutrition-inflammation complex is crucial as malnutrition in the absence of inflammation is not clearly detrimental to cardiovascular health (Stenvinkel *et al*., 2000); this may underline the greater importance or specificity of inflammation as a predictor for prognosis. What does appear to be clear is that immune dysfunction and chronic inflammation have negative outcomes and there is a great need for interventions that may improve the systemic inflammatory environment. Furthermore, it is clear that these patients are highly vulnerable and that all interventions should be wary of exacerbating the apparently fragile immunological and pro-inflammatory state.

1.6 Physical activity and health

There is now overwhelming evidence showing physical inactivity as one of the greatest risk factors for mortality and CVD in the general population. Low levels of physical fitness are associated with an equal or greater risk of all-cause mortality than other more established risk factors such as hypertension, high cholesterol and smoking (Aerobics Centre Longitudinal Study, Wei *et al*., 1999).

Epidemiological evidence suggests that regularly partaking in physical activity is protective against CVD, type II diabetes mellitus, certain cancers, metabolic syndrome and dementia amongst many others (Hardman and Stensel, 2009; Pedersen and Saltin, 2006). The protection that exercise confers is in a dose response pattern (within normal levels) and independent of obesity; even a small increase in weekly activity levels appears to offer a large health benefit in highly inactive populations (Lee and Skerrett, 2001).

There is more to health than simply living longer. In addition to protection against premature mortality, regular physical activity is likely to give the greatest benefits in terms of preserved functional capacity and living without illness in the later years of life (Hardman and Stensel, 2009). Exercise is recommended by the UK health authorities with a general consensus that 5x 30 min of moderate activity or 5x 15 min vigorous activity per week is the minimum required, but that more is desirable (American College of Sports Medicine (ACSM), 2009; Department of Health, 2011).

1.7 Exercise programmes and end-stage renal disease

The first records of exercise intervention studies in ESRD patients were published over three decades ago (Goldberg *et al*., 1980). Initially exercise training was completed at non-dialysis times, before intradialytic and home-based exercise programmes were developed. Each programme type has benefits and drawbacks in terms of effectiveness, cost, convenience and time. Konstantinidou and colleagues (2002) compared the efficacy of the three programme styles and concluded that non-dialysis times gave the greatest benefits in terms of exercise capacity (43% increase in maximum oxygen uptake ($\dot{V}O_{2max}$) vs. 24% with intradialytic and 17% with home-based). Later studies supported these findings; interestingly, the drop-out rates were much lower in those participating in

intradialytic exercise programmes (Kouidi *et al*., 2004). Therefore, for a long-term sustainable programme exercise during HD (intradialytic) may be more advantageous.

Risks of exercise

With numerous co-morbidities and high cardiac risk the potential risks of exercise in this population must be considered. Presently, no serious cardiac events have been reported related to exercise (Johansen and Painter, 2012). An exercise programme in Brazil with 34 patients completing 3,077 sessions over a 5-y period reported only a few minor hypotensive events with no serious haemodynamic repercussions; there were no major complications during this time (Reboredo *et al*., 2011), nor were there in over 28,000 patient-hours of exercise in a meta-analysis of the literature (Smart and Steele, 2011). Anecdotally at least, there appears to be no increased risk of cardiac events. The most common injury associated with exercise is musculoskeletal injury. CKD patients with bone disease and parathyroid disorders may be more susceptible; on the other hand, strengthening due to exercise may arguably reduce the risk of falls or fracture (Johansen, 2008).

It should be stressed that most studies screen patients to ensure they are eligible to exercise, and exercise is most frequently prescribed at a moderate-intensity. The cardiovascular vulnerability of HD patients would make it likely that the risk of a cardiovascular event during exercise is elevated in this population compared with a healthy individual but this may be no more so than an individual taking part in any other rehabilitation programme. There appears to be no evident reason why CKD patients would be contraindicated to exercise beyond any co-morbidity they may have (e.g. hypertension and type II diabetes mellitus). In the absence of any set guidelines specific to CKD, individual risk assessment of patients appears wise taking into account any guidelines related to co-morbidities.

Specific to intradialytic exercise, it is generally recommended that exercise is completed in the first 2 h of treatment (Smart *et al*., 2013). This is because when fluid removal is high, exercise may be precluded after 3 h of treatment due to HD-induced declines in blood pressure and cardiac output leading to hypotension (Moore *et al*., 1998).

Effects of an exercise intervention in end-stage renal disease

Over 100 studies have now been published with results regarding the effects of an exercise intervention programme on ESRD patients. Recent reviews describe the findings in greater depth (Heiwe and Jacobson, 2014; Johansen, 2008; Kosmadakis *et al*., 2010; Smart and Steele, 2011). Exercise training of differing modalities consistently improves muscle function and exercise capacity. The extent of physical improvements varies depending upon the type and duration of the exercise programme. $\dot{V}O_{2peak}$ has been shown to improve within 6 months by 13-43% (Carney *et al*., 1983; Deligiannis *et al*., 1999), and even bigger improvements (70%) have been reported after 4 y of exercise at non-dialysis times (Kouidi *et al*., 2004). It is therefore well established that regular exercise can improve exercise capacity in this population.

Regular exercise appears to improve many aspects of cardiovascular health in ESRD patients. High blood pressure, a frequent co-morbidity associated with kidney disease, may be reduced by training and anti-hypertensive medications may be lowered (Anderson, Boivin and Hatchett, 2004; Hagberg *et al*., 1983; Miller *et al*., 2002; Painter *et al*., 1986). Elsewhere, improvements in heart-rate variability, left ventricular ejection fraction, signal averaged electrocardiogram, reduced arterial stiffness and pulse pressure have been observed, all of which suggest a reduced risk of cardiac death (Deligiannis *et al.*, 1999; Kouidi, Grekas and Deligiannis, 2009; Mustata *et al*., 2004; Nam *et al.*, 2010; Toussaint, Polkinghorne and Kerr, 2008). Circulating lipids are altered to a more favourable profile, triglycerides and total and LDL cholesterol levels are reduced whilst HDL cholesterol concentrations are increased (Goldberg *et al*., 1980; Goldberg *et al*., 1983).

Other areas that regular exercise may improve include psychological parameters that are often disturbed in ESRD patients such as depression and quality of life, and markers of malnutrition (e.g. albumin) (Heiwe and Jacobson, 2011). Finally, intradialytic exercise theoretically may improve dialysis efficiency; however, empirical evidence is unclear (Bennett *et al*., 2007; Kirkman *et al*., 2013; van Vilsteren, de Greef and Huisman, 2005).

Overall, it appears clear that regular exercise has the potential to have a number of benefits to HD patients to various different facets of health and wellbeing.

1.8 Exercise and inflammation in the general population

In the general population there is a growing body of evidence to suggest that markers of systemic inflammation are lower in individuals that are regularly physically active (Kasapi and Thomson, 2005). Cross-sectional observational studies in healthy populations have shown inverse relationships between markers of systemic inflammation and physical activity levels or fitness (Church *et al.,* 2002; Colbert *et al.,* 2004; Ford, 2002). This is further supported by a large cohort (n = 998) observational study which included a large range of BMI and age (Shanley *et al.,* 2011). They found CRP, IL-6 and TNF-α were significantly lower in the high tertile of leisure time frequency and perceived level of fitness compared to the low tertile even after adjustment for BMI, age, sex and smoking. Despite this, BMI was the greatest influencing factor for CRP and IL-6; likewise, age was for TNF-α.

Another large cohort study with a 10-year follow-up also suggested that regular exercise has a role in lowering circulating markers of inflammation (Hamer et *al.,* 2012). Those individuals that met an average of 2.5 h per week of moderate physical activity had lower CRP and IL-6, this was maintained after 10 years and those that increased physical activity had a decrease in IL-6 and CRP at follow-up. It is also of note that these relationships were independent of waist circumference.

Research into the mechanisms by which exercise may elicit an anti-inflammatory effect has largely focused on adiposity and exercise-induced changes in body composition. Adipose tissue can play a role in chronic inflammation through the secretion of inflammatory cytokines (e.g. IL-1β, IL-6, TNF-α, CCL5) (Uguccioni *et al.,* 1995; Kintscher *et al.,* 2008). Furthermore, the accumulation of monocytes as macrophages in adipose tissue is also purported to be a major source of increased concentrations of inflammatory cytokines (Keophiphath *et al.,* 2010). However, as exercise appears to have a beneficial effect in the absence of changes to adiposity, and that non-obese individuals that are inactive have elevated plasma markers of inflammation compared to non-obese active

individuals, it appears that the mechanisms through which increases in physical activity have a positive impact upon inflammation are not restricted to changes in adiposity.

Evidence has emerged for several other potential mechanisms by which regular exercise may lower circulating markers of systemic inflammation. One possible mechanism that has been the focus of much recent attention is the increased production and release of cytokines from contracting skeletal muscle ('myokines') during exercise (Pedersen and Febbraio, 2008). Muscle and circulating levels of IL-6 increase in response to exercise loads of sufficient duration and to a lesser extent, intensity. A major stimulus for IL-6 release is a fall in muscle glycogen content (Keller *et al.,* 2005) but increases in intracellular calcium levels and formation of reactive oxygen species also play a role in activating transcription factors known to regulate synthesis of IL-6 (Fischer, 2006). Increases in circulating levels of IL-6 appear to stimulate the release of anti-inflammatory cytokines IL-10 and IL-1ra and inhibits the release of the pro-inflammatory cytokines IL-1β and TNF-α (Pedersen and Febbraio, 2008), creating a circulating 'anti-inflammatory' environment with every bout of exercise. It should also be noted that the half-life of IL-6 is prolonged by combining with the soluble IL-6 receptor (sIL-6R), the expression of which on the muscle membrane is also increased by exercise training (Keller *et al.,* 2005).

However, the muscle release of IL-6 and subsequent cascade of anti-inflammatory cytokines cannot be the sole mechanism underlying the purported anti-inflammatory effects of exercise because short durations of low to moderate intensity of exercise are associated with reductions in circulating concentrations of markers of inflammation, yet do not appear to stimulate IL-6 release (Fischer, 2006). A recent review of further mechanisms thought to be involved in the beneficial effects of exercise on inflammation (Gleeson *et al.,* 2011) highlighted reduced expression of toll-like receptors on monocytes and macrophage with subsequent inhibitory effects on monocyte/macrophage IL-6 and TNF-α production, inhibition of monocyte/macrophage infiltration into adipose tissue, phenotypic switching of macrophages from a pro-inflammatory phenotype to an anti-inflammatory phenotype within adipose tissue, a reduction in the circulating numbers of pro-inflammatory monocytes and an increase in the circulating numbers of Tregs as having a potential role. It appears that regular exercise may elicit a number of potential mechanisms that favour a more anti-inflammatory (or less inflammatory) environment.

1.9 Unanswered questions and aims

The immune dysfunction observed in HD patients is an important area for therapeutic intervention because the high levels of inflammation are implicated with increased risk of CVD, cachexia and mortality. The effect of regular exercise in HD patients on markers of inflammation has given mixed results (Table 1.8) and there are a number of limitations of the research completed to date (for a review: Dungey *et al*., 2013). Evidence is limited to one or two circulating factors that are often secondary outcome measures. Consequently, no studies have explored the effect of exercise on cellular markers of inflammation that have shown to adapt favourably to training in healthy cohorts. In controlled trials, with the exception of one research group (Afshar *et al.*, 2010 and 2011), there is presently very limited evidence of an anti-inflammatory effect of regular exercise in ESRD patients.

Furthermore, to the author's knowledge, no studies have reported the acute effects of exercise on any markers of inflammation. Due to the immune dysfunction prevalent in this cohort this is important to assess the safety of exercise from an immunological perspective. Exercise has the capacity to induce marked transient increases in circulating IL-6 and a subsequent cytokine cascade and suppress various aspects of immune function (Pedersen and Febbraio, 2008; Walsh *et al*., 2011). These are normal anti-inflammatory responses in healthy individuals but may have different consequences in ESRD patients. Assessment of the immediate as well as long-term response to exercise is therefore required in this cohort.

Presently very little is known about the expression of GHS-R1a in ESRD patients. Recent data suggesting that an acute bout of exercise may increase the proportion of peripheral blood mononuclear cells (PBMCs) expressing GHS-R1a suggests this may be a mechanism through which exercise exerts anti-inflammatory effects (Bishop *et al.,* 2013). This represents a novel area of potential interest.

The aims of this thesis were to explore the impact of habitual physical activity, an acute bout of exercise and a long-term exercise training programme on markers and mechanisms of systemic inflammation in HD patients. The primary aims were to ascertain the safety of exercise in this cohort from a novel immunological perspective and

determine whether exercise can have beneficial effects for the cardiovascular and immunological health and general wellbeing of these patients.

Table 1.8 - Exercise intervention studies in haemodialysis patients reporting markers of systemic inflammation.

Study	**Design** (No. of patients)	**Training**	**Outcome measures** *Exercise vs. Control (unless stated)*
Controlled Trials			
Afshar *et al.,* 2010	RCT (7 Aerobic vs. 7 Resistance vs. 7 CON)	Aerobic: ID cycling: RPE 12-14 10-30 min, 3x/wk, 8 wk Resistance exercises: RPE 15-17; 3x/wk, 8 wk	Aerobic vs. Resistance vs. CON CRP: - 83.9% vs. - 67.9% vs. + 1.5% Albumin: NC
Afshar *et al.,* 2011	RCT (14 EX vs. 14 CON)	ID cycling @ RPE 12-14 10-30 min 3x/wk, 8 wk	Serum Leptin: - 19.9% vs. + 29.2% CRP: - 83.2% vs. + 1.2%
Cheema *et al.,* 2007 and Cheema *et al.,* 2011	RCT (24 EX vs. 25 CON)	ID PRT: 2 sets 10 exercises @ RPE 15-17 using free weights. 3x/wk, 12 wk	Log CRP: -0.08 vs. +0.24 TNF-α: NC IL-1β: NC IL-6: NC IL-10: NC IL-12: NC
Daniilidis *et al.,* 2004	RCT (20 EX vs. 14 CON)	NDT Aerobic mixed interval exercises @ 75-85% HR_{peak} 60 min, 3x/wk, 6 months	IL-2: NC IL-4: NC IL-6: NC
Kopple *et al.,* 2007	RCT (10 End-EX vs. 15 Str-EX vs. 12 Com-EX vs. 14 CON vs. 20 Healthy-CON)	End-EX: ID cycling up to 40 min @ approx. 50% $\dot{V}O_{2peak}$ Str-EX: NDT leg resistance exercise 3 sets of 6-8reps @ 80% of 5RM Com-EX: half End-EX/half Str-EX All: 3x/wk, 18 wk	CRP: NC TNF-α: NC IL-6: NC
Molsted *et al.,* 2014	Crossover control (n = 23)	NDT intense resistance training 3x/wk, 16 wk	IL-6: NC
Oliveros *et al*., 2011	Controlled (5 EX vs. 6 CON)	ID cycling and resistance 3x/wk, 16 wk	CRP: NC IL-6: NC TNF-α: NC
Toussaint, Polkinghorne and Kerr, 2008	Randomised crossover control (n = 10 + 9)	ID cycling 30 min 3x/wk 3 months (1 month washout)	3-months EX vs. 3 months non-EX CRP: NC
Wilund *et al.,* 2010	RCT (8 EX vs. 9 CON)	ID cycling, 45 min @ RPE 12-14 3x/wk, 4 months	CRP: NC IL-6: NC

Table 1.8 continued.

Study	**Design** (No. of patients)	**Training**	**Outcome measures** *Exercise vs. Control (unless stated)*
Uncontrolled designs			
Golebiowski *et al.,* 2012	Uncontrolled (29 EX)	ID cycling 3x/wk 3 months	CRP: NC IL-6: NC
Moraes *et al*., 2014	Uncontrolled (37 EX)	ID resistance using Therabands 3x/wk, 6 months	IL-6: NC CRP: 2.3 to 1.6 $mg{\cdot}L^{-1}$ TNF-α: NC
Nindl *et al.,* 2004	Uncontrolled (10 EX)	NDT: PRT using 9 resistance machine exercises 1-3 sets 15x reps 2x/wk, 12 wk	CRP: week -6: 12.42 ± 2.96 $mg{\cdot}L^{-1}$; week 0: 10.37 ± 2.71 $mg{\cdot}L^{-1}$; week 6: 7.55 ± 1.57 $mg{\cdot}L^{-1}$; week 12: 6.12 ± 1.07 $mg{\cdot}L^{-1}$
Załuska *et al.,* 2002	Uncontrolled (10 EX)	ID cycling 30 min, 3x/wk, 6 months	CRP: decrease ($P < 0.046$)

Com, Combined; CON, Control; CRP, C-reactive protein; End, Endurance; EX, Exercise; HR_{peak}, Peak heart-rate; ID, Intradialytic; IL, Interleukin; NC, No significant changes; NDT, Non-dialysis time; PRT, Progressive resistance training; RCT, Randomised controlled trial; RM, Repetition maximum; RPE, Rating of perceived exertion; Str, Strength; TNF-α, Tumour necrosis factor-alpha.
Updated from Dungey *et al.,* 2013.

Chapter 2

General Methods

2.1 Research design

The research presented in this thesis was a collaborative project between Loughborough University and the University Hospitals of Leicester (UHL). The studies feature NHS patients that were recruited from renal departments within the UHL NHS trust and healthy volunteers recruited from Loughborough University and the local area.

The work reported in this thesis is a compilation of three separate study protocols. The studies carried out are described in their relevant chapters including a detailed discussion of the findings. The data presented here is distinct to this thesis.

Study one is a cross-sectional observational study detailed in Chapter 3: Characterisation of Physical Activity and Circulating Markers of Inflammation in Haemodialysis Patients and Healthy Age-Matched Controls.

Study two is an investigation into the acute effects of intradialytic exercise and is presented in two chapters, Chapter 4: The Acute Effects of a Bout of Intradialytic Exercise on Circulating Markers of Inflammation and Immune Function, and after some preliminary data required further investigation, Chapter 5: An Investigation into the Effects of an Acute Bout of Exercise during Haemodialysis on Haemodynamic Parameters and Markers of Cardiac Injury.

The final study is a training study reported in Chapter 6: The Effect of a 6-month Intradialytic Exercise Programme on Circulating Markers and Leucocyte Phenotypes associated with Inflammation.

A general discussion of the full body of work follows the results chapters.

The specific protocols and participant characteristics can be found in each chapter. The general methods that are applicable to the whole thesis follow in this chapter.

2.2 Ethical approval and the recruitment process

All research was conducted with full ethical approval by the relevant governing body. The reader is requested to refer to each study chapter for further information regarding the study protocol and ethical approval.

The University Hospitals of Leicester NHS trust acted as the research governance sponsor for the NHS approved studies involved in this research project. The managers and matrons of each HD unit gave their permission for research to be carried out in their unit prior to any research taking place and the Consultant Nephrologist and GP of each patient recruited was informed of their participation in research. All personnel directly involved with the study protocol completed 'Good Clinical Practice' training before any research was started and all researchers involved in the patient consenting process passed biennial UHL consent assessments.

Patients were only approached to participate in research once a Consultant Nephrologist had confirmed their eligibility and asked the patient if they were happy to discuss research with a member of the study team. Patients were approached if they took part in (or were about to take part in) the exercise rehabilitation programme offered to all eligible patients at the HD unit and satisfied the study exclusion criteria (Table 2.1) or, for the non-exercising control subjects, if they were deemed eligible to take part in such a programme were it available at their unit. The study protocol was explained in full and every patient was given at least 48 h to consider participation prior to being asked if they wished to take part in the study. Full written consent was then obtained and letters detailing the study were sent to their GP.

For the recruitment of healthy controls in the cross-sectional study, participants volunteered after responding to an e-mail, poster or leaflet and had the study explained to them in full prior to written consent.

All participants involved in any study were permitted to drop out at any stage without giving any reason. All aspects of the research conformed to the ethical principles outlined in the Declaration of Helsinki (World Medical Association, 2013).

Exclusion criteria

The general exclusion criteria used for all NHS patients involved in any of the study protocols is described in Table 2.1. These criteria were used because these conditions would currently preclude patients from taking part in the exercise, have a direct impact upon outcome measures or put undue extra demands upon the study (e.g. translators).

Table 2.1 – Exclusion criteria for end-stage renal disease patients

Exclusion criteria
Age < 18 years
Lower limb vascular access
Cardiovascular event [myocardial infarction, unstable angina, stroke, transient cerebral ischaemic attack] in last 3 months
Severe heart failure (NYHA III-IV)
Severe chronic obstructive pulmonary disease (COPD)
Active liver disease
Uncontrolled diabetes mellitus (HbA1c >9%)
Severe lower limb orthopaedic problems
Severe lower limb neuromuscular disease
Clinically overt infection in last 6 weeks
Pregnancy
Insufficient command of English to understand the patient information sheet and give consent

HbA1c, Glycated haemoglobin.
NYHA New York Heart Association functional classification system: moderate (III) and severe (IV) – Stages III and IV describe marked inhibition of physical activity.

For the healthy participants recruited in the cross-sectional arc of this project a more stringent exclusion criterion was utilised to exclude volunteers who were not deemed 'healthy' (Table 2.2). Specifically, potential participants were asked whether they reported a number of different symptoms associated with kidney disease or any other recurrent health issues. A health screen questionnaire ensured participants reported any previous health concerns and medications.

Each participant was assessed on an individual basis. For the more elderly individuals minor health issues were assessed case-by-case; for example, well-controlled blood pressure in an 80-y-old who took anti-hypertensive medication was deemed satisfactory if no further conditions were reported.

Table 2.2 – Exclusion criteria for healthy participants

Exclusion criteria
Any evidence of kidney disease (e.g. urinary problems)
Not aged between 18 and 85 years
Any personal history of cardiovascular disease, metabolic disease, high blood pressure or dyslipidaemia (abnormal blood fat (triglyceride) or cholesterol)
Dieting or have extreme dietary habits
Diabetic
Current smoker
Recent infection (last 6 weeks)
Taking drugs known to affect digestion or metabolism, medical or illegal (e.g. anabolic steroids, marijuana, amphetamines, thyroid prescription drugs).
Pregnancy
Insufficient command of English to understand the patient information sheet and give consent

2.3 Haemodialysis treatment

No aspect of the research studies interfered with the patients' routine care. HD, medications and dietary advice were continued as normally prescribed.

Both HD units used in the studies followed a similar treatment procedure. Patients were allocated to a time-slot (morning, afternoon or evening) that they attended thrice weekly, either on a Monday, Wednesday and Friday or on Tuesday, Thursday and Saturday. The first session of the week had an additional day of toxin and fluid build-up over the weekend; consequently, outcome measures were never taken on the first HD session of the week.

Patients arrived at the renal unit via transport and weighed themselves. From this weight and predefined target weight an UF goal was set and this amount of fluid was removed during HD. Once at their bed resting blood pressure was taken using an automated sphygmomanometer before the arterial and venous needles were inserted (for AVF) or the catheter was connected for HD. The pump speed was determined by the quality of the access, the greater speeds allowing a greater blood flow and better quality dialysis treatment. Once the prescribed HD time had elapsed the patient's access was disconnected and the new 'dry' weight checked against the target weight.

If the patient was prescribed erythropoietin or iron sucrose these were given intravenously by the nurses during HD after all patients from that shift were connected to HD; this was not at a set time but occurred at similar times on each shift. Patients were asked to take the rest of their medications in their usual routine manner.

The HD machines used (4008 series; Fresenius Medical Care, Bad Homburg, Germany) check arterial and venous pressure throughout treatment and alarm when unexpected changes occur and allow bicarbonate to be added during treatment to correct acidosis. Polysulfone high-flux dialysers were used. The dialysate, dialyser size, needle size and dialysis duration were prescribed according to patient size, UF targets, tolerance and blood biochemistry results; these were recorded, although rarely changed.

2.4 Exercise during haemodialysis

Two HD units were used to recruit patients to studies in this thesis. One HD unit did not have the facilities or staff to provide an exercise programme to the patients; this group were recruited for the non-exercising aspects of the research. In the second HD unit an exercise programme was prescribed to patients as part of routine clinical care, this was available to all patients regardless of their participation in research.

All patients who were clinically eligible to cycle were offered the opportunity to take part in the intradialytic exercise programme. Exercise was provided in the form of a specially designed recumbent cycle ergometer (Letto series; Motomed, Reck, Germany; Figure 2.1) which could be set up for the patient while they were dialysing. The ergometer features a motor that allows passive cycling at the lowest gears and at higher gears the patient cycled against the force of the motor; the gears ranged from 0 to 20. Patients were encouraged to warm-up and cool-down for 5 min at a low gear before and after each exercise session. To reduce the risk of complications with HD, exercise was not offered in the first 30 min of treatment to allow treatment to settle or in the last 90 min in line with previous suggestions (Moore *et al.,* 1998).

Figure 2.1 – Patients exercising during a haemodialysis session.

Upon initiation of training patients began at a low gear and a short duration until comfortable with the cycling motion. The exercise programme was then increased in duration up to 30 min and in gear to induce a greater resistance. Patients were familiarised with the rating of perceived exertion scale (RPE; Borg, 1973) through careful explanation and practice of the different relative exertion scores (i.e. how breathless they feel and how long they may be able to persist with exercise at different levels of exertion). Patients were asked to find a gear that represented "somewhat hard" effort, or 12-14 on the RPE scale. Due to the pragmatic nature of the studies the exercise was not standardised, exercise was personalised for each patient; however, in most cases 30 min per session was recommended. Progression up the gears (and therefore increasing resistance) was encouraged regularly, particularly when RPE scores were reported below 12.

Prior to enrolling in the research study patients were allowed to become familiar with the cycling programme. This gave patients a couple of weeks of cycling to ensure they were confident exercising during HD; this minimised drop-out rates. Patients were withdrawn from the study if they were unable to exercise for a prolonged period of time (> 2 months) or if they became ineligible for the study (Table 2.1).

The opportunity to cycle during HD was usually offered two or three times per week. Patients were entitled to refuse exercise, and were not offered exercise if deemed temporarily unfit to exercise by their consultant, dialysis nurse or the physiotherapist. Monthly blood test results were checked for abnormalities and blood pressure and UF goal were checked prior to every exercise session. Exercise was not recommended if the patient presented with any of the following: temporary anaemia (haemoglobin < 8 $g \cdot dL^{-1}$), hyper/hypotension (SBP > 190 or < 90 mmHg), fluid accumulation (UF goal > 3.5 L or overt oedema), injury or abnormal monthly blood results (e.g. elevated potassium).

The following output was collected from the cycle ergometer after every exercise session: the gears used, the duration of exercise, the average cycle speed (rpm), mean power output and peak power output (watts). In addition, an estimated distance cycled (km) and estimated energy expenditure of the exercise (kcal) was recorded.

2.5 Medical records

The medical history of each patient was extracted from the medical notes and checked with the patient. Aetiology, co-morbidities, dialysis vintage, transplant history and HD prescription were logged. All medications or supplements prescribed to patients by clinicians were noted and updated on a monthly basis. Patients taking medications known to have immunosuppressant activity (e.g. glucocorticoids) were omitted from analysis of their circulating inflammatory markers, topical anti-inflammatories and anti-histamines were permitted.

As part of the routine clinical care each HD patient had blood tests taken each month; typically, these occurred during the first week of the month on the second session of the week (i.e. Wednesday or Thursday). From these tests biochemistry and haematology results were accessed from the patients' medical records; for all studies involving patients the test dated closest to the day of the trial was used. Records of serum albumin, bicarbonate, creatinine, potassium, sodium, urea, platelets, haemoglobin, red and white blood cell counts, phosphate and week-averaged blood pressures were documented where available.

2.6 Blood sampling and outcome measures

All blood samples taken from HD patients were collected using a sterile dry syringe drawn directly from the HD access; this blood was immediately dispensed into prepared monovettes (Sarstedt, Nümbrecht, Germany) to prevent coagulation.

Blood samples from healthy participants were taken from an antecubital vein using a butterfly needle and drawn directly into prepared monovettes.

Blood was gently inverted in the pre-treated tubes and prepared for analysis.

Complete Cell Count

Blood from a K_3 ethylenediaminetetraacetic acid (EDTA) monovette was analysed using an automated cell-counter ($A^c.T^{TM}$5diff haematology analyser, Beckman Coulter, High

Wycombe, UK). Measurements of red blood cell count, white blood cell count, haematocrit, haemoglobin, neutrophils, lymphocytes and monocytes were recorded; plasma volume was estimated from haematocrit (i.e. 1 – haematocrit).

Assessment of kidney function

Blood from healthy participants was collected into a serum gel monovette and allowed to coagulate for 30 min prior to centrifugation for 15 min at 1500 x *g*. This tube was transported on ice to the UHL pathology laboratory for independent on-the-day analysis of the urea and electrolyte profile (urea, creatinine, potassium, sodium). Creatinine was assessed using ADVIA 1800 and ADVIA 2400 analysers using the Jaffe principle (producing a red-coloured creatinine-picrate complex) (Husdan and Rapaport, 1968).

$$Creatinine + Picric\ acid \xrightarrow{OH^-} Creatinine\ Picrate$$

From the creatinine result, age and ethnicity the eGFR was calculated using the simplified MDRD equation (Levey *et al.,* 2000). Assessment in this manner emulates the way kidney function is assessed in the primary care setting in the UK.

$$\begin{aligned} \text{eGFR} = 32788 \text{ x Serum Creatinine } (\mu\text{mol} \cdot \text{L}^{-1})^{-1.154} \text{ x Age}^{-0.203} \\ \text{x } [1.21 \text{ if Black}] \\ \text{x } [0.742 \text{ if Female}] \end{aligned}$$

eGFR was also calculated in the HD patients from monthly blood tests. However, all patients recruited to the research had been receiving HD treatment for more than three months and chronic ESRD had been well established.

Bacterial stimulation

1 mL of heparinised blood was added to an eppendorf tube containing 50 µL of 10 $mg \cdot mL^{-1}$ bacterial extract solution (84015-1VL, Sigma-Aldrich, Gillingham, UK) and another 1 mL of blood was aliquot into a separate empty eppendorf tube. Both samples were mixed through gentle inversion and incubated on a dry block heater at 37°C for 60 min with another gentle inversion after 30 min. After incubation the samples were centrifuged at 13,000 x *g* for 2 min and the supernatant harvested into eppendorf tubes and frozen at < -20°C for later analysis.

Neutrophil degranulation assay

Neutrophil elastase secretion was assessed in bacterially-stimulated plasma and the non-stimulated plasma using a commercially available enzyme-linked immunosorbent assay (ELISA) kit specific for polymorphonuclear cell elastase (RM 191021100, BioVendor GmbH, Heidelberg, Germany). Bacterially-stimulated samples were diluted 1:1000 (5 + 45 µL followed by 5 + 495 µL) and unstimulated samples diluted 1:100 (5 + 495 µL) using the dilution buffer provided in the kit. All samples from the same individual were analysed on the same plate.

The bacterially-stimulated release of elastase was calculated by subtracting the unstimulated values from the stimulated values. These quantities were adjusted for haematocrit and divided by the neutrophil cell count to give the bacterially-stimulated release of elastase per neutrophil.

Plasma collection

Blood in the K_3EDTA monovettes was centrifuged at 1,500 x *g* for 10 min at 4°C. The separated plasma was drawn off and immediately frozen in 1 mL measures in eppendorf tubes at < -20°C ready for later analysis.

Enzyme-linked immunosorbent assays

Plasma concentrations of IL-6, IL-10, TNF-α, IL-1ra, IL-1β (R&D systems, Abingdon, Oxfordshire, UK) and CRP (IBL International GmbH, Hamburg, Germany) were measured using individual commercially available ELISA kits. High-sensitivity ELISAs were used for the determination of IL-6, IL-10 and TNF-α. The manufacturer's instructions were followed carefully. Specific details pertaining to sample dilutions and coefficients of variation (CV) can be found in the relevant chapter methods.

2.7 Flow cytometry

Staining for Regulatory T cells

A 120 µL aliquot of whole blood from a heparinised monovette was added to an eppendorf tube containing 10 µL cocktail of conjugated antibodies: CD4-FITC (fluorescein isothiocyanate), CD25-PE-CY7 (phycoerythrin-cyanide dye) and CD127-AlexaFluor647 (Becton Dickinson Biosciences, Oxford, UK). Another eppendorf tube containing 240 µL of whole blood remained unstained. Samples were mixed and incubated on ice in the dark for 20 min. Erythrocytes were lysed by adding 1.5 mL of lysing solution (FACS lysis buffer, Becton Dickinson Biosciences, Oxford, UK) and incubated for 10 min in the dark. Samples were centrifuged at 3,500 rpm, 4°C for 6 min and the supernatant aspirated. The remaining cells were then washed through addition of phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) and 2 $mmol \cdot L^{-1}$ EDTA and centrifuged at 3,800 rpm, 4°C for 6 min. The supernatant was aspirated and the cells resuspended in 400 µL chilled (4°C) PBS (containing 0.5% BSA and 2 $mmol \cdot L^{-1}$ EDTA) and analysed by flow cytometry (FACSCalibur, Becton Dickinson Biosciences, Oxford, UK). 50,000 events within a lymphocyte gate (determined by morphology) were acquired per analysis.

Staining for monocyte phenotypes

A 120 µL aliquot of whole blood from the heparinised tube was added to an eppendorf tube containing 10 µL CD14-FITC and 10 µL CD16-PE (phycoerythrin) (Becton Dickinson

Biosciences, Oxford, UK). Another tube containing 240 µL whole blood remained unstained. Samples were mixed and incubated on ice, lysed, washed and analysed in the same manner as described above; 100,000 events in total were acquired per analysis.

Staining for GHS-R1a on T cells and monocytes

A 100 µL aliquot of whole blood from the heparinised tube was added to two duplicate eppendorf tubes, containing 5 µL CD3-FITC and 5 µL CD14-PE conjugated antibodies; 25 µL anti-GHS-R1a (Santa Cruz Biotechnology Inc.) was added to one of these tubes. Another eppendorf tube containing 250 µL of whole blood remained unstained. Samples were mixed and incubated on ice in the dark for 20 min. Erythrocytes were lysed by adding 1.5 mL of lysing solution (FACS lysis buffer, Becton Dickinson Biosciences, Oxford, UK) and incubated for 10 min in the dark. Samples were centrifuged at 3,500 rpm, 4°C for 6 min and the supernatant aspirated. 25 µL of 1:100 diluted allophycocyanin (APC) conjugated anti-donkey IgG F(ab')2 in Hank's Balanced Salt Solution (HBSS) was added to the two labelled samples and incubated in the dark for 10 min. Remaining cells were then washed in PBS containing 0.5% BSA and 2mmol·L^{-1} EDTA, resuspended and analysed in the same manner as above; 100,000 events in total were acquired per analysis.

Flow cytometry analysis

Regulatory T cells

Tregs are defined as T cells ($CD3^+$ lymphocytes) that express CD4, high levels of the IL-2 receptor (CD25) and high levels of intracellular fork-head box protein 3 (FoxP3). The IL-7 receptor (CD127) can be used to more readily quantify FoxP3 as an inverse relationship exists between CD127 and FoxP3 expression (Liu *et al.,* 2006; Seddiki *et al.,* 2006).

$CD4^+CD25^+CD127^{low/-}$ cells (Tregs) were ascertained using CellQuest software (Becton Dickinson Biosciences, Oxford, UK). The unstained sample was used to quadrant boundaries to allow accurate acquisition of stained samples. The proportion of lymphocytes expressing $CD4^+$ (subsequently referred to as the proportion of $CD4^+$ lymphocytes) was measured by finding the lymphocyte population based on morphology (forward scatter vs. side scatter); on a new dot plot gated on lymphocytes, CD4 was plotted against side scatter. The proportion of $CD4^+$ expressing $CD25^+CD127^{low/-}$ were identified by plotting the gated $CD4^+$ lymphocytes on a density dot plot of CD25 against CD127 (Figure 2.2; subsequently referred to as the proportion of regulatory T cells).

$CD4^+$ lymphocyte count was calculated by multiplying the lymphocyte cell count by the proportion of lymphocytes that were $CD4^+$. $CD4^+CD25^+CD127^{low/-}$ counts were found by multiplying $CD4^+$ lymphocyte count by the fraction of $CD4^+$ that were $CD25^+CD127^{low/-}$.

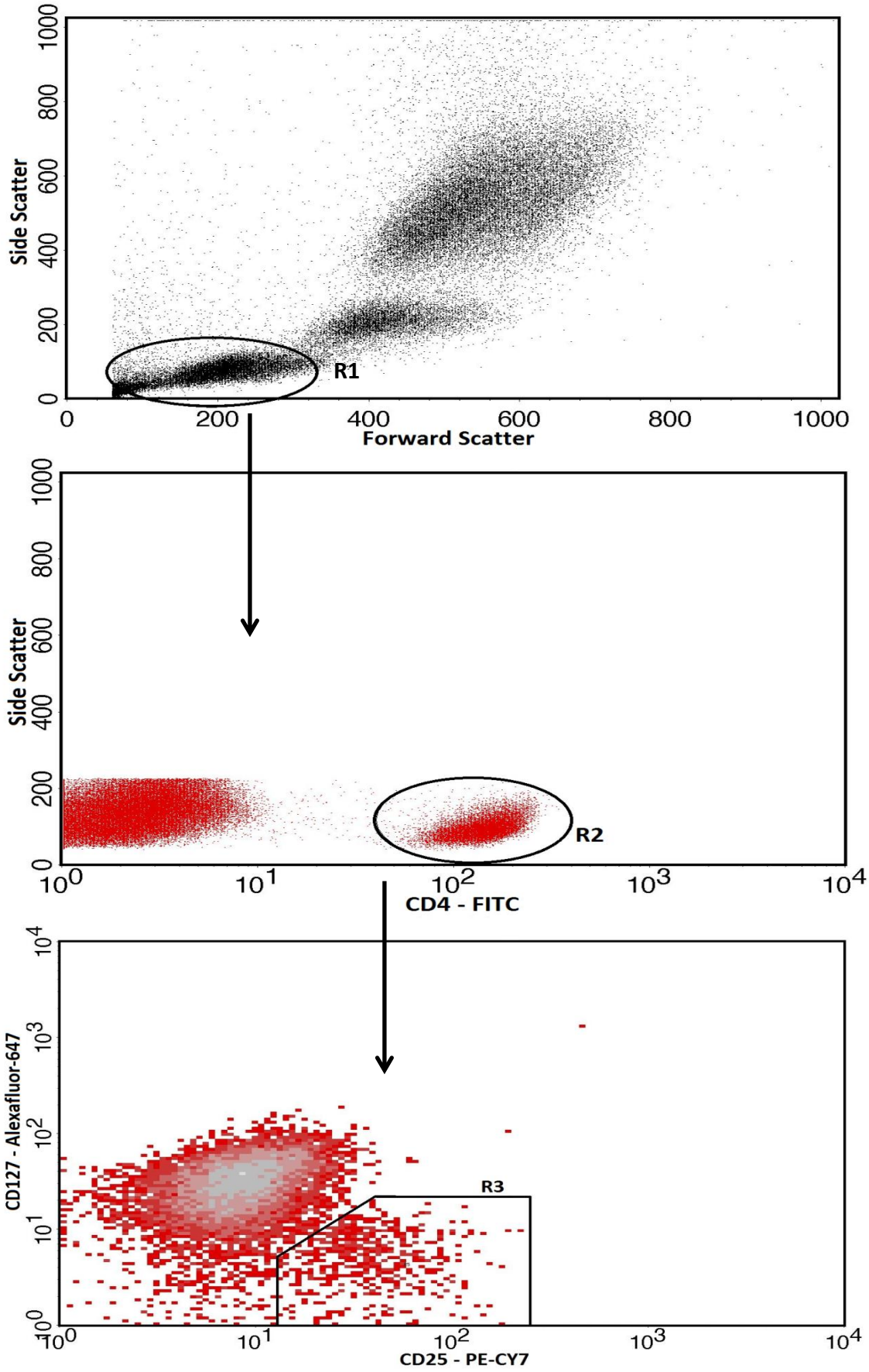

Figure 2.2 - Analysis of regulatory T cells by flow cytometry. R1 shows identification of lymphocytes based on morphology. R2 shows identification of those lymphocytes that are $CD4^{+}$. R3 denotes $CD25^{+}CD127^{low/-}$ on a density plot.

Monocyte phenotypes

Monocyte populations were identified using CellQuest software. Firstly, neutrophils were identified using side-scatter vs. CD16 and eliminated from further analyses. A group of monocytes was then identified based on morphology (forward-scatter vs. side-scatter). A new dot-plot gated on the selected monocytes then grouped cells into the three monocyte phenotypes (CD14 vs. CD16); monocytes were identified as $CD14^{++}CD16^{-}$, $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{++}$ (Figure 2.3; as recommended by Ziegler-Heitbrock and Hofer, 2013). $CD14^{-}CD16^{-}$ were disregarded as non-monocytes. The percentage of selected cells that were $CD14^{++}CD16^{-}$, $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{++}$ cells was added together (total monocytes); the proportion of each subset from the total monocytes was then calculated. Counts were determined by multiplying the proportion of each subpopulation by the overall monocyte count.

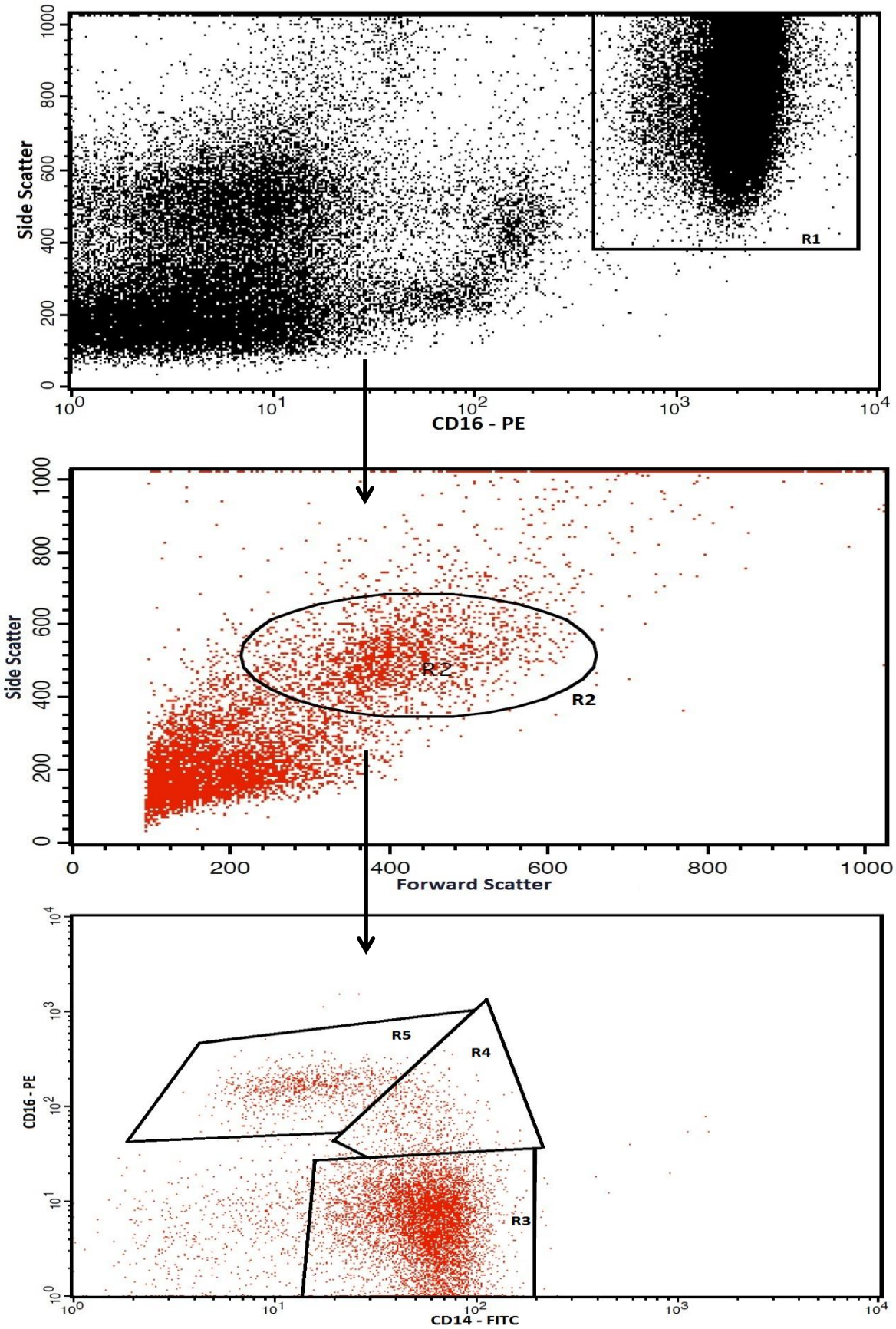

Figure 2.3 - Analysis of monocyte phenotypes by flow cytometry. R1 shows identification of neutrophils based on $CD16^{++}$ and side scatter. R2 shows identification of monocytes based on morphology after omission of neutrophils. $CD14^{++}CD16^{-}$ (R3), $CD14^{++}CD16^{+}$ (R4) and $CD14^{+}CD16^{++}$ (R5) monocytes were identified from this population of monocytes.

GHS-R1a on T cells and CD14+ Monocytes

T cells ($CD3^+$ lymphocytes) were identified in a two-step manner: 1) lymphocytes were identified based on morphology; 2) $CD3^+$ cells were identified on a dot-plot of CD3 vs. side scatter. These gates were used in combination to isolate $CD3^+$ lymphocytes (T cells). $CD14^+$ monocytes were identified via dot plot (CD14 vs. side scatter). Histogram plots of cells incubated with the isotype control antibody were used to define the threshold of positive staining for GHS-R1a on each cell type. This threshold was then used to assess the extent of positive staining for GHS-R1a on cells that had been conjugated to the IgG-APC antibody; the difference between the two values representing the proportion of cells positively expressing GHS-R1a (Figure 2.4). The geometric mean of the fluorescence intensity was recorded for the GHS-R1a expression.

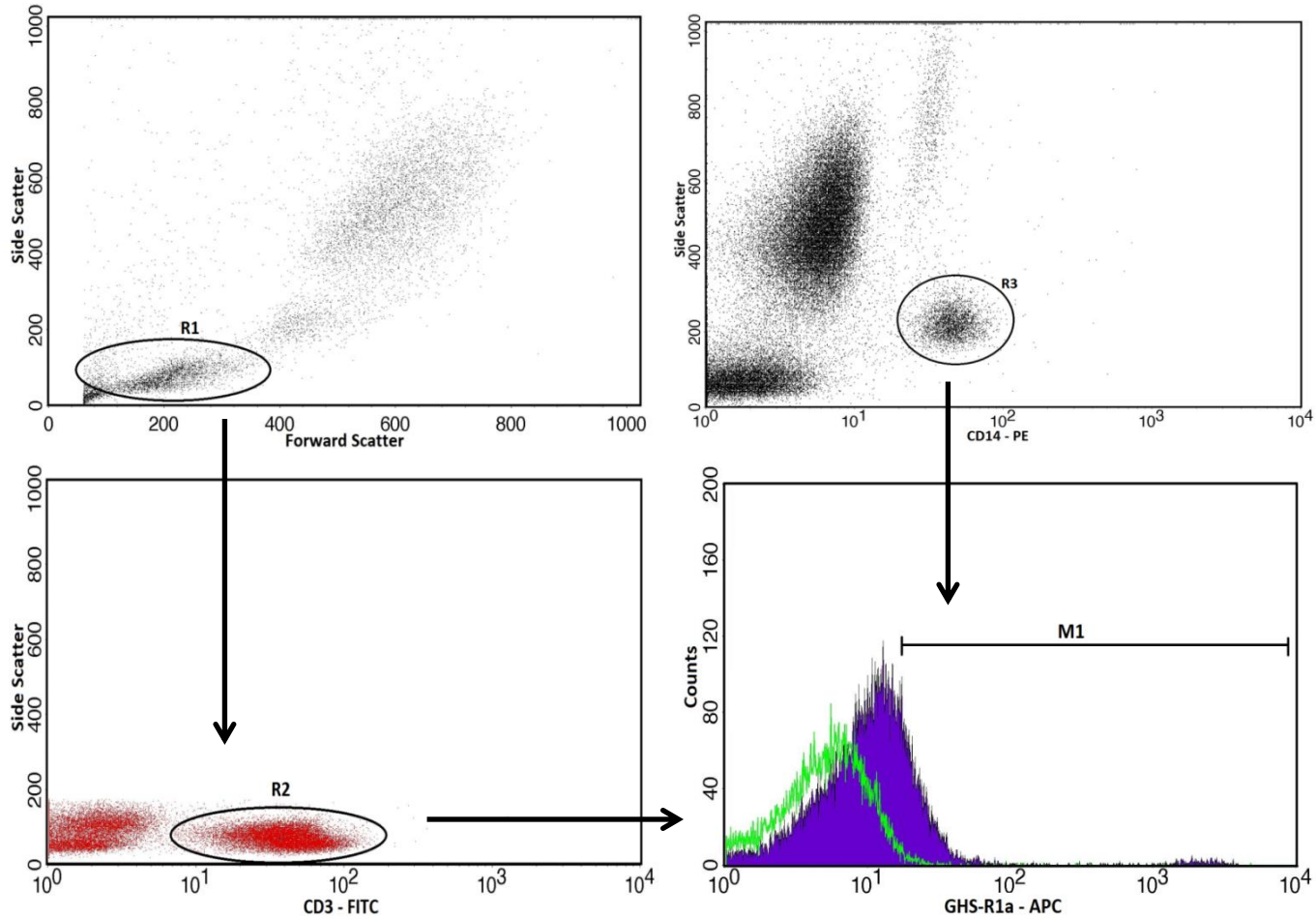

Figure 2.4 - Analysis of GHS-R1a by flow cytometry. R1 shows identification of lymphocytes based on morphology. R2 shows identification of $CD3^+$ T cells within the gated lymphocytes. R3 shows $CD14^+$ monocytes identified on the bases of CD14 vs. Side scatter. For both cell types GHS-R1a positive threshold was determined on the isotype negative control (M1 gate on the green outline population) and again on the conjugated antibody (M1 gate on the purple filled population).

2.8 Questionnaires

Questionnaires were completed on the same day of a blood sample once the patient had begun HD treatment. All questionnaires were explained to the subject. When AVF access precluded writing the researcher wrote verbatim for the patient. For those patients who could write during dialysis, the questionnaires were explained to them and were left to complete the questionnaires on their own, healthy participants completed the questionnaires in a similar manner. Sample questionnaires can be found in the appendices.

Anxiety and Depression

The Hospital Anxiety and Depression Scale (HADS; Zigmond and Snaith, 1983) was designed for diagnosing depression in somatically ill patients and is validated in ESRD patients (Loosman *et al.,* 2010). The questionnaire asks seven questions relating to anxiety and seven with depression with a score of 0-3 available for each answer; yielding a total score out of 42. A score of ≥ 12 has been suggested as the cut-off point for depression in this population (Loosman *et al.,* 2010). The HADS questionnaire was used to assess psychological wellbeing.

Symptom burden

Health-related quality of life was measured in terms of uraemic symptoms using the Leicester Uraemic Symptom Score tool (LUSS). Patients reported the frequency and intrusiveness of eleven different symptoms. The questionnaire is scored 0-4 for frequency and 0-4 for intrusiveness and the total number of symptoms identified is counted; generating a composite total score out of 99. The LUSS was used to allow careful analysis of individual symptoms as well as giving an overall picture of the impact of ESRD on quality of life.

Self-reported functional capacity

The Duke Activity Status Index (DASI) is a self-administered questionnaire that predicts physical function and exercise capacity (Hlatky *et al.,* 1989). The DASI is a 12-item questionnaire concerning functional ability to which the patient answers yes if they feel they are able to do an activity or no if not. Each question has a different weighting based upon the metabolic equivalent of task (MET) value of each task. The DASI has been widely used in renal disease patients and validated in pre-dialysis CKD patients (Ravani *et al.,* 2012). The DASI was used to assess the patients' self-reported physical functional ability.

Physical activity levels

The general practice physical activity questionnaire (GPPAQ) is an NHS screening tool used in primary care to assess adult physical activity levels (Department of Health, 2009). It is a validated and easy to use method to determine whether an individual is sedentary and may be at risk of co-morbidities associated with inactivity. The primary outcome from the GPPAQ is a 'Physical Activity Index', a product of activity at work and regular exercise that clusters individuals into 'inactive', 'moderately inactive', 'moderately active' and 'active' groups (Table 2.3); these groups correlate with CVD risk. The GPPAQ was designed for use in healthy individuals in a primary care setting and was completed by the healthy control participants in this project.

Table 2.3 – The calculation of the physical activity index from occupational and other physical exercise

Physical Exercise and / or Cycling ($h \cdot wk^{-1}$)	**Occupation**			
	Sedentary	**Standing**	**Physical**	**Heavy Manual**
0	Inactive	Moderately Inactive	Moderately Active	Active
Some but < 1	Moderately Inactive	Moderately Active	Active	Active
1 – 2.9	Moderately Active	Active	Active	Active
≥ 3	Active	Active	Active	Active

(Department of Health, 2009)

Food recall

Prior to all blood samples the participant was asked to recall all food or drink ingested so far on that day. A standard food recall form was used; this requested approximate quantities and timings of each item. Participants were prompted when further information was necessary.

2.9 Physical Activity

SenseWear™ physical activity monitors use a three-axis accelerometer as well as sensors detecting heat flux, skin temperature and galvanic skin response. SenseWear™ monitors have been validated using studies with doubly labelled water (Johannsen *et al.,* 2010) and are shown to adequately replicate energy expenditure and steps taken (Andre *et al.,* 2006; King *et al.,* 2004). These sensors have been used previously in HD patients (Avesani *et al.,* 2012; Mafra *et al.,* 2011).

Participants were given the accelerometers and instructed to wear the activity monitor for 7 days. The accelerometer was worn on the centre of the left triceps brachii; for patients with an AVF the non-fistula arm was always used. Participants were asked to remove the activity monitor when washing and for a short period of time each day; they were also asked to make sure this time was not a particularly active time. Otherwise, activity monitors were to be worn all day and night for 7 consecutive days. Any moderate or strenuous exercise bouts were recorded (e.g. cycling, gym class).

Data was downloaded onto the BodyMedia™ software alongside the participant's height, weight (dry weight for HD patients), smoking status and age. Minute-by-minute data was exported into a spreadsheet where short gaps in the data were filled using individual average values. Where large gaps (> 2 h) in waking hours existed or if the monitor was worn for less than a total 18 h, the day was excluded from analysis. If night-time data were missing these gaps were filled with average night-time activity for the participant on other days.

The accelerometer measured the time spent in different activity zones defined by METs. Sedentary behaviour was defined as 0-3 METs, moderate activity as 3-6 METs and vigorous as > 6 METs. In addition the number of steps taken per day was calculated and energy expenditure was estimated by the software and this was adjusted to kcal per kg body mass. Each parameter was adjusted to represent a 24 h period (i.e. steps·day^{-1}). At least one HD treatment day and one non-HD treatment day of usable data was required from each patient or all data was omitted.

2.10 Muscular endurance (STS 60)

Muscular endurance was assessed using the sit-to-stand 60 (STS 60) test. The STS 60 is widely used in CKD and chronic obstructive pulmonary disease (COPD) patients and is validated against more detailed and lengthy methods of assessment (McIntyre *et al.,* 2006; Ozalevli *et al.,* 2007). After discussing a 6 minute incremental walking test with a number of HD patients it became clear that patients were reluctant to spend additional time doing assessments. The STS 60 was used to assess functional ability, represented by muscular endurance.

For the patients the STS 60 was completed either prior-to or post-HD on a day when blood samples were not taken, the timing of which was replicated on subsequent tests. All participants had the procedure explained and demonstrated and were allowed a short practice before completing the test. Participants were asked to stand from a seated position in a chair of standardised height with their arms folded across their chest and return to a seated position as many times as comfortably possible within 60 s. Each stand was counted aloud and the participant was informed when half the time was completed, no other feedback was given during the STS 60. The test was terminated early if the participant was unable to complete any further stands or if they felt any undue discomfort.

2.11 Statistical analysis

The specific statistical analysis for each study is described in detail in the relevant chapter.

All data was assessed for normality of distribution using the Shapiro-Wilk test. Analysis of variance (ANOVA) was considered robust to minor departures of normality, where data was significantly non-normal two-factor ANOVA was performed on the logarithmic transformation of the data and reported in their original form.

When using ANOVA, if Mauchly's test indicated a violation of the assumption of sphericity the degrees of freedom were corrected using Greenhouse-Geisser method or Huynh-Feldt equation if the epsilon was greater than 0.75 (Atkinson, 2001).

P values derived from *post hoc* tests were adjusted for multiple comparisons using the Holm-Bonferroni method (Holm, 1979).

Effect sizes (ES) were calculated using Cohen's D and pooled standard deviation to adjust for sample size (Hedges, 1981); 0.3 was considered a small effect, 0.5 a medium effect and 0.8 a large effect (Cohen, 1988).

All statistical analysis was performed on Statistical Package for Social Sciences (SPSS v.21, IBM, New York, USA). Graphs were drawn using GraphPad Prism (v6. GraphPad Software Inc, CA, USA). Data is presented as mean ± standard deviation (SD), median (interquartile range) or count (percentage) as described. Statistical significance was accepted at the $P < 0.05$ level.

Chapter 3

Characterisation of Physical Activity and Circulating Markers of Inflammation in Haemodialysis Patients and Healthy Age-Matched Controls

3.1 Abstract

HD patients have substantially elevated risk of CVD and mortality that is partly attributed to the harmful effects of uraemia on the immune system. The immune system is chronically over-activated leading to inflammation and dysfunction. HD patients are reported to be a highly sedentary population but the extent to which inactivity is associated with inflammation and immune dysfunction is not clearly defined. The aim of this study was to characterise HD patients in terms of both their physical activity and function and various circulating markers and mechanisms of inflammation. This study also sought to analyse the interactions between these factors.

Thirty HD patients and 16 age-matched healthy controls volunteered to take part in the study (63.9 ± 13.8 vs. 61.4 ± 10.9 y; 60% vs. 50% male). Participants wore an accelerometer for 7 days to evaluate habitual physical activity levels and the STS 60 test was completed for physical function. Circulating concentrations of IL-6, CRP, TNF-α and IL-10 were assessed via ELISA and proportions and numbers of monocyte phenotypes, Tregs and PBMC GHS-R1a expression were assessed via flow cytometry. Neutrophil degranulation response to a bacterial challenge was determined. Finally, aspects of quality of life were evaluated via LUSS, DASI and HADS questionnaires.

HD patients had significantly elevated CRP, IL-6 and TNF-α concentrations (4.90 (2.68-9.95) vs. 0.83 (0.21-1.90) $mg \cdot L^{-1}$; 4.53 (2.71-6.98) vs. 0.81 (0.43-1.76) $pg \cdot mL^{-1}$; 3.89 (2.93-4.27) vs. 0.96 (0.60-1.73) $pg \cdot mL^{-1}$ respectively; all $P < 0.001$). HD patients were less active than the healthy controls (2994 (2067-4459) vs. 7594 (5847-9539) $steps \cdot day^{-1}$; $P < 0.001$) and this was exacerbated on days when they dialysed (HD 2100 (1649-2923) vs. non-HD 3279 (2393-4997) $steps \cdot day^{-1}$; $P < 0.001$). The STS 60 score was the strongest correlate of IL-6 and CRP levels in HD patients (rho = -0.559, $P = 0.007$ and rho = -0.499, $P = 0.018$); activity levels correlated with circulating markers of inflammation in the healthy controls but not HD patients.

Distribution of monocyte populations shifted toward intermediate ($CD14^{++}CD16^{+}$: 6.55 (5.74-7.52) % vs. 4.34 (3.74-4.95) %, $P < 0.001$) and non-classical phenotypes ($CD14^{+}CD16^{++}$: 14.5 (10.6-17.9) % vs. 6.5 (4.2-8.5) %, $P < 0.001$) in HD patients. The proportion, but not the number, of CD4+ lymphocytes that were Tregs was lower in HD patients (6.55 ± 1.34% vs. 7.67 ± 0.98%, $P = 0.006$), GHS-R1a expression on T cells and monocytes was not different between groups, although the number of GHS-R1a+ monocytes in the HD cohort was greater ($P < 0.024$). When adjusted to take into account circulating cell numbers, the neutrophil degranulation response to bacterial stimulation was blunted in the patient group (215 ± 52 vs. 281 ± 85 $fg \cdot cell^{-1}$, $P = 0.043$).

Patients experienced a greater number and overall burden of symptoms and reported a greater degree of depression.

The high levels of pro-inflammatory markers and mechanisms found in the circulation of HD patients in this study likely underlies the increased risk of CVD experienced by HD patients as a consequence of their immune over-activation and dysfunction. There appears a great potential to improve physical activity in HD patients. The impact of increasing physical activity in HD patients warrants further investigation, particularly as exercise may have the capacity to address various aspects of chronic inflammation, as well as a wide range of symptoms associated with ESRD.

3.2 Background

ESRD patients are associated with a highly elevated risk of all-cause and, specifically, cardiovascular mortality (Sarnak *et al.,* 2003.). Traditional risk factors for cardiovascular mortality are less applicable in this vulnerable population and a reverse epidemiology of traditional prognostic markers is observed (BMI, hypertension, cholesterol; Kalantar-Zadeh *et al.,* 2005). Non-traditional markers including chronic inflammation are particularly important in predicting mortality in this population (Kalantar-Zadeh *et al.,* 2003; Stenvinkel, 2001).

Various aspects of immune dysfunction have been reported in ESRD, and even more so in HD patients. The underlying causes of this dysfunctional immune system are multifaceted and likely include the presence of recurrent infections, a uraemic milieu and the effects of regular HD treatment, amongst other factors (Cheung, Paik and Mak*,* 2010). The result is a compromised immune system that is both chronically over-activated but also dysfunctional against antigen challenge (Vaziri *et al.,* 2012).

In the general population, the CKD population, and in other chronic diseases, regular physical activity is purported to have a plethora of beneficial properties. Regular exercise is associated with reduced mortality and morbidity in various chronic diseases (Pedersen and Saltin, 2006). Moreover, habitual physical activity and exercise training are associated with an anti-inflammatory response that manifests itself through a number of different mechanisms. Reduced circulating concentrations of pro-inflammatory cytokines have been reported but additionally, alterations in visceral fat mass, adrenal hormones, monocyte and macrophage phenotype, function and receptor expression further to increased proportions of circulating regulatory lymphocytes have been found (Gleeson *et al.,* 2011).

Within the HD population, patients are frequently inactive and the greater levels of inactivity are associated with an increased risk of mortality (Johansen *et al.,* 2000; O'Hare *et al.,* 2003). However, the extent to which inactivity is associated with inflammation and immune dysfunction is not clearly defined (Dungey *et al.,* 2013). Furthermore, studies in this cohort have remained focussed on circulating cytokine concentrations rather than cellular mechanisms of inflammation.

HD patients suffer from a wide range of symptoms associated with their disease (Murtagh, Addington-Hall and Higginson, 2007). Consequently, not only is life expectancy reduced but the quality of life is diminished. Simply reporting the presence of a symptom does not explore the level of intrusiveness felt by the patient; the symptoms that afflict HD patients need to be fully understood in order find methods to amend them.

The main purpose of this study was to characterise the HD population in terms of markers and mechanisms underlying systemic inflammation, and levels of physical activity and function in comparison with a group of healthy age-matched individuals. Further, this study aimed to explore symptoms that may affect the quality of life that HD patients experience. Finally, this study sought to determine the potential interactions between these factors.

3.3 Methods

Ethics statement

The HD patients recruited to this study were consented under NHS ethics; all aspects of this study were included in the protocol approved by the NHS trust Research and Development office (ref. UHL 11045) and the NHS Research Ethical committee (ref. 11/EM/0149). The healthy participants were recruited under Loughborough University local ethical approval (ref. R13-P112).

Recruitment

HD participants were a subset of the exercise training study. All patients who took part in the longitudinal study and were able to complete this protocol did so; of the 38 patients recruited to the training study 8 were lost to drop out or completion prior to this study. HD patients were recruited prior to enlisting healthy participants.

Healthy participants were subsequently recruited in an age-matched fashion. The HD patients who had already completed the protocol were grouped into age ranges (< 40, 40-50, 50-60, 60-70, 70-80 and 80 < y) and recruitment of healthy participants was designed so as to recruit a similar proportion of people from each age range.

On arrival at the laboratory a thorough medical history was taken using a health screen questionnaire and any conditions that became apparent were investigated further. Exclusion criteria can be found in the general methodology (Tables 2.1 and 2.2). If the participant was deemed eligible informed consent was gained.

Protocol

The healthy participants arrived at the laboratory between 10am and midday having not eaten in the previous 2 h and having abstained from caffeine, alcohol and meat for at least 12 h. Resting blood pressure was taken using an automated blood pressure reader (Omron M5–1, Omron Healthcare, Hoofddorp, The Netherlands), and height and weight

were measured. A resting blood sample (25 mL) was then taken from an antecubital vein directly into pre-treated monovettes. The participant completed 5 questionnaires: the HADS, DASI, LUSS, GPPAQ and a food recall form. Upon completion a STS 60 test was carried out and the accelerometer was given to the participant to wear.

After 7 days the participant returned the accelerometer to the laboratory. The participant was asked to recall any specific exercise bouts during the week and any problems with the activity monitor, these were noted.

In the HD patients, the blood sample was taken prior to dialysis through the HD access into a dry syringe that was dispensed immediately into pre-treated monovettes. The DASI, HADS, LUSS and food recall form were completed while the patient was dialysing. The accelerometer was given to the patient to wear on a non-fistula arm before collection 7 days later; the STS 60 was performed in the week prior or after activity monitoring. Medical histories were accessed from the patient's medical notes and the results from the most recent blood test were recorded.

Food, caffeine, and alcohol intake was not restricted in the HD patients. Diet recall found no alcohol consumption and minimal food and caffeine consumption in the 2 h prior to blood sampling.

Outcome measures

The following outcome measures were determined, for detailed methods the reader is asked to refer to the general methodology (Chapter 2).

Kidney function was derived using the simplified MDRD equation from creatinine results (Levey *et al*., 2000). For healthy participants, serum from a serum gel monovette was collected and sent to the University Hospitals of Leicester pathology department for independent assessment of creatinine.

Heparinised blood was used to determine the quantity and proportion of Tregs ($CD4^{+}CD25^{+}CD127^{low/-}$), distribution of monocyte phenotypes (classical: $CD14^{++}CD16^{-}$, intermediate: $CD14^{++}CD16^{+}$, non-classical: $CD14^{+}CD16^{++}$) and the expression of the GHS-R1a on T cells and $CD14^{+}$ monocytes using flow cytometry.

Circulating concentrations of CRP (intra-assay CV 6.6%, inter-assay CV 10.0%), IL-6 (10.2% and 10.0%), TNF-α (8.2% and 9.6%), and IL-10 (intra-assay CV 10.8%) were assessed using commercially available ELISA kits and plasma from K_3EDTA treated monovettes. Two healthy CRP values were below the lowest standard of the assay (< 0.1 $mg \cdot L^{-1}$); the value of the lowest standard is reported (0.1 $mg \cdot L^{-1}$). Neutrophil elastase degranulation in response to a bacterial challenge was measured as described earlier (Chapter 2) on a single plate (intra-assay CV 2.2%), samples were excluded when unstimulated plasma elastase values exceeded 500 $\mu g \cdot L^{-1}$.

Anxiety and depression and a total score from the HADS questionnaire, a total symptom burden score and individual symptom scores from the LUSS, and a perceived physical function score from the DASI were calculated in both populations. The physical activity index was defined using the GPPAQ questionnaire in the healthy group.

Daily energy expenditure adjusted for body mass, steps per day, and time spent in sedentary (0-3 METs) and physically active ranges ('moderate', 3-6 METs; 'vigorous', > 6 METs) were established from the accelerometer data.

Participant characteristics

Thirty HD patients and 16 healthy participants were recruited to the study (Table 3.1); all participants completed all the questionnaires. Healthy participants provided complete usable accelerometer data and STS 60 tests. Twenty-four HD patients provided complete and usable accelerometer data, 3 declined or were unfit to wear the monitor and 3 wore the monitor but data was incomplete and unusable upon analysis; 23 HD patients completed a STS 60 test. Blood samples were taken from all patients, plasma concentrations of inflammatory factors were measured from all patients, where antibody staining was unsuccessful in the flow cytometry the participant numbers are stated. Only 300 µL of blood was collected from one healthy participant; therefore, for all blood measures other than Tregs, n = 15.

Table 3.1 - Participant characteristics

	HD patients (n = 30)	Healthy participants (n = 16)	*P* value
Age (y)	63.9 ± 13.8	61.4 ± 10.9	0.547
Gender ‡			
Male	18 (60%)	8 (50%)	0.515
Ethnicity ‡			
White	22 (73%)	16 (100%)	0.023*
Indian	8 (27%)	0 (0%)	
Body mass (kg)	78.0 ± 22.1	75.3 ± 16.6	0.673
BMI (kg·m^{-2})	27.8 ± 6.1	26.1 ± 5.0	0.349
Aetiology ‡ #			
Glomerulonephritis	6 (20%)		
Cystic/poly	5 (17%)		
Renal vascular disease	3 (10%)		
Pyelonephritis	5 (17%)		
Interstitial	1 (3%)		
Diabetes	2 (7%)		
Uncertain	5 (17%)		
Other	3 (10%)		
Haemodialysis vintage † **(y)**	2.38 (1.46-4.34)		
Immunosuppressive therapy ‡ [a]	3 (10%)		

BMI, Body Mass Index.
Data presented as mean ± SD, † Data presented as median (interquartile range),
‡ Data presented as n (%).
*Significant difference between groups.
based on UK Renal Registry classification (UK Renal Registry, 2010).
[a] excluded from analysis of immune function and markers of inflammation.

Healthy and HD participants were well matched for age. There were also no significant differences between the two groups in terms of gender, body mass and BMI. Ethnicity varied between the groups; comparisons between ethnicities found significant differences in height, body mass and age (all lower in Indian patients) but not in any outcome measures of interest; consequently all data is reported as a single cohort. Three HD patients were excluded from analysis of immune and inflammatory parameters due to the immunosuppressive medication they were prescribed; all were previous recipients of a renal transplant.

Statistical analysis

Simple comparisons between HD and healthy groups were completed using independent samples t-tests or, where data was non-normally distributed, the non-parametric Mann-Whitney test. Physical activity levels on HD treatment days compared with non-dialysis days were assessed using repeated measures paired t-tests or Wilcoxon signed-rank test for non-parametric data. Chi square was used to assess differences in gender and ethnicity between groups.

Correlation analysis was used to assess relationships between physical activity, inflammatory markers and other parameters. Normally distributed data were correlated using Pearson's correlation coefficient (*r*); Spearman's rho was used when data was non-parametric.

3.4 Results

Disease condition and biochemistry

The key biochemistry and disease states for both populations are reported in Table 3.2. Kidney function was normal in the healthy participants and significantly greater than the eGFR of the HD group. One healthy participant had an eGFR value below 60 (55 $mL \cdot min^{-1} \cdot 1.73m^{-2}$), this elderly gentleman (79.8 y) had no renal symptoms and normal blood pressure; the lower eGFR was considered normal ageing, his data is included.

Circulating electrolytes and metabolic waste products were different between groups. Resting SBP was not different between groups although the diastolic blood pressure (DBP) was higher in the healthy group; this may be because this group was less medicated. HD patients were more anaemic and had a higher leucocyte count, mainly accounted for by elevated numbers of circulating neutrophils (ES = 0.82).

Morbidities reported in the healthy group included temporary ailments (e.g. conjunctivitis, nail infection, uveitis), depression and stress, and conditions often associated with older age such as deafness, osteoporosis and arthritis, and hypertension (n = 2). HD patients had a large number of various co-morbidities including hypertension (n = 27), dyslipidaemia, thyroid and parathyroid conditions, type II diabetes mellitus and ischaemic heart disease.

On the basis of this biochemistry the HD patients could be considered as a representative HD population, the control population were adjudged a regular healthy cohort with all measured biochemistry and renal function fitting within normal healthy ranges (Kumar and Clark, 2012).

Table 3.2 – Serum biochemistry, haematology, resting blood pressure and disease condition in healthy and haemodialysis participants

	HD patients (n = 30)	Healthy controls (n = 16)	*P* value	Normal range
eGFR† (mL·min^{-1}·1.73m^{-2})^{n1}	6 (5-7)	106 (84-117)	< 0.001*	> 90
Creatinine (µmol·L^{-1})^{n1}	745 ± 140	69 ± 22	< 0.001*	79 – 118
Urea† (mmol·L^{-1})^{n1}	17.4 (13.7-19.7)	4.9 (4.3-6.3)	< 0.001*	2.5 – 6.7
Sodium† (mmol·L^{-1})^{n1}	135 (133-138)	139 (137-140)	0.005*	135 – 146
Potassium† (mmol·L^{-1})^{n1}	5.20 (4.55-5.78)	4.60 (4.35-4.90)	0.029*	3.5 – 5.0
Systolic BP (mmHg)	135 ± 17	129 ± 13	0.212	< 130
Diastolic BP (mmHg)	74 ± 14	83 ± 7	0.006*	< 85
Red blood cells (x10^{12}·L^{-1})^{n1}	3.70 ± 0.54	4.49 ± 0.44	< 0.001*	3.9 – 6.5
Haemoglobin† (g·dL^{-1})^{n1}	10.8 (10.3-12.2)	13.5 (12.2-14.6)	< 0.001*	11.5 – 17.5
Leucocytes (x10^{9}·L^{-1})^{n1}	6.64 ± 2.16	5.14 ± 0.95	0.002*	4 – 11
Neutrophils (x10^{9}·L^{-1})^{n1}	3.95 ± 1.73	2.85 ± 0.80	0.006*	2.0 – 7.5
Monocytes† (x10^{9}·L^{-1})^{n1}	0.60 (0.53-0.70)	0.50 (0.45-0.70)	0.185	0.2 – 0.8
Lymphocytes (x10^{9}·L^{-1})^{n1}	1.72 ± 0.67	1.49 ± 0.36	0.233	1.5 – 4.0
Co-morbidities† (count per person)	4 (2-5) (range 1 – 7)	1 (1-1) (range 0 – 4)	< 0.001*	
Medications† (count per person)	10 (7-14) (range 4 – 19)	1 (0-1) (range 0 – 4)	< 0.001*	

BP, Blood Pressure; eGFR, estimated Glomerular Filtration Rate; HD, haemodialysis.
Data presented as mean ± SD, † Data presented as median (interquartile range).
n1 n = 15 in healthy participants.
* Significant difference between groups.
Normal ranges derived from Kumar and Clark (2012).

Physical activity

The healthy participants reported a range of activity levels, 10 of the participants had a Physical Activity Index score eligible for a 'Brief Intervention in Physical Activity' (Department of Health, 2009).

Healthy participants were significantly more active than the HD patients (Table 3.3 and Figure 3.1). HD patients spent less time active (ES = 0.56), expended less energy per kg body mass (ES = 0.74) and took far fewer steps during the day (ES = 1.25). In the STS 60 test, a surrogate measure of physical function, healthy participants significantly outscored their HD counterparts (ES = 0.79).

Table 3.3 - Indices of physical activity and function in healthy and haemodialysis participants

	Haemodialysis patients (n = 24)	**Healthy controls (n = 16)**	***P* value**
Physical Activity Index [#]			
Active	-	6 (37.5%)	
Moderately active	-	2 (12.5%)	
Moderately inactive	-	2 (12.5%)	
Inactive	-	6 (37.5%)	
Energy Expenditure (kcal·day^{-1})	2258 ± 566	2511 ± 357	0.121
Adjusted [†] **(kcal·kg^{-1}·day^{-1})**	28.2 (24.9-31.9)	33.3 (30.0-35.0)	0.006*
Steps [†] **(per day)**	2994 (2067-4459)	7594 (5847-9539)	< 0.001*
Sedentary behaviour [†] **(min·day^{-1})**	1385 (1335-1411)	1335 (1295-1354)	0.011*
Moderate activity [†] **(min·day^{-1})**	55 (28-104)	104 (85-137)	0.011*
STS 60 (reps) [n1]	18 ± 9	28 ± 13	0.026*

STS 60, Sit-to-Stand 60.
Data presented as mean ± SD, † Data presented as median (interquartile range),
Data presented as count (percentage).
[n1] n = 23 for haemodialysis patients.
* Significant difference between groups.

HD patients were significantly less active on days when they had HD treatment (Figure 3.1). They spent more time being sedentary and less time in the moderately active range (3-6 METs; 51.8 (16.0-92.4) vs. 57.2 (27.8-105) min·day^{-1}, *P* = 0.040, ES = 0.05). Moreover, patients took fewer steps on treatment days (*P* < 0.001, ES = 0.38) and expended less energy (*P* = 0.014, ES = 0.16).

In the healthy group the Physical Activity Index was shown to be a good indicator of physical activity as it correlated with steps taken (rho = 0.659, *P* = 0.006), STS 60 (rho = 0.626, *P* = 0.010), DASI score (rho = 0.594, *P* = 0.015), time physically active (rho = 0.651, *P* = 0.006), energy expenditure adjusted for body mass (rho = 0.563, *P* = 0.023) as well as age (rho = -0.770, *P* < 0.001) and BMI (rho = -0.626, *P* = 0.010). Conversely, energy expenditure without adjustment for body mass was not a good correlate with other measures of physical activity (e.g. time physically active in healthy: rho = 0.053, *P* = 0.846; and HD: rho = 0.109, *P* = 0.613).

The STS 60 score was not associated with direct measures of physical activity in the HD patients, but related to steps taken per day (*r* = 0.639, *P* = 0.008) and time physically active (rho = 0.587, *P* = 0.017) in the healthy group. In addition, STS 60 negatively correlated with age in both cohorts (HD: *r* = -0.687, *P* < 0.001; healthy: *r* = -0.528, *P* = 0.035).

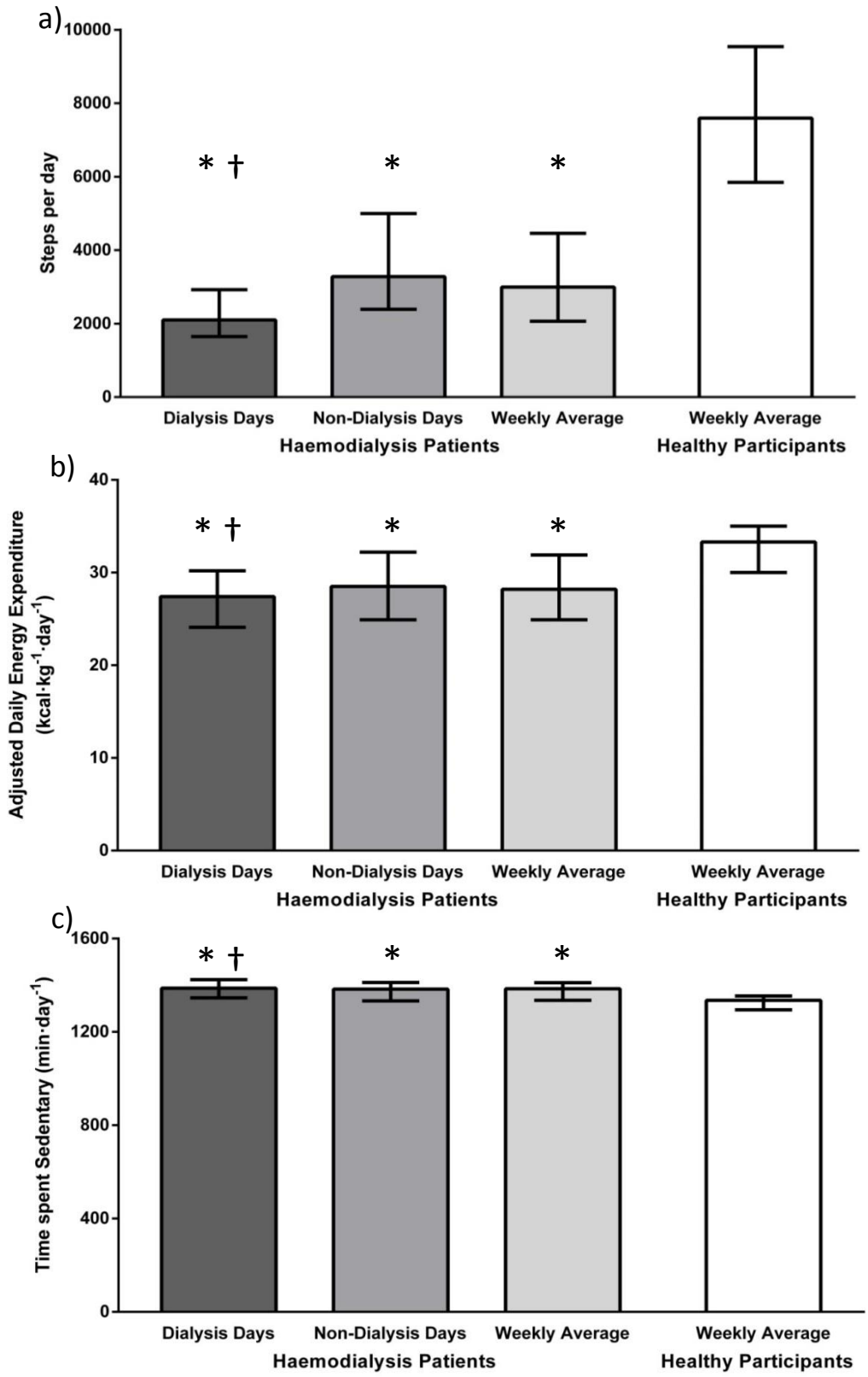

Figure 3.1 - Parameters of physical activity in haemodialysis patients on treatment and non-treatment days and in healthy participants. Figure **a)** shows steps per day, **b)** daily energy expenditure adjusted for body mass, **c)** time spent being inactive per day (< 3 METs).
* denotes significantly different from the healthy group,
† denotes significantly different from the non-dialysis days.
Data is presented as median and interquartile range.
Haemodialysis patients n = 24, healthy controls n = 16.

Circulating markers of inflammation

Elevated circulating concentrations of IL-6, TNF-α and CRP (all $P < 0.001$, ES = 1.37, 1.62 and 1.26 respectively) were observed in HD patients (Figure 3.2). There was difficulty detecting IL-10 in both groups, the proportion of samples greater than the low standard of the assay was higher in the HD groups but no statistical differences were evident between groups ($P = 0.293$).

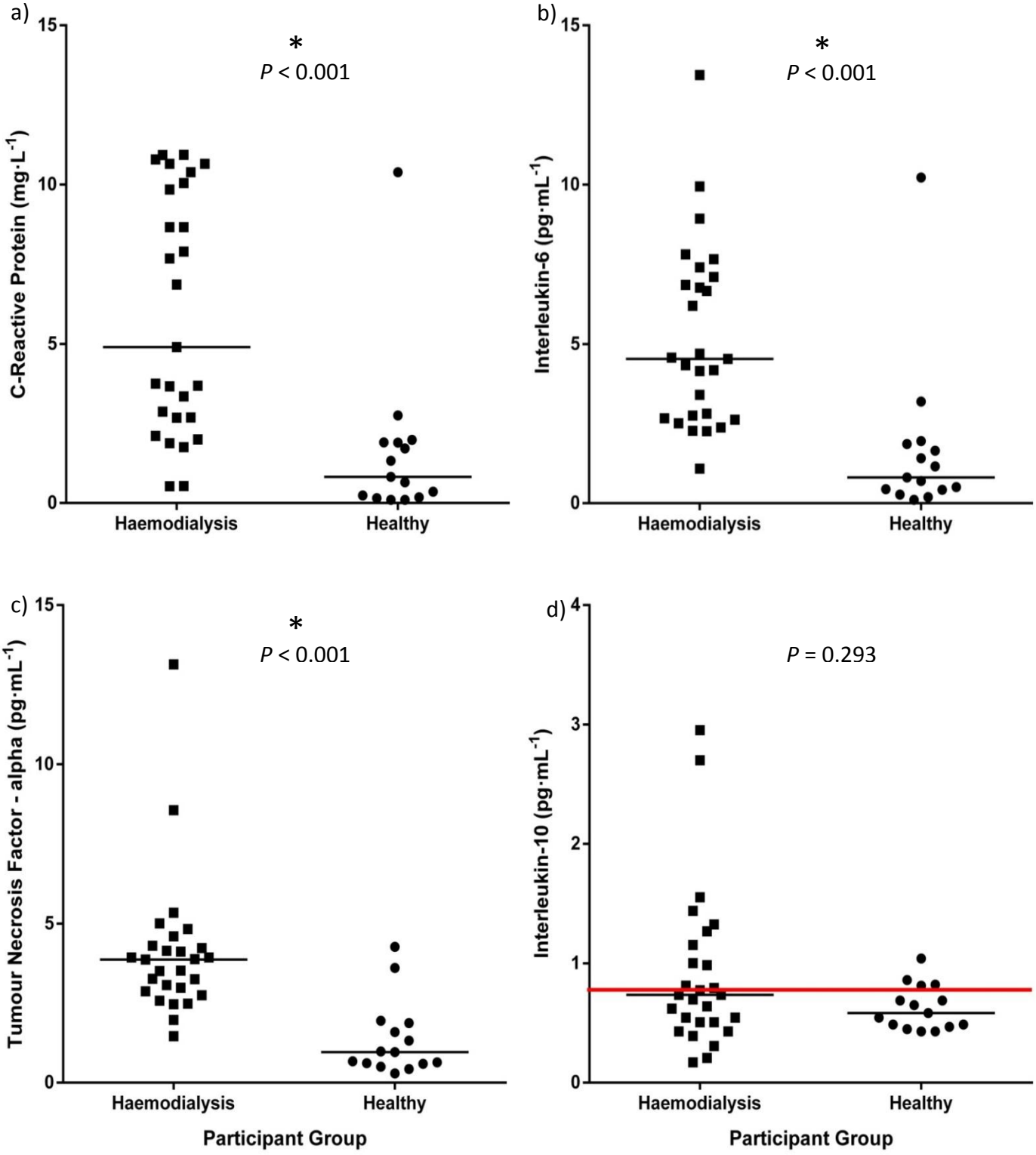

Figure 3.2 - Circulating concentrations of **a)** C-reactive protein, **b)** Interleukin-6, **c)** Tumour necrosis factor alpha, and **d)** Interleukin-10 in the haemodialysis patients and healthy controls. Black symbols represent each individual, the black line denotes the median and the red line is the low standard of the assay for interleukin-10.
* denotes a significant difference between groups.
Haemodialysis patients n = 27, healthy controls n = 15.

Monocyte subpopulations were shifted towards intermediate and non-classical phenotypes in HD patients in both the proportion (ES = 1.17 and 1.37) and count (ES = 1.33 and 1.62) of each monocyte subset (Table 3.4); example phenotype distributions are shown in Figure 3.3. The proportion of $CD4^+$ lymphocytes that were Tregs was lower in HD patients (ES = 0.95); although the number of Tregs was not dissimilar to the healthy controls as HD patients had higher counts of total $CD4^+$ lymphocytes (ES = 0.75). The proportion of T cells and monocytes expressing the GHS-R1a was not different between groups. The number of $CD14^+$ monocytes expressing GHS-R1a was greater in HD patients compared with healthy controls (ES = 0.70).

Table 3.4 - Circulating leucocyte subpopulations and expression of GHS-R1a in healthy and haemodialysis participants

	Haemodialysis patients (n = 26)	Healthy controls (n = 15)	*P* value
Classical monocytes			
Proportion (%) †	77.1 (74.2-83.7)	89.5 (87.0-92.1)	< 0.001*
Count (cells·μL^{-1})	510 ± 107	505 ± 130	0.908
Intermediate monocytes †			
Proportion (%)	6.55 (5.74-7.52)	4.34 (3.74-4.95)	< 0.001*
Count (cells·μL^{-1})	42.7 (34.5-53.3)	21.7 (19.4-29.1)	< 0.001*
Non-classical monocytes †			
Proportion (%)	14.5 (10.6-17.9)	6.4 (4.2-8.5)	< 0.001*
Count (cells·μL^{-1})	101 (74-110)	30 (23-50)	< 0.001*
CD4$^+$ Lymphocytes			
Proportion (%) [n1]	24.9 ± 6.9	21.5 ± 6.9	0.127
Count (cells·μL^{-1}) † [n2]	393 (294-543)	264 (227-369)	0.010*
Regulatory T cells			
Proportion (%) [n1]	6.55 ± 1.34	7.67 ± 0.98	0.006*
Count (cells·μL^{-1}) † [n2]	24.0 (19.3-36.2)	20.1 (18.6-25.0)	0.212
CD3+ Lymphocytes † [n2]			
Proportion (%)	41.3 (31.1-52.5)	34.6 (33.4-43.7)	0.300
Count (cells·μL^{-1})	689 (452-904)	605 (437-665)	0.194
GHS-R1a$^+$ T cells † [n2]			
Proportion (%)	10.2 (9.1-13.4)	14.9 (8.8-17.4)	0.229
GMFI	5.60 (4.31-7.03)	4.38 (3.12-5.59)	0.109
Count (cells·μL^{-1})	86.9 (41.6-108.4)	68.2 (44.5-89.5)	0.408
GHS-R1a$^+$ CD14$^+$ monocytes † [n3]			
Proportion (%)	11.6 (7.9-15.3)	8.4 (6.7-9.8)	0.219
GMFI	4.78 (3.83-6.56)	3.08 (2.69-3.90)	0.023*
Count (cells·μL^{-1})	73.3 (52.0-97.1)	42.1 (33.6-59.4)	0.024*

GHS-R1a, Growth hormone secretagogue receptor-1a; GMFI, Geometric mean florescence intensity; HD, haemodialysis patients.

Classical monocytes, CD14^{++}CD16$^-$; Intermediate monocytes, CD14^{++}CD16$^+$; Non-classical monocytes, CD14$^+$CD16^{++}.

Regulatory T cells, proportion of CD4$^+$ lymphocytes expressing CD25$^+$CD127$^{low/-}$.

Data presented as mean ± SD, † Data presented as median (interquartile range).

[n1] n = 16 for healthy controls; [n2] n = 27 for haemodialysis patients; [n3] n = 13 for healthy controls.

* Significant difference between groups.

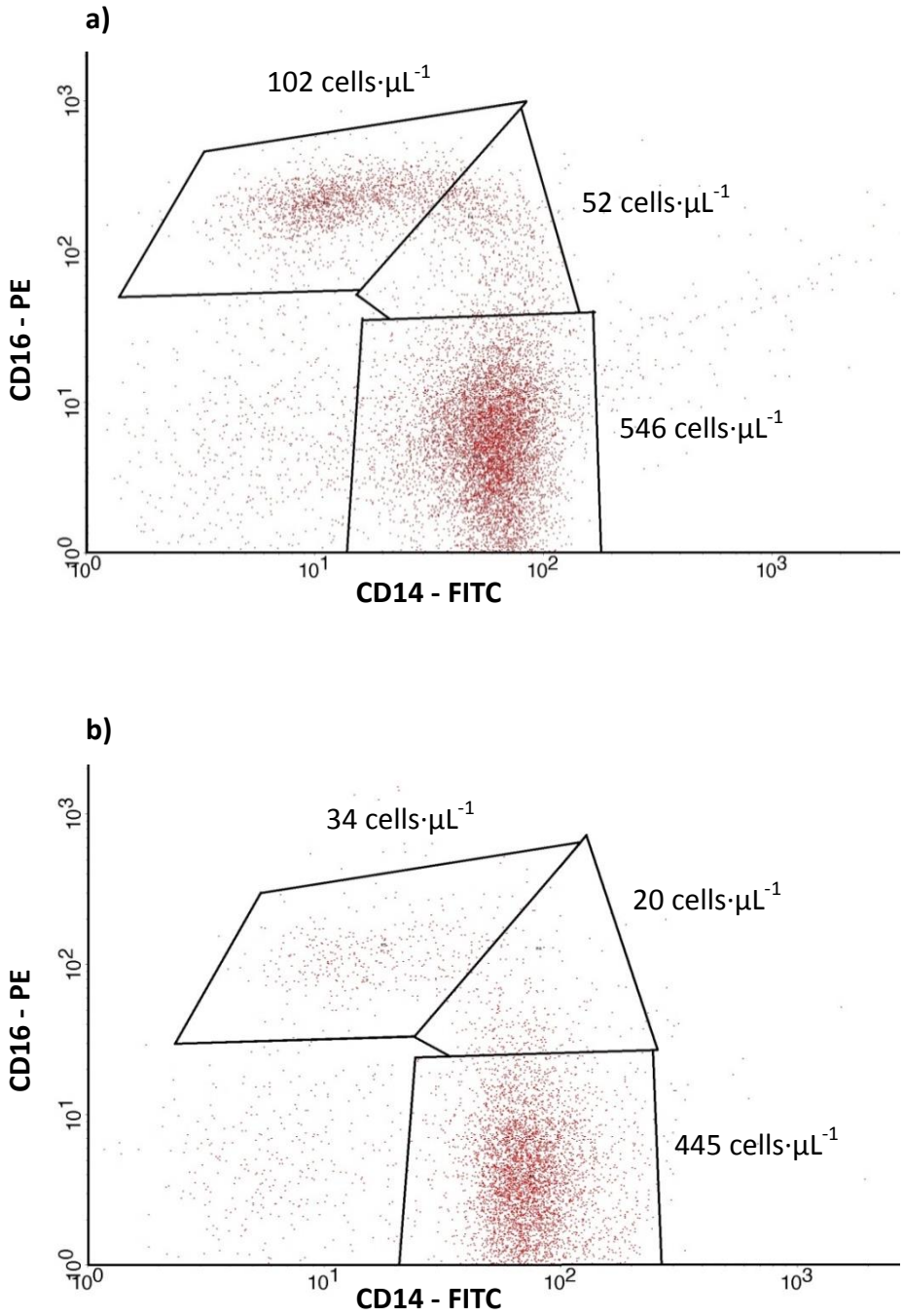

Figure 3.3 - Representative distribution of monocyte phenotypes shown by flow cytometry in **a)** a haemodialysis patient **b)** a healthy individual. Numbers denote the concentration of cells in each specific phenotype.

Within both the HD and healthy cohorts IL-6 and CRP levels correlated significantly (rho = 0.562, *P* = 0.002; rho = 0.688, *P* = 0.005 respectively). Further, both cohorts demonstrated a positive correlation between the concentrations of TNF-α with the proportion of intermediate monocytes (HD: rho = 0.402, *P* = 0.042; healthy: rho = 0.611, *P* = 0.016). BMI was positively correlated with IL-6 and CRP (rho > 0.815, *P* < 0.001) and older age was associated with elevated IL-6 (rho = 0.529, *P* = 0.043) in the healthy cohort but neither were related to markers of inflammation within the HD population (*P* > 0.05).

Within the HD group, STS 60 was the strongest correlate of IL-6 and CRP. In the healthy controls IL-6 and CRP correlated with a number of parameters of physical activity (Table 3.5); whereas in the HD group, other than the STS 60, no associations were seen between physical activity levels and cytokines (all *P* ≥ 0.218).

There was a trend towards an association in time physically active and proportion of Tregs in the HD patients (rho = 0.421, *P* = 0.051) but no significant associations between monocyte or lymphocyte subsets or GHS-R1a expression and activity levels were found.

Table 3.5 - Associations between physical activity parameters and markers of inflammation

	Haemodialysis patients		**Healthy controls**	
	IL-6	**CRP**	**IL-6**	**CRP**
STS 60	rho = -0.559 *P* = 0.007*	rho = -0.499 *P* = 0.018*	rho = -0.519 *P* = 0.047*	rho = -0.491 *P* = 0.063
Steps per day	rho = 0.111 *P* = 0.622	rho = 0.020 *P* = 0.930	rho = -0.711 *P* = 0.003*	rho = -0.349 *P* = 0.203
Adjusted energy expenditure	rho = -0.001 *P* = 0.998	rho = -0.025 *P* = 0.913	rho = -0.775 *P* = 0.001*	rho = -0.624 *P* = 0.013*
Time physically active	rho = -0.016 *P* = 0.942	rho = -0.273 *P* = 0.218	rho = -0.711 *P* = 0.003*	rho = -0.697 *P* = 0.004*

CRP, C-reactive protein; IL-6, Interleukin-6; STS 60, Sit-to-stand 60.
* *P* < 0.05.

Neutrophil degranulation

Circulating concentrations of elastase were not statistically different between the groups under normal conditions (P = 0.328; Figure 3.4a). Elastase released after incubation with a bacterial stimulant was not dissimilar between groups (HD patients: 1357 ± 627 vs. healthy participants: 1315 ± 715 $\mu g \cdot L^{-1}$, P = 0.870) and this correlated strongly in both groups with the neutrophil count (HD patients: r = 0.903, P < 0.001; controls: r = 0.824, P < 0.001). After adjusting for the number of neutrophils, a blunted response per cell was observed in the HD patients (ES = 0.93; Figure 3.4b).

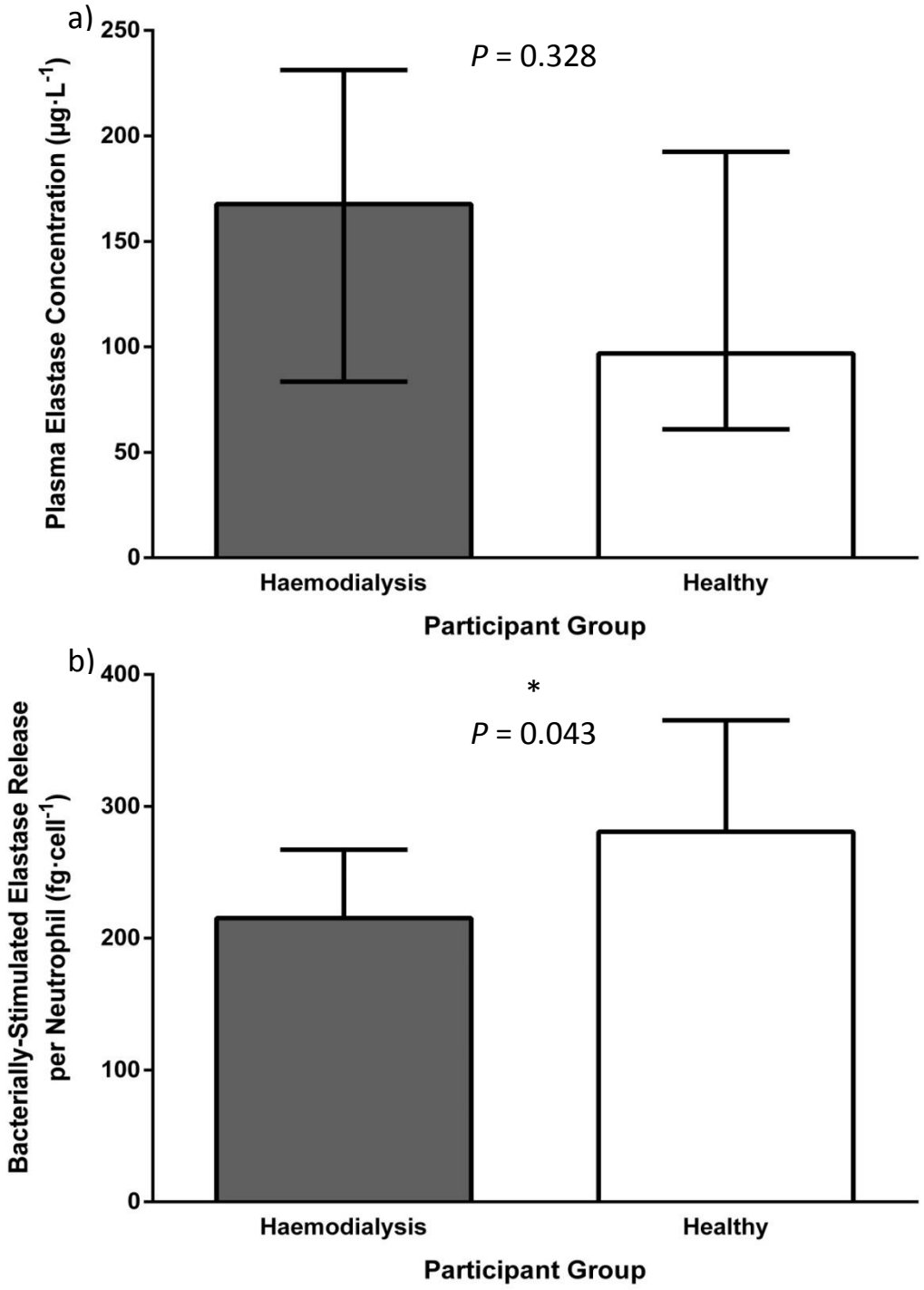

Figure 3.4 - Plasma elastase concentrations in haemodialysis patients and healthy participants **a)** under normal conditions, **b)** after stimulation with bacteria and adjusted per neutrophil count. Data is presented as a) median and interquartile range, b) mean and standard deviation.
* denotes a significant difference between groups.
Haemodialysis patients n = 20, healthy controls n = 10.

Quality of life parameters

HD patients scored higher on the depression aspect of the HADS questionnaire (4 (2-7) vs. 1 (1-2), $P = 0.003$, ES = 1.07); however, no significant difference in anxiety score arose (3 (1-6) vs. 4 (3-7), $P = 0.101$). HD patients scored lower in the DASI questionnaire (29.1 (21.1-35.5) vs. 58.2 (44.2-58.2), $P < 0.001$, ES = 2.46), suggesting a lower perceived ability to complete physical tasks.

HD patients reported a greater number of symptoms on the LUSS (7 ± 3 vs. 4 ± 3, $P = 0.002$, ES = 1.02) and the total symptom score was higher (37 (25-47) vs. 11 (7-25), $P < 0.001$, ES = 1.65). A graphical representation of the symptom burden of the HD patients and healthy cohort is shown in Figure 3.5.

In the healthy group a loss of muscle strength or power was associated with age (rho = 0.641, $P = 0.007$); conversely, no symptoms correlated with age in the HD patients. In both groups BMI positively correlated with total LUSS score (HD: rho = 0.468, $P = 0.009$; healthy: rho = 0.591, $P = 0.016$).

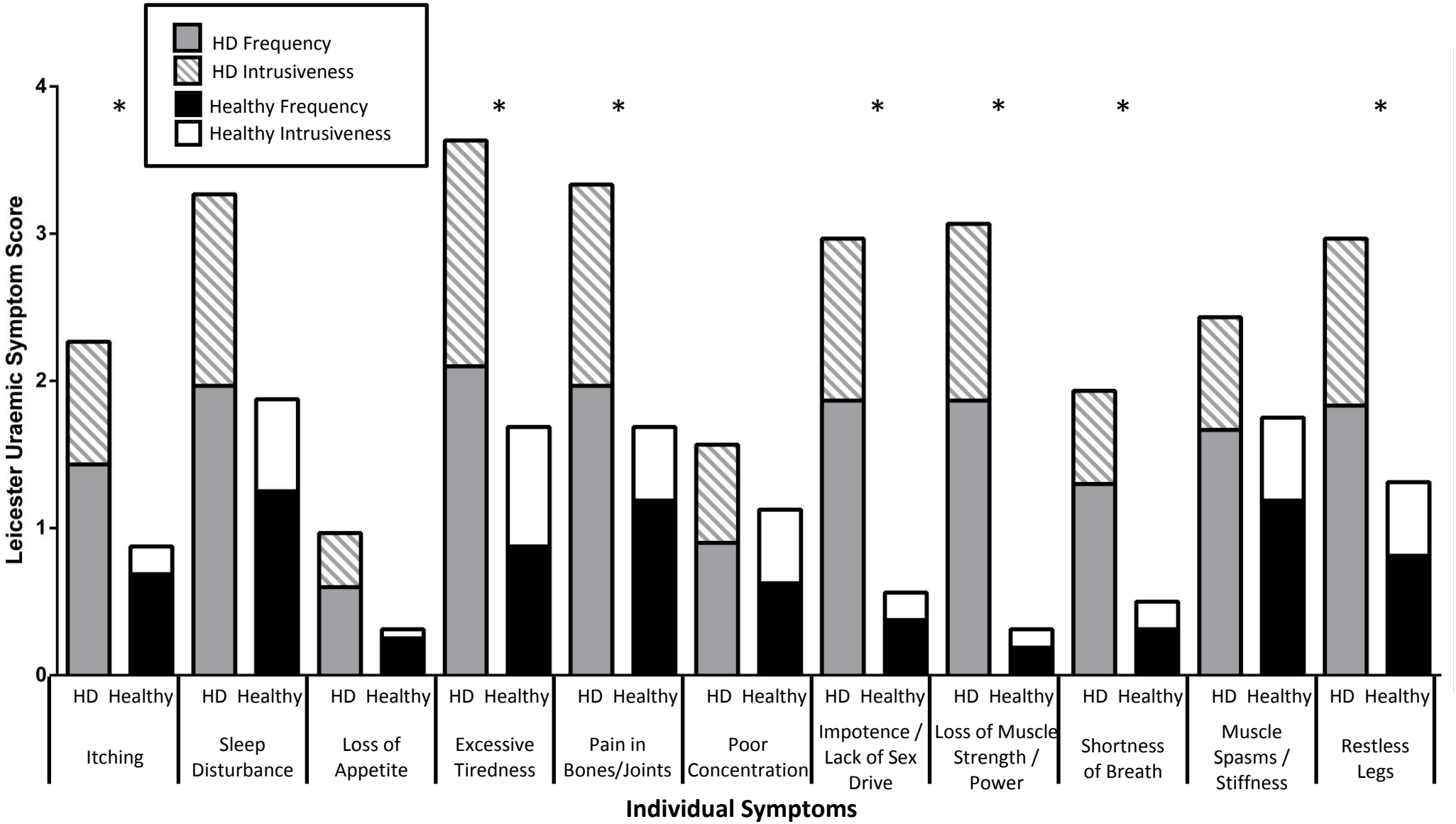

Figure 3.5 - Total symptom score for each individual symptom for the healthy and haemodialysis population. Total scores are a composite of frequency (black / grey bars) and intrusiveness (white / striped bars) of the symptom with each bar representing the mean total symptom score for that population for each individual symptom. Haemodialysis patients n = 30, healthy controls n = 16.
*denotes significant differences between groups ($P < 0.05$).

3.5 Discussion

This study aimed to characterise the HD population in terms of physical activity, markers of inflammation and quality of life in order to improve the effectiveness of future intervention studies. The HD patients displayed significant differences from the age-matched healthy cohort in almost all of the characteristics measured. Patients were less active, demonstrated a greater degree of systemic inflammation, immune dysfunction, and were afflicted with depression and an increased symptom burden. Relationships between physical function and markers of inflammation were observed in both groups.

Physical inactivity

Despite the healthy cohort reporting modest levels of activity, 10 of which were eligible for a brief intervention in physical activity (Department of Health, 2009), the difference between the healthy population and the HD patients was still marked. It is not surprising that HD patients are inactive (physical inactivity is a risk factor for development of CKD and progression to ESRD [Hallan *et al*., 2006; Robinson-Cohen *et al*., 2014; Stengel *et al*., 2003]); however, the extent of their inactivity is particularly concerning; 80% of this cohort achieved less than 5,000 steps per day. Current guidance, of which specific advice for HD patients is scarce, suggests CKD patients should aim to complete the same amount of physical activity as healthy individuals (30 min of moderate activity repeated 5 times a week; ACSM, 2009).

These findings support others that report activity levels of HD patients lower than sedentary healthy controls (Baria *et al*., 2010; Johansen *et al*., 2000). In 134 HD patients wearing similar accelerometers to the present study a higher daily activity level was reported (median 5,660 steps·day^{-1}); this may be a result of a difference in age (54.9 ± 15.9 y; Avesani *et al*., 2012). HD patients experience an accelerated age-related decline in activity levels seen in healthy people (Johansen *et al*., 2000); consequently larger differences in activity are likely to be observed in older populations.

Low physical activity levels are clinically significant as they are associated with poor outcome. This is demonstrated in two papers taken from subsets of the Dialysis

Morbidity and Mortality Wave 2 study. ESRD patients who self-reported sedentary behaviour had a 1.62 fold increased risk of mortality in the following year (O'Hare *et al*., 2003). Moreover, moderately active ESRD patients who recounted exercising 2-3 or 4-5 times per week (18% and 6% of the 2,507 patients followed) had a reduced relative risk of mortality (hazard ratio 0.74 and 0.70 respectively; Stack *et al*., 2005). Elsewhere, regular activity pre-transplantation is associated with a reduction in post-transplant mortality risk (hazard ratio 0.52); emphasising the importance of improving physical activity in ESRD (Rosas *et al*., 2012). These results all suggest a shift in sedentary behaviour to a more active lifestyle would have survival benefits in individuals with ESRD.

The cause of the ultra-sedentary behaviour found is unclear but likely to be multifaceted. Physiologically, anaemia and muscle wasting are conceivable causes of inactivity. EPO therapy may improve exercise capacity (Lundin *et al*., 1991); however, not as much as would be expected in ESRD (Akiba *et al*., 1995; Robertson *et al*., 1990) and HD patients cease exercise before oxygen transport limitations are met suggesting a different source of fatigue (Moore *et al*., 1993b).

Uraemia is associated with muscle wasting due to heightened metabolic acidosis, inflammation, malnutrition and hormone resistance (Chen *et al*., 2013). Progressive resistance exercise studies have demonstrated muscular hypertrophy in ESRD (Kirkman *et al.,* 2014); thus, the catabolic environment can be overcome by exercise. Further, in the present study physical function (STS 60) did not correlate with physical activity levels in HD patients (with steps: rho = 0.050, P = 0.835) unlike within the healthy group suggesting that physical function may not have been the limiting factor for physical activity. Conversely, the DASI correlated with time physically active (rho = 0.440, P = 0.031) and energy expenditure (rho = 0.673, $P < 0.001$) implying that patients perception of what they can do may be a more important factor in determining activity.

Beyond the physiological reasons for inactivity, patients are confronted by a number of psychosocial barriers. Despite substantial interest in exercise, patients report a number of barriers including 'fatigue', 'shortness of breath', 'lack of time', 'pain', 'too many medical problems' and 'fear of getting hurt' (Delgado and Johansen, 2012; Young *et al*., 2012). It is perceptible that a number of these barriers may be overcome or improved by exercise

itself; exercise counselling by general practitioners, nephrologists and physiotherapists may help enhance physical activity.

One commonly cited barrier to exercise in HD patients is the reduction in free time due to the time commitments of thrice-weekly HD treatment. Like others, the present study found habitual physical activity levels were lower on days when patients have HD treatment (Avesani *et al*., 2012; Baria *et al*., 2010; Majchrzak *et al* 2005); this may be due to the 4 h of enforced sedentary behaviour during treatment or post-dialysis fatigue syndrome (Sklar *et al*., 1996). However, despite the adverse impact HD has upon physical activity, it cannot be solely responsible for sedentary behaviour because activity levels on non-HD days are still substantially lower than the healthy group (Figure 3.1). Exercise during HD should help correct the imbalance in activity levels between treatment and non-treatment days. Although in order to fully redress inactivity it is clear physical activity would need to be increased in all aspects of the patients' lifestyles. Because of the variety of potential factors that determine sedentary behaviour, applying the Biopsychosocial model to consider psychological and social barriers in addition to biomedical factors for each patient may be beneficial (Engel, 1981).

Markers of inflammation

HD patients displayed an increased inflammatory burden in almost every aspect of the circulating markers and mechanisms measured.

Cytokines and C-reactive protein

Large elevations in the concentration of the pro-inflammatory cytokines IL-6 and TNF- α and the acute-phase protein CRP were demonstrated in the HD patients. It is well established that circulating cytokines are persistently elevated in HD patients (Jacobs *et al*., 2004; Kimmel *et al*., 1998). Cytokines are elevated in circulation partly due to a reduction in renal clearance demonstrated by the extended plasma half-life of cytokines in animal models of CKD (Poole *et al*., 1990). Moreover, increased secretion of cytokines

results from the activation of leucocytes in response to the uraemic milieu (see Table 1.5). For example, IL-6 mRNA in peripheral blood mononuclear cells (PBMCs) is reported to be higher in HD patients than PD patients or healthy controls suggesting a greater secretion in this population due to chronic activation (Yamaguchi *et al*., 1996).The amplified pro-inflammatory cytokine concentrations illustrate that this HD cohort endures chronic systemic inflammation; a condition strongly associated with atherosclerosis, cachexia and mortality (Kalantar-Zadeh *et al.*, 2003; Stenvinkel, 2001). CRP and particularly IL-6 are powerful prognostic markers of cardiovascular mortality in ESRD (Stenvinkel 2001; Stenvinkel *et al*., 2002). IL-6 and TNF-α appear directly involved in the pathogenesis of atherosclerosis and accelerated protein catabolism (Table 1.6). CRP may also have pro-atherogenic properties itself.

Of the measured cytokines, only the anti-inflammatory IL-10 was not significantly different between groups. Despite the substantial pro-inflammatory environment shown in the HD patients IL-10 was not elevated, perhaps indicating an impairment of the anti-inflammatory response. Elsewhere, low amounts of IL-10 in HD have been associated with immune impairment and CVD (Seyrek *et al*., 2005) and patients with the high IL-10 producing genotype are protected from immune dysfunction and CVD (Girndt *et al*., 2002). It should be noted that these low IL-10 concentrations are in contrast to others who report raised circulating IL-10 in HD patients (Mortia *et al*., 1997; Yilmaz *et al*., 2014). Further, the majority of samples were recorded below the low standard of the assay despite using a high-sensitivity kit; these results should be interpreted with caution.

In large cohorts of healthy populations higher physical activity levels are associated with lower CRP and IL-6 (Colbert *et al*., 2004; Ford, 2002). Our findings in this small control cohort (not adjusted for age or BMI) support this. Regular activity over a prolonged period has been shown to maintain low markers of inflammation; over a 10-y follow-up period the most active individuals preserved lower levels of inflammatory cytokines and those individuals that increased activity levels saw a decrease in IL-6 and CRP (Hamer *et al*., 2012). Whether this relationship can be extended to HD patients is unclear.

Intriguingly, the strongest correlate of IL-6 and CRP in the HD patients was the STS 60 score suggesting that physical function and inflammation may be interrelated. High circulating IL-6 may lead to muscle wasting and lower physical function; on the other

hand, maintaining physical function may be a promising means of restricting inflammation. This finding lends support to Mafra and colleagues who found that HD patients with elevated CRP (> 5 $mg \cdot L^{-1}$) had lower total energy expenditure and physical activity than those with lower CRP and healthy controls (Mafra *et al.,* 2011). Self-reported activity levels in patients starting HD also inversely correlated with CRP (Anand *et al.,* 2011). On the other hand, no clear relationships were found between markers of systemic inflammation (although not IL-6) and activity or fitness levels in two other studies (Hung *et al.,* 2003; Zamojska *et al.,* 2006); the authors cited high inter-individual variability in cytokine levels, small sample size or a true lack of association as possible explanations. The present study found no associations between activity levels and cytokines in HD patients.

The independence of physical function and physical activity levels is perhaps surprising. It is conceivable that physical function is a better marker of long term fitness and the high level of variance in physical activity explain the contradictory results and why these markers do not correlate in this cohort of HD patients. There are also a multitude of confounding factors associated with ESRD, co-morbidities and medications that may overshadow any benefit of greater daily physical activity.

In the healthy controls BMI and age were strongly associated with IL-6 and CRP, but no such relationship was observed in HD patients. These traditional risk factors for CVD have less importance in the ESRD setting that is defined by premature ageing and muscle wasting. Chronological and biological ages appear disparate this may explain why, in this small cohort, age is not related to a number of factors in HD patients but is in the healthy group.

Monocytes

Chronic exposure to a uraemic environment appears to cause adaptations in the distribution and characteristics of circulating leucocytes in the HD population. This patient group have an increased proportion and number of intermediate and non-classical monocytes compared with the healthy group. This finding has been previously illustrated (Heine *et al*., 2008; Merino *et al*., 2011; Nockher and Scherberich, 1998), with

similar differences reported in HD and healthy groups: classical 439 ± 219 vs. 504 ± 184, intermediate 53 ± 33 vs. 29 ± 13, and non-classical 130 ± 76 vs. 70 ± 33 cells·μL^{-1} (Heine *et al*., 2008).

It is uncertain as to why a shift in monocyte subsets occurs. Current opinion is that monocyte subsets derive from the same precursor and differentiate from classical to intermediate to non-classical monocytes (Heine *et al*., 2012). It is plausible that in ESRD the uraemic environment and chronic activation of monocytes causes an acceleration of this differentiation leading to the greater proportion of intermediate and non-classical monocytes. Moreover, non-classical monocytes show molecular and phenotypic evidence of senescence (Merino *et al*., 2011), that has been shown to be exacerbated on this phenotype in HD patients (Ramirez *et al*., 2005).

The association between intermediate monocytes and TNF-α in both groups is in agreement with others that suggest intermediate monocytes are responsible for a large proportion of TNF-α, IL-1β and IL-6 secreted in response to LPS stimulation (Cros *et al*., 2010; Rossol *et al*., 2012; Shantsila *et al*., 2011), i.e. that intermediate monocytes are potent pro-inflammatory cells. This is further supported by earlier work that found CD14^{+}CD16^{+}HLA-DR^{++} monocytes were major producers of TNF-α (Belge *et al.,* 2002) combined with recent findings that intermediate monocytes have the greatest expression of HLA-DR (Wong *et al.,* 2011). Furthermore, intermediate monocytes exhibit spontaneous ROS production, elevated adhesion molecule expression (CCR5 and CX_3CR1) and aid T cell proliferation and activation. Thus, the increase in intermediate monocytes seen here provides cellular evidence of chronic inflammation and immune dysfunction.

Non-classical monocytes have also been suggested as a major source of TNF-α and IL-6 (Wong *et al*., 2011); however, Cros and colleagues demonstrate these cells primarily produce pro-inflammatory cytokines in response to virus via TLR7-TLR8-MyD88-MEK pathway and may be a major source of IL-1ra (Cros *et al*., 2010; Rossol *et al.,* 2012). There is agreement that non-classical monocytes exhibit patrolling behaviour, are prone to extravasation and aid in T cell proliferation and activation, signifying a role in plaque development and atherosclerosis.

Classical monocytes are purported to be the greatest producers of IL-10 and are strong phagocytes (Cros *et al*., 2010; Wong *et al*., 2011). The proportion of classical monocytes

was reduced in the HD patients, but the number of these cells was similar in both groups. However, it has long been established that the function of monocytes is inhibited by the uraemic environment as shown by reduced antigen-presenting capacity, B7-CD28 interaction and non-responsiveness to vaccination (Girndt *et al*., 1993; Girndt *et al*., 1995; Meuer *et al*., 1987).

The distribution of monocyte subpopulations had no association with activity levels or physical function in either group. Elsewhere, $CD16^+$ monocytes were more prevalent in an inactive elderly healthy cohort than active healthy age-matched individuals (Timmerman *et al*., 2008). However, a significant difference in BMI may have confounded these findings as higher BMI is associated with a greater number of $CD16^+$ monocytes (Rogacev *et al*., 2010). Interestingly, 12 weeks of exercise training corrected monocyte subsets in the inactive group to the levels seen in the active group and this is suggested as one mechanism through which regular exercise may exert an anti-inflammatory effect (Timmerman *et al.,* 2008). Consequently, the low physical activity levels and high concentrations of intermediate and non-classical monocytes in HD patients would suggest great potential for improvement.

Regulatory T cells

There was a lower proportion of Tregs in the HD population, as previously reported (Hendrikx *et al*., 2009); however, the absolute counts of Tregs were not dissimilar between groups due to higher lymphocyte counts in the HD population. This is in contrast to other studies that have reported a reduction in the absolute number of $CD4^+CD25^+FoxP3^+$ and $CD4^+CD25^+$ in ESRD patients (Hendrikx *et al*., 2009; Topal *et al*., 2013). Whether HD patients have a reduced Treg population is contentious, as others have observed a higher proportion of $CD4^+CD25^+$ cells (Lisowska *et al*., 2014) and $FoxP3^+$ cells (Libetta *et al*., 2010). A potential reason for these divergent findings is the variability in nomenclature used to define Tregs. Those simply measuring $CD4^+CD25^{bright}$ may find elevated numbers because CD25 (IL-2 receptor) is present on activated T cells, the proportion of which are higher in ESRD patients (Meier *et al*., 2005; Stachowski *et al*., 1991). It is therefore of particular importance to use FoxP3 or CD127 in accurately

defining Tregs. There are large gaps in the literature about the effect of HD membranes, co-morbidities, medications and other clinical factors (e.g. age and ethnicity) on the Treg population; this may account for some of the variability in the current literature.

The lower proportion of Tregs observed in this study may be due to an increased susceptibility for T cell apoptosis as a consequence of repeated activation by uraemic toxins and HD (Meier *et al*., 2002). Further, the suppressive capacity of Tregs is inhibited in ESRD patients (Hendrikx *et al*., 2009). The important role of Tregs in regulating the immune response of T lymphocytes and secretion of anti-inflammatory IL-10 means that a reduction in proportion and suppressive function of Tregs in HD patients have an important role in chronic inflammation (Shalev *et al*., 2011).

The proportion of Tregs within the $CD4^+$ lymphocyte population is lower in healthy sedentary students compared with recreationally active or trained individuals suggesting that regular exercise may help maintain or enhance the Treg population (Handzlik *et al*., 2013). In the HD patients a trend for an association between Tregs and physical activity levels was seen, a bigger cohort is required to fully clarify this association. Whether increasing activity levels can rectify Treg proportions in HD patients is yet to be seen, but has been observed in a non-CKD animal model (Wang *et al*., 2012).

Growth hormone secretagogue receptor expression

There were no significant differences in the number of T cells but an increase was seen in the number of monocytes expressing GHS-R1a. GHS-R1a expression is up-regulated upon activated leucocytes (Dixit *et al*., 2004) therefore an increase in GHS-R1a expression could be expected to be seen in HD patients. Expression of GHS-R1a on leucocytes has not previously been reported in CKD patients, large inter-individual variability was found and this may explain the lack of significant differences in GHS-R1a expression on T cells in these small groups. The geometric mean fluorescence intensity and number of $GHS\text{-}R1a^+$ monocytes was higher in HD patients than the healthy group. This may be a genuine up-regulation of GHS-R1a on monocytes or due to the altered monocyte phenotype populations described earlier. The $CD14^+$ category used included both classical and intermediate monocytes that likely express different amounts of GHS-R1a due to their

differing functional roles (Cros *et al*., 2010; Wong *et al*., 2011); although this has yet to be formally defined.

Little is currently known about the effect of lifestyle or chronic disease on GHS-R1a. Recent data suggests sedentary behaviour is associated with a lower expression of GHS-R1a on lymphocytes and an acute bout of exercise is associated with a significant increase in the number of both T cells and monocytes expressing GHS-R1a; this may be another means by which exercise has an anti-inflammatory effect (Bishop *et al*., 2013). An increase in GHS-R1a expression and inhibition of pro-inflammatory secretion would appear beneficial in reducing chronic immune over-activation in HD patients. Whether exercise can exert this effect in HD patients merits future research.

Neutrophil degranulation

A specific blunting of elastase release per neutrophil in response to a bacterial challenge was observed in the HD patients. Spontaneous secretion of elastase into plasma at rest and during HD treatment has previously been reported in this population (Costa *et al*., 2008b; Polanska *et al*., 2010; Yoon, Pahl and Vaziri, 2007). Neutrophils are the primary defence against invading bacteria and elastase secretion is a potent bactericidal activity. A reduction in neutrophil killing ability is likely to contribute to the increased susceptibility to infections in these patients (Dalrymple *et al*., 2010).

Neutrophils in uraemia are chronically activated and exist in a 'primed' state leading to an elevated basal release of ROS and granules (Costa *et al*., 2008b; Sela *et al*., 2005). Primed neutrophils in ESRD are associated with reduced migration (Pindjakova and Griffin, 2011), phagocytosis (Mahajan *et al*., 2005) and bactericidal activity (Anding *et al*., 2003), abnormal production of ROS (Yoon, Pahl and Vaziri, 2007) and a greater propensity for apoptosis (Majewska *et al*., 2003). This blunted neutrophil response may, in part, be compensated for by greater neutrophil counts as observed in this study. However, this may lead to a greater degree of spontaneous ROS secretion into circulation. Consequently, neutrophils in HD patients may contribute to systemic inflammation and, apparent here, be less responsive to bacteria.

Quality of life parameters

The complex condition of ESRD and co-morbidities leads to a wide variety of symptoms. HD patients reported a greater symptom frequency and intrusiveness than the healthy individuals. Tiredness and trouble falling and staying asleep are commonly reported symptoms, along with pain in joints and loss of muscle strength (Abdel-Kader *et al*., 2009; Kimmel *et al*., 2003). This greater symptom burden implies a reduced quality of life. A systematic review found that HD patients report multiple symptoms with fatigue, pruritus, constipation, anorexia, pain and sleep disturbance the most frequently reported, although the intrusiveness of each symptom was not accounted for (Murtagh, Addington-Hall and Higginson, 2007).

HD patients scored higher on the depression element of the HADS questionnaire suggesting a poorer mental wellbeing. Depression in ESRD is commonly reported, although prevalence varies hugely depending on the patient demographics and the screening method (Kimmel, 2002); a recent estimate of 20-30% prevalence in CKD suggests a greater frequency than in other chronic diseases (Hedayati and Finkelstein, 2009).

In this small cohort no clear associations were found between activity levels and symptom burden. However, in a large cohort of ESRD patients (n = 1678), those patients who reported the lowest physical activity levels were more likely to report insomnia, depression and restless legs (Anand *et al*., 2013). Furthermore, recent evidence from the DOPPS (Dialysis Outcomes and Practice Patterns Study) show aerobic activity is associated with greater quality of life, reduced depression symptoms and mortality (Lopes *et al.,* 2014). An increase in physical activity could feasibly improve the symptom burden that afflicts HD patients.

Limitations

This study is purely observational and descriptive in nature; consequently, causation cannot be determined. However, this study does provide useful information in defining the HD patients and observing differences with the general population. This allows the generation of hypotheses that can be tested in future intervention studies.

One limitation to this study is that all recruited HD patients were eligible to exercise; therefore patients with worse disease condition were excluded. It is possible that the extent of inactivity and inflammation is underestimated in the current cohort compared with the overall HD population. Further, the cohort is too small to be able to declare this group as representative of all HD patients. However, the cohort was large enough to observe the substantial differences in a physical activity, quality of life and markers of systemic inflammation and immune function between HD patients and healthy controls.

Implications

Overall, this study has demonstrated that HD patients have an immune system that is both pro-inflammatory and dysfunctional. These findings suggest that HD patients are a highly vulnerable population. The extremely high mortality rates due to CVD are unacceptable (Schiffrin, Lipman and Mann, 2007) and interventions to improve aspects of their immune system and cardiovascular health are required.

The low levels of activity and physical function in HD patients indicates a great potential for improvement. The broad capacity of exercise to enhance physical function, overall quality of life as well as aspects of cardiovascular and immune health has attracted attention to a number of chronic diseases (Pedersen and Saltin, 2006). From the observational data presented here a link between physical function and circulating markers of inflammation was found; this warrants further investigation to understand whether changes in physical function are associated with improvements in inflammation in HD patients. On the other hand, due to the immune vulnerability in these patients it is also important to verify whether any interventions are safe with regard to any possible adverse effects on the already high markers of systemic inflammation and immune dysfunction.

3.6 Conclusions

HD patients are substantially less active and have reduced physical function than age-matched healthy controls. Inactivity is exacerbated on days when patients have HD treatment but remains low on non-treatment days. The patients exhibit a greater number of symptoms than the control subjects and their overall symptom burden is considerably higher.

In the HD patients pro-inflammatory cytokines were elevated in circulation, monocytes were shifted in distribution toward intermediate and non-classical subsets associated with pro-inflammatory and pro-atherogenic properties, and the proportion of Tregs was reduced demonstrating an alteration in the immune system toward a chronically activated pro-inflammatory environment. Additionally, neutrophil degranulation to bacterial challenge was suppressed suggesting immune dysfunction. The findings reported in this study likely underlie the increased risk of CVD and infection experienced by HD patients as a consequence of their immune over-activation and dysfunction.

Physical function was the strongest predictor of IL-6 and CRP in the HD group. There appears a great potential to improve physical activity in HD patients. The impact of increasing physical activity in HD patients warrants further investigation, particularly as exercise may have the capacity to address a wide range of symptoms associated with ESRD.

Chapter 4

The Acute Effects of a Bout of Intradialytic Exercise on Circulating Markers of Inflammation and Immune Function

4.1 Abstract

Regular exercise is reported to have numerous benefits for ESRD patients. Exercise during HD is feasible and compliance and drop-out rates are improved compared to at other times, but little is known about the acute impact of exercise during HD. HD patients are very vulnerable and exercise has the potential to have profound transient effects on leucocytes, markers of systemic inflammation, and immune function. The main aims of this study were to analyse the immediate effects of an exercise bout during HD on circulating markers of inflammation and aspects of immune function compared with a routine care HD session; primarily, to assess the safety of intradialytic exercise.

Fifteen HD patients (57.9 ± 10.5 y) volunteered to take part in two trials separated by a week and organised in a randomised crossover design. On the control trial the patients rested throughout HD. On the exercise trial patients completed 30 min of cycling exercise during HD, 1 h into the treatment, at a perceived exertion of "somewhat hard". Blood samples were taken pre-exercise (60 min), post-exercise (100 min), 1 h post-exercise (160 min) and at the end of HD (240 min). Circulating concentrations of IL-6, TNF-α and IL-1ra and neutrophil degranulation response to bacterial stimulant were assessed via ELISA. The proportions and numbers of monocyte phenotypes and GHS-R1a expression on PBMCs were assessed via flow cytometry.

Exercise had no significant effect on plasma IL-6, IL-1ra or TNF-α, although the degree of TNF-α decline during the exercise trial correlated with the power output achieved during exercise (rho = -0.674, P = 0.023). Trends were observed in the distribution of monocytes after exercise, non-classical monocytes appeared to increase immediately after exercise and decrease by the end of HD compared with the control trial (interaction: P = 0.080), which is a normal response to exercise. Intermediate monocytes decreased during both trials, but a trend for a greater decrease was seen on the exercise trial (interaction: P = 0.085). Neutrophil degranulation in response to bacterial stimulation appeared suppressed immediately after exercise compared with the control trial (interaction: P = 0.027); this effect was transient and post-exercise responsiveness was not significantly lower than pre-exercise responsiveness (60 min: 305 ± 76 vs. 100 min: 288 ± 77 $fg \cdot cell^{-1}$, P = 0.332, ES = 0.22).

Exercise at an intensity that can be routinely carried out on HD appears to have relatively little impact upon markers of systemic inflammation or immune function. Intradialytic exercise did not exacerbate the pro-inflammatory environment associated with HD nor induce abnormal

alterations to leucocytes. From an immunological perspective, moderate-intensity cycling exercise during HD is well tolerated and appears safe.

4.2 Background

As seen in the previous chapter, HD patients present with many aspects of chronic inflammation and immune dysfunction. A number of factors influence HD patients' immune system including the disease itself and the environment that is associated with uraemia (Betjes, 2013; Vaziri *et al.*, 2012).

HD treatment involves the circulation of blood *ex vivo* via a needle or catheter access and the diffusion of solutes into highly purified water across a membrane in a dialyser. This provides many increased opportunities for endotoxin influx, recurring infections and immune activation (Table 1.5). Regular dialysis, required for long-term survival in ESRD patients without transplantation, is associated with a chronic over-activation of leucocytes leading to the changes in leucocyte populations, circulating inflammatory markers and anergy to bacterial stimulation described in the previous chapter.

The patients observed in the previous study were also highly inactive and this was exacerbated on days when they had HD treatment. As with most populations, healthy or diseased, regular exercise is reported to have numerous benefits for ESRD patients. These benefits include improved exercise capacity, muscle function, quality of life and various other cardiovascular health benefits (Heiwe and Jacobson, 2014; Smart and Steele, 2011; Smith and Burton, 2012).

Exercise 'rehabilitation' in this population is challenging due to low physical capacity and function and numerous complications associated with the disease and treatment. Structured exercise programmes held on non-dialysis days or based at home give functional benefits to patients (Bulckaen *et al*., 2011; Carney *et al*., 1987; Deligiannis *et al*., 1999). However, compliance is a persistent problem especially for outpatient exercise programmes. Exercise during HD is feasible and compliance and drop-out rates are significantly improved compared with outpatient programmes (Kouidi *et al.*, 2004; Nonoyama *et al.*, 2010). Intradialytic exercise can therefore be suggested as an effective means to change exercise behaviour in the maximum number of patients and at a time when they are usually sedentary.

In the healthy population exercise is reported to induce an anti-inflammatory environment through the secretion of IL-6 from contracting muscles into circulation and the subsequent anti-inflammatory cytokine cascade (Pedersen and Febbraio, 2008). Exercise also causes a number of other transient anti-inflammatory alterations including the distribution of monocyte phenotypes and decreased toll-like receptor expression on monocytes, as well as in adipose, hepatic and muscle tissue (Gleeson *et al.*, 2011; Oliveira *et al.*, 2011).

Little is known about the acute impact of exercise during HD. However, HD patients are very vulnerable and exercise has the potential to have profound transient effects on leucocytes, circulating markers of inflammation, and immune function that the dysfunctional immune system in HD patients may not respond to adequately. It is important to investigate the immediate impact of exercising during HD, a time where these patients may be at an even greater susceptibility to infection and immune activation.

The main aims of this study were to analyse the immediate effects of an exercise bout during HD on circulating markers of inflammation and aspects of immune function compared with a routine care HD session. Primarily this study sought to check the safety of intradialytic exercise, and secondly to assess any potential improvements exercise may have.

4.3 Methods

Ethics statement

All aspects of the following protocol and study documentation were approved by NHS trust R&D ethical committee (ref. UHL 10903) and NHS Research Ethical Committee (ref. 10/H0406/36).

Recruitment

Patients were deemed fit to exercise by a Consultant Nephrologist prior to giving written informed consent to participate in the study. Patients were approached if they took part in the exercise rehabilitation programme offered to all eligible patients at the HD unit and satisfied the study exclusion criteria (Table 2.1). Two patients were reluctant to donate the amount of blood asked for; the numbers for each outcome measure therefore vary (Figure 4.1).

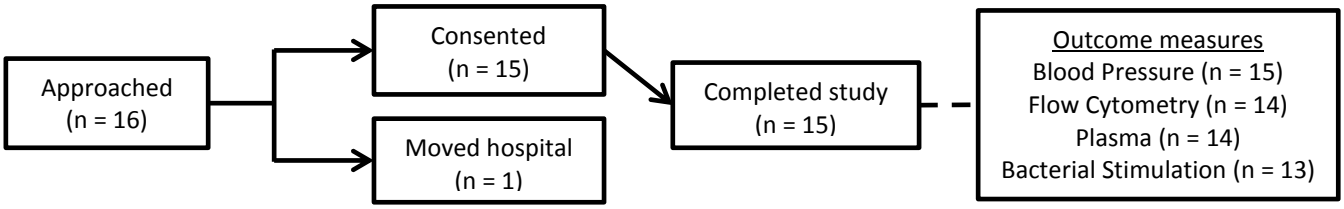

Figure 4.1 – Recruitment flow diagram showing the number of patients that were approached, recruited and completed the study and the number of each type of outcome measure collected.

Protocol

Patients participated in two trials during HD treatment separated by a week and carried out on the same day of the week. Trials were organised in a counterbalanced randomised-crossover design with the order determined using a website designed specifically for research randomisation (Urbaniak and Plous, 2012). On the exercise trial patients performed a 30-min bout of intradialytic exercise 60 min into their HD session similar to normally prescribed intradialytic exercise. On the control trial the patient remained rested throughout HD. Blood pressure was measured at the beginning of HD and then with blood samples prior to and immediately after exercise, 1 h post-exercise

and at the end of HD and at the equivalent times during the resting control trial (Figure 4.2). Due to the warm-up and time taken to set up the exercise the immediate post-exercise sample occurred at approximately 100 min.

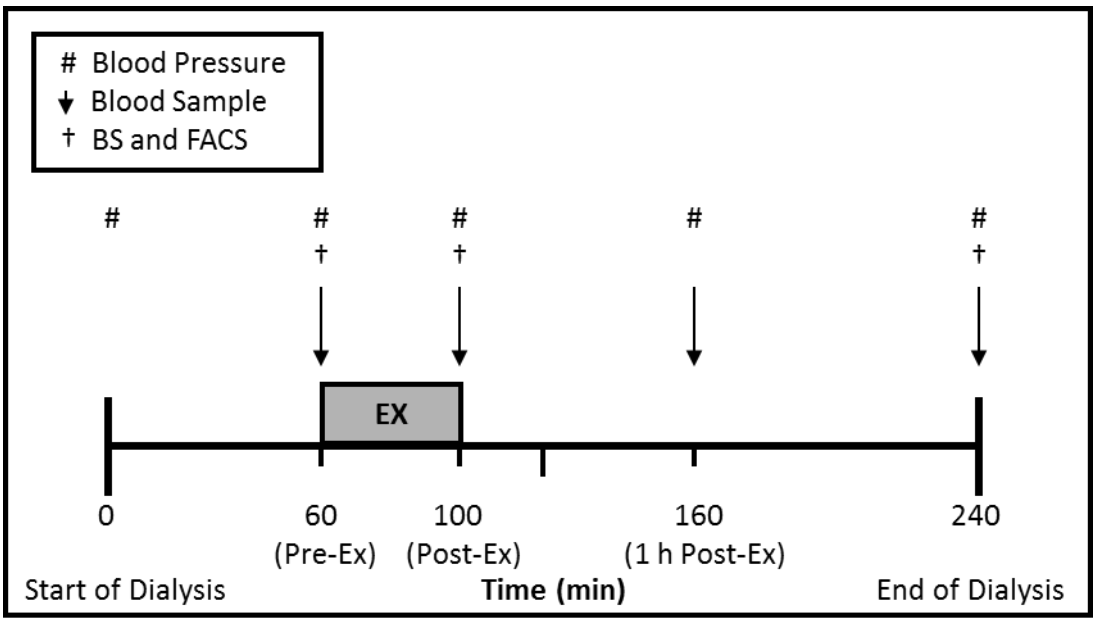

Figure 4.2 - Trial schematic. The arrows represent times when blood samples were taken; daggers represent times when blood was taken for flow cytometry analysis and bacterial stimulation; the hashes represent blood pressure readings.
During the exercise trial, exercise was completed at the time shown by the grey box, during the control trial the patient rested throughout.
BS, Bacterial stimulation; FACS, Flow cytometry.

Exercise

Exercise was completed on a specially designed cycle ergometer (Letto series; Motomed, Reck, Germany). Patients completed a 5 min warm-up at a self-selected low gear and speed prior to cycling for 30 min. Patients were asked to cycle at an intensity they perceived to be "somewhat hard" using the RPE scale (Borg, 1973). RPE scores, the gear and speed were recorded every 5 min and the participant was encouraged to adjust the gear if the reported RPE was outside the required range. All participants were familiar with the cycle ergometer and RPE scale from routinely taking part in an exercise programme at the renal unit for a minimum of 3 months. Patients were accustomed to adjusting the gear (resistance) or pedal speed to maintain the desired intensity. Upon completion of the 30 min exercise, after the post-exercise blood sample was collected, a

5 min cool-down was performed at a low gear and speed. Average and peak power outputs were noted along with estimated distance cycled and energy expenditure.

Blood samples

A 20 mL sterile dry syringe was used to draw blood directly from the HD access. Blood samples were then immediately aliquoted into heparin and K_3EDTA treated monovettes.

Outcome measures

Cell counts and plasma samples were collected at all 4 time-points. Bacterial stimulation and flow cytometry analysis was carried out on 3 of the 4 time-points (see Figure 4.2). GHS-R1a expression on T cells and monocytes and the distribution of monocyte phenotypes (classical: $CD14^{++}CD16^{-}$, intermediate: $CD14^{++}CD16^{+}$, non-classical: $CD14^{+}CD16^{++}$) were determined.

Plasma concentrations of cytokines associated with inflammation were examined via ELISA. IL-1β (intra-assay CV: 2.2%), IL-1ra (7.4%), IL-10 (12.2%), IL-6 (9.8%) and TNF-α (8.8%) were analysed using commercially available assays following the manufacturer's instructions. All individual patient samples were run on the same plate.

Pilot work found intradialytic plasma samples often contained a concentration of IL-6 greater than the top standard used in the kit (10 $pg \cdot mL^{-1}$). To ensure samples were within the dynamic range of the assay plasma samples were diluted 1:2 in the calibrator diluent provided (R&D Systems Europe Ltd., Abingdon, UK) and analysis repeated.

For analysis of IL-1ra (R&D Systems Europe Ltd., Abingdon, UK) plasma samples were diluted 1:3 in the calibrator diluent. A single plate for IL-1ra was completed with no discernible pattern, therefore only 10 patients were analysed.

TNF-α, IL-10, and IL-1β (R&D Systems Europe Ltd., Abingdon, UK) were analysed in undiluted plasma following the manufacturer's instructions. Only 1 patient had detectable IL-10 concentrations and no patients had detectable IL-1β, therefore results are not reported.

CRP (CV: 6.1%; IBL International GmbH, Hamburg, Germany) was measured in the 60 min plasma sample of both trials to give a reference measure of inflammation.

Neutrophil elastase degranulation in response to a bacterial stimulation and plasma elastase concentrations were analysed as described earlier (Chapter 2; intra-assay CV: 6.6%).

Omitted samples

One participant was excluded from cytokine data due to a difference of > 5 $mg \cdot L^{-1}$ in CRP between trials. Also, one patient was excluded from cytokine data as IL-6 concentrations were consistently more than two orders of SD above the group mean, an additional outlier was excluded from analysis of TNF-α and IL-1ra. One patient was excluded from analysis of neutrophil degranulation due to a plasma elastase of > 500 $\mu g \cdot L^{-1}$. Finally, one patient was excluded from analysis of monocyte phenotypes due to populations that were too unclear to define accurately.

Participant characteristics

Fifteen HD patients volunteered to take part in the study and completed both trials, the basic participant characteristics are described in Table 4.1. The cohort included a mix of ethnicity, gender, disease aetiology, HD vintage and age (range 38.6 – 75.3 y).

Co-morbidities

All patients had one or more significant non-renal co-morbidity reported in their medical history. Specifically, 2 of the 15 patients had type II diabetes mellitus (13%) and 14 were diagnosed with hypertension (93%); other common co-morbidities included psoriasis and thyroid conditions.

Table 4.1 – Participant characteristics

Age (y)	57.9 ± 10.5
Gender ‡	
Male	9 (60%)
Ethnicity ‡	
White British	7 (47%)
Indian	8 (53%)
Height (m)	1.66 ± 0.09
Post-haemodialysis weight (kg)	76.5 ± 20.0
BMI (kg·m^{-2})	27.4 ± 6.5
Haemodialysis vintage (y) †	3.62 (1.77-3.82)
Access ‡	
AVF	13 (87%)
Catheter	2 (13%)
Aetiology ‡ #	
Glomerulonephritis	5 (33%)
Cystic / Poly	3 (20%)
Pyelonephritis	1 (7%)
Diabetes	1 (7%)
Uncertain	3 (20%)
Other	2 (13%)
Number of co-morbidities	3 ± 2 (range 1 – 7)
Number of medications	10 ± 4 (range 4 - 16)

AVF, Arteriovenous fistula; BMI, Body Mass Index. (n = 15)
Data presented as mean ± SD, † Data presented as median (interquartile range),
‡ Data presented as n (%).
# based on UK Renal Registry classification (UK Renal Registry, 2010).

Medications

Patients were prescribed a large number of different medications. HD requires administration of an anticoagulant; additionally, 10 patients were receiving intravenous erythropoietin treatment for anaemia. 11 patients (73%) were taking a form of anti-hypertensive medication; these include a number of different forms of medication (ACE inhibitors, calcium channel blockers, diuretics). Other frequent medications included

vitamin D supplementation (Alfacalcidol, 93%), phosphate binders (calcium acetate and Sevelamer, 80%), analgesics (Paracetamol and Aspirin, 60%), proton-pump inhibitors (Lansoprazole and Omeprazole, 53%) and statins (Atorvastatin, Rosuvastatin and Simvastatin, 53%). Nutritional supplementation was also common (e.g. vitamin and energy supplements). Prescriptions were not altered between the two trials.

Statistical analysis

Two factor repeated measures ANOVA was used to analyse data: trial (exercise vs. control) x time (60 vs. 100 vs. 160 vs. 240 min; or 60 vs. 100 vs. 240 min where applicable). Where a significant effect was found *post hoc* paired t-tests and repeated contrasts were used.

For comparing treatment conditions between trials paired t-tests were used; non-normally distributed data was compared using Wilcoxon signed-ranks tests.

Correlation analysis was used to assess relationships in changes in inflammatory markers and other parameters. Normally distributed data were correlated using Pearson's correlation coefficient (*r*); Spearman's rho was used when data was non-parametric.

4.4 Results

Haemodialysis treatment

There were no significant differences in the HD treatment between the exercise and control trials (Table 4.2). All patients were prescribed 4 h HD treatment. Target weights and dialysis duration were not changed between trials, pre- and post-HD weight, UF and pump speeds remained similar between trials; there were also no changes to prescribed medications.

Table 4.2 - Haemodialysis treatment parameters on exercise and control trials

	Exercise Trial	**Control Trial**	***P* value**
Pre-HD weight (kg)	77.8 ± 19.9	77.9 ± 20.3	0.714
Post-HD weight (kg)	76.4 ± 19.9	76.6 ± 20.0	0.192
Target weight (kg)	76.0 ± 19.8	76.0 ± 19.8	0.334
UF goal (L)	1.76 ± 0.65	1.73 ± 0.74	0.783
Dialysis fluid removal (L)	1.90 ± 0.71	1.79 ± 0.77	0.463
Pump speed † (mL·min^{-1})	320 [300-360]	325 [300-400]	0.553

HD, haemodialysis; UF, ultrafiltration. (n = 15)
Data is presented as mean ± SD,
† Data is presented as median (interquartile range).

Exercise

All patients successfully completed exactly 30 min of cycling at the prescribed time (60 min into HD). The average pedal speed recorded was 63 ± 10 rpm on an average gear of 14 ± 3. The patients reported the difficulty as “somewhat hard”; specifically, an RPE of 13 ± 1. The ergometer measured the mean power output at 21.5 ± 8.1 W with a range of 10 to 35 W, the average peak power was 33.8 ± 14.8 W. The estimated distance calculated on the ergometer was 9.62 ± 1.63 km with an estimated energy expenditure of 73.4 ± 17.3 kcal.

Plasma volume

As would be expected, ANOVA revealed an effect of time on plasma volume ($F_{(3,39)}$ = 15.749, P < 0.001; Table 4.3). *Post hoc* analysis found that plasma volume on the first sample (60 min) was significantly greater than all subsequent times ($P \leq 0.008$, ES range 0.41–0.85) and the final sample (240 min) was lower than all previous times ($P \leq 0.015$, ES range 0.26–0.85). No effect of trial ($F_{(1,13)}$ = 0.579, P = 0.460) or interactions were found ($F_{(2,23)}$ = 0.647, P = 0.647). All following blood results were adjusted for changes in the plasma volume (Dill and Costill, 1974).

Cell counts

ANOVA revealed an effect of time on total leucocytes ($F_{(2,22)}$ = 8.293, P = 0.003; Table 4.3). *Post hoc* tests revealed cell counts at 100 min (post-exercise) were significantly higher than 160 min (1 h post-exercise: P = 0.015, ES = 0.18) and 240 min (end of dialysis: P = 0.012, ES = 0.37). No effect of trial ($F_{(1,13)}$ = 3.289, P = 0.093) or time*trial interaction ($F_{(3,39)}$ = 1.043, P = 0.385) was found.

This pattern was replicated in the neutrophil counts; an effect of time was observed ($F_{(2,18)}$ = 7.84, P = 0.006), but no significant trial ($F_{(1,12)}$ = 1.703, P = 0.216) or time*trial interaction ($F_{(3,36)}$ = 0.357, P = 0.785). *Post hoc* tests found that more neutrophils were found in circulation at 100 min (post-exercise) than at 160 min (P = 0.045, ES = 0.17) and 240 min (P = 0.024, ES = 0.34).

In lymphocyte counts ANOVA found no main effects of trial ($F_{(1,12)}$ = 0.793, P = 0.391), time ($F_{(2,19)}$ = 1.881, P = 0.185), or time*trial interaction ($F_{(3,36)}$ = 2.121, P = 0.115).

Table 4.3 - Leucocyte subsets during exercise and control trials

	Exercise Trial				Control Trial			
	Pre-Ex	Post-Ex	1 h post	End	Pre-Ex	Post-Ex	1 h post	End
Time (min)	*60*	*100*	*160*	*240*	*60*	*100*	*160*	*240*
Plasma volume (%)	62.2 ± 4.1	59.6 ± 5.3	58.8 ± 7.2	56.9 ± 8.0	62.5 ± 5.0	61.2 ± 4.9	59.48 ± 7.1	57.6 ± 6.9
Leucocytes ($x10^9 \cdot L^{-1}$) [Lg n = 14]	6.16 ± 1.89	6.36 ± 1.90	5.90 ± 1.87	5.49 ± 1.66	5.71 ± 1.74	5.74 ± 1.71	5.55 ± 1.79	5.31 ± 1.77
Neutrophils ($x10^9 \cdot L^{-1}$) [Lg]	4.07 ± 1.83	4.20 ± 1.86	3.83 ± 1.70	3.53 ± 1.50	3.69 ± 1.56	3.70 ± 1.57	3.50 ± 1.49	3.28 ± 1.48
Lymphocytes ($x10^9 \cdot L^{-1}$) [Lg]	1.44 ± 0.51	1.49 ± 0.48	1.43 ± 0.58	1.36 ± 0.57	1.42 ± 0.53	1.38 ± 0.63	1.42 ± 0.79	1.41 ± 0.76
Monocytes ($x10^9 \cdot L^{-1}$) [Lg]	0.47 ± 0.13	0.50 ± 0.19	0.49 ± 0.17	0.46 ± 0.17	0.51 ± 0.17	0.48 ± 0.17	0.48 ± 0.20	0.46 ± 0.24

Data is presented as mean ± SD. (n = 13; except for total leucocytes where n = 14)

[Lg] Statistical analysis performed on log-transformed data.

Circulating markers of inflammation

Interleukin-6

ANOVA found no significant effects of trial ($F_{(1,11)}$ = 0.021, *P* = 0.888), or time*trial interaction ($F_{(3,33)}$ = 0.281, *P* = 0.839; Figure 4.3a). An effect of time was detected ($F_{(1,15)}$ = 5.26, *P* = 0.027). *Post hoc* analysis suggested a difference between concentrations at the end of dialysis and all previous times (60 min: *P* = 0.029, ES = 0.81, 100 min: *P* = 0.028, ES = 0.82, 160 min: *P* = 0.032, ES = 0.45); however, these differences did not remain statistically significant after adjustment for multiple comparisons (*P* ≥ 0.168).

Interleukin-1 receptor antagonist

ANOVA found no effects of trial ($F_{(1,8)}$ = 4.610, *P* = 0.064), time ($F_{(3,24)}$ = 0.335, *P* = 0.800) or interaction effects ($F_{(2,13)}$ = 2.252, *P* = 0.151; Figure 4.3b).

Tumour necrosis factor-alpha

Despite a perceptible difference, TNF-α concentrations at 60 min were not statistically significantly different between trials (*P* = 0.156, ES = 0.59; Figure 4.3c).

ANOVA found an effect of time ($F_{(2,17)}$ = 8.349, *P* = 0.004). *Post hoc* analysis found that TNF-α concentrations at 60 min (pre-exercise) were significantly different from all subsequent samples (100 min: *P* = 0.011, ES = 0.51, 160 min: *P* = 0.011, ES = 0.61, 240 min: *P* = 0.007, ES = 0.68). However, only the difference between end of dialysis and pre-exercise remained statistically significant after adjustment for multiple comparisons (*P* = 0.042).

No main effect of trial ($F_{(1,10)}$ = 1.335, *P* = 0.275), or time*trial interaction ($F_{(2,15)}$ = 2.818, *P* = 0.103) were observed. However, the change in TNF-α from pre-exercise to the end of HD correlated with the mean power output (rho = -0.674, *P* = 0.023), suggesting the greater the power output during exercise the greater the subsequent reduction in TNF-α during HD.

C-reactive protein

CRP was measured at the 60 min time-point as a reference marker of systemic inflammation. Despite average CRP concentrations being similar between trials (exercise: 3.77 (2.56-4.49) vs. control: 3.93 (2.43-4.52) $mg \cdot L^{-1}$, P = 0.695; Figure 4.3d), a degree of variation was detected and the average intra-individual CV between trials was 29.8%.

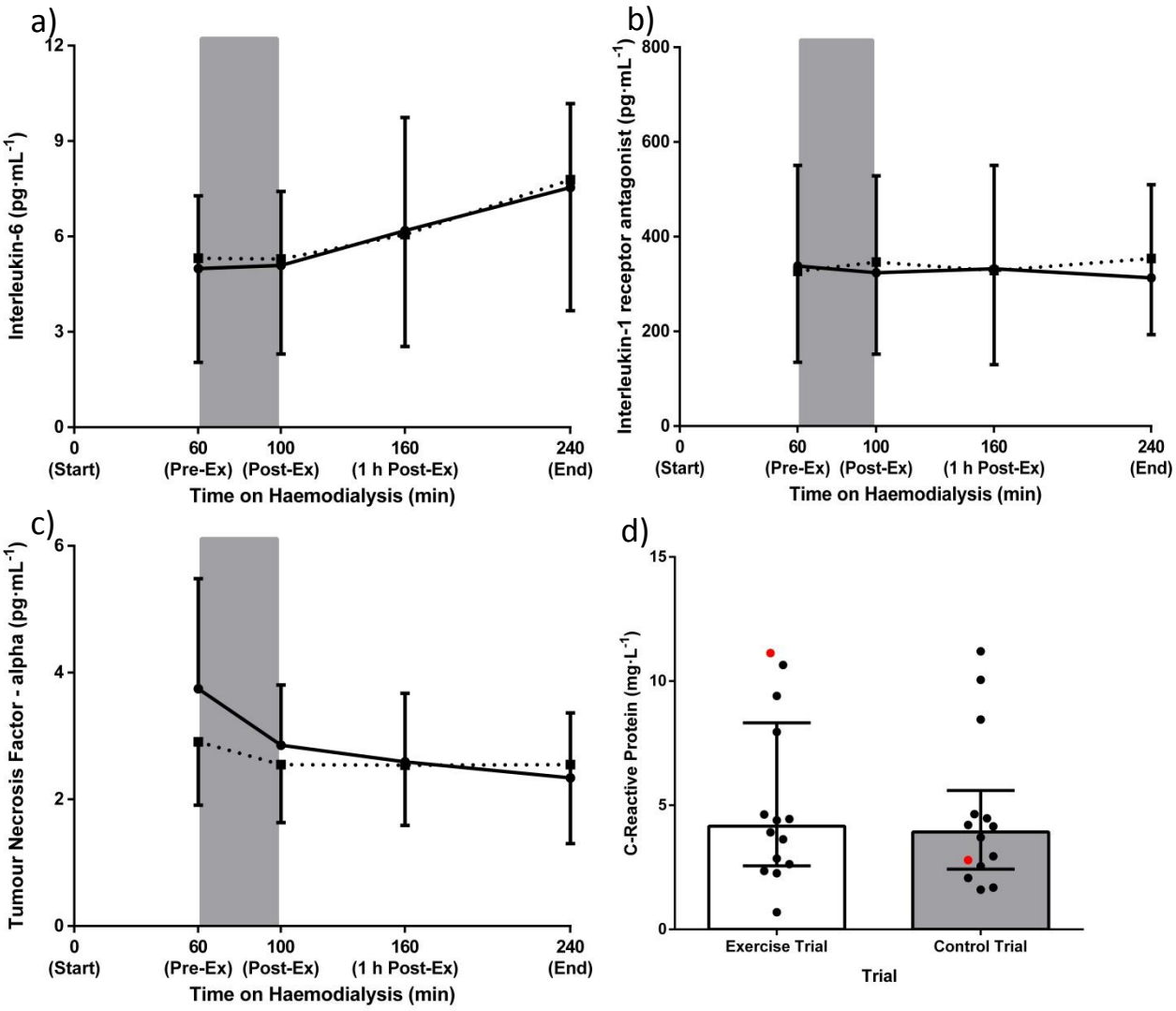

Figure 4.3 - Circulating concentrations of **a)** Interleukin-6 (n = 12), **b)** Interleukin-1 receptor antagonist (n = 9) and **c)** Tumour necrosis factor alpha (n = 11) during haemodialysis on the exercise (—●—) and control (···■···) trials.
The grey bar represents the 30 min of exercise completed on the exercise trial.
Data is presented as mean ± standard deviation.
Statistical analysis performed on log-transformed data. Main effect of time was found in IL-6 and TNF-α ($P < 0.05$).
d) Shows reference C-reactive protein concentrations on both trials for each participant. Data is presented as median ± interquartile range, circles represent each individual patient. Red dots indicate one patient that was excluded due to high variation between the two trials.

Monocytes

Classical monocytes

There were no effects of trial, time or time*trial interaction in either the proportion or the number of classical monocytes ($P > 0.05$; Table 4.4).

Intermediate monocytes

There was no main effect of trial in the proportion or number of intermediate monocytes. An effect of time was found on the proportion ($F_{(2,24)} = 12.544$, $P = 0.002$; Table 4.4) and the number of intermediate monocytes ($F_{(2,24)} = 12.72$, $P < 0.001$). *Post hoc* analysis adjusted for multiple comparisons found a significantly lower proportion of intermediate monocytes at the end of dialysis than at the previous times (60 min vs. 240 min: $P = 0.006$, ES = 0.80; 100 min vs. 240 min: $P = 0.006$, ES = 0.75); similarly, counts followed the same pattern (60 min vs. 240 min: $P = 0.001$, ES = 0.75; 100 min vs. 240 min: $P = 0.003$, ES = 0.64).

There was a trend toward a time*trial interaction in the proportion of intermediate monocytes ($F_{(2,24)} = 2.733$, $P = 0.085$). *Post hoc* contrasts suggested a trend for a different interaction from 60 min to 240 min across the trials, i.e. a greater reduction in the proportion of intermediate monocytes in the exercise trial, although this was not quite statistically significant ($P = 0.056$).

Non-classical monocytes

No main effects of trial or time in the proportion or number of non-classical monocytes ($P > 0.05$; Table 4.4). A trend for an interaction in the proportion of non-classical monocytes was noted ($F_{(2,24)} = 2.808$, $P = 0.080$). *Post hoc* contrasts suggested the change in proportion from 100 min to 240 min was different across trials ($P = 0.046$); however, after adjustment for multiple comparisons this did not remain statistically significant ($P = 0.138$). No interaction effect was seen in the number of non-classical monocytes ($P > 0.05$).

Table 4.4 - Monocyte phenotypes during exercise and control trials

	Exercise Trial			Control Trial		
	Pre-Ex	Post-Ex	End	Pre-Ex	Post-Ex	End
Time (min)	*60*	*100*	*240*	*60*	*100*	*240*
Classical monocytes						
Proportion (%) [Lg]	82.2 ± 5.3	80.7 ± 6.1	83.9 ± 4.3	82.9 ± 5.6	82.1 ± 5.6	82.8 ± 4.7
Count (cells·µL^{-1}) [Lg]	368 ± 120	375 ± 148	376 ± 172	400 ± 137	379 ± 141	359 ± 199
Intermediate monocytes						
Proportion (%)	7.50 ± 2.36	6.84 ± 1.98	5.48 ± 1.88	6.59 ± 1.48	6.92 ± 1.68	5.72 ± 1.37
Count (cells·µL^{-1}) [Lg]	32.0 ± 10.2	30.7 ± 14.4	22.4 ± 6.8	32.2 ± 15.8	31.8 ± 14.7	24.0 ± 13.5
Non-classical monocytes						
Proportion (%)	10.3 ± 3.9	12.5 ± 4.8	10.6 ± 3.9	10.5 ± 4.7	11.0 ± 4.5	11.5 ± 4.1
Count (cells·µL^{-1}) [Lg]	45.5 ± 22.8	55.9 ± 29.6	44.5 ± 19.9	52.3 ± 37.0	50.6 ± 30.3	48.6 ± 29.6

Data presented as mean ± SD. (n = 13)

[Lg] Statistical analysis performed on log-transformed data.

Growth hormone secretagogue receptor expression

$CD3^+$ Lymphocytes

No main effects were found in the proportion and number of leucocytes that were T cells ($CD3^+$ lymphocytes) and no interactions were found ($P > 0.05$; Table 4.5).

GHS-R1a expression on T lymphocytes

The proportion of T cells that expressed GHS-R1a was not different between trials and no interaction effect was found ($P > 0.05$; Figure 4.4a). A main effect of time was observed ($F_{(2,26)} = 5.284$, $P = 0.012$); *post hoc* analysis revealing a significant increase between 60 min and 240 min (adjusted for multiple comparisons: $P = 0.006$, ES = 0.65). There were no main effects found on actual number of GHS-$R1a^+$ T cells ($P > 0.05$) or on the GMFI of the GHS-R1a on T cells ($P > 0.05$; Table 4.5).

GHS-R1a expression on $CD14^+$ monocytes

ANOVA found no effect of trial ($F_{(1,13)} = 0.001$; $P = 0.974$) or time*trial interaction ($F_{(2,26)} = 0.782$; $P = 0.468$) on the proportion of $CD14^+$ monocytes expressing GHS-R1a (Figure 4.4b). An effect of time was seen ($F_{(2,26)} = 4.849$, $P = 0.016$), with *post hoc* analysis showing an increase in GHS-R1a expression from 60 min to 240 min (adjusted for multiple comparisons: $P = 0.045$, ES = 0.53). There were no effects of trial, time or time*trial interaction on the absolute number of $CD14^+$ monocytes expressing GHS-R1a or on the GMFI of GHS-R1a on $CD14^+$ monocytes ($P > 0.05$; Table 4.5).

Table 4.5 - Expression of growth hormone secretagogue receptor on T cells and monocytes during the exercise and control trials.

	Exercise Trial			**Control Trial**		
	Pre-Ex	Post-Ex	End	Pre-Ex	Post-Ex	End
Time (min)	*60*	*100*	*240*	*60*	*100*	*240*
$CD3^+$ lymphocytes						
Proportion (%)	43.6 ± 9.4	43.0 ± 7.3	43.4 ± 13.4	41.7 ± 9.9	40.3 ± 10.1	37.8 ± 11.6
Count (cell·µL^{-1}) ^{Lg}	629 ± 251	666 ± 282	636 ± 378	606 ± 295	591 ± 365	581 ± 412
GHS-R1a$^+$ $CD3^+$ lymphocytes						
GMFI ^{Lg}	9.3 ± 5.9	9.6 ± 4.6	11.3 ± 6.2	9.2 ± 4.1	9.0 ± 4.1	9.5 ± 5.0
Count (cells·µL^{-1}) ^{Lg}	98 ± 83	113 ± 70	124 ± 106	99 ± 89	101 ± 87	123 ± 128
GHS-R1a$^+$ $CD14^+$ monocytes						
GMFI ^{Lg}	9.6 ± 6.5	10.1 ± 6.5	10.2 ± 7.0	9.4 ± 5.0	9.4 ± 4.9	9.2 ± 4.0
Count (cells·µL^{-1}) ^{Lg}	69 ± 49	100 ± 84	89 ± 59	84 ± 60	81 ± 45	102 ± 85

GHS-R1a, Growth hormone secretagogue receptor 1a; GMFI, Geometric mean florescence intensity. (n = 14)
Data is presented as mean ± SD.
^{Lg} Statistical analysis performed on log-transformed data.

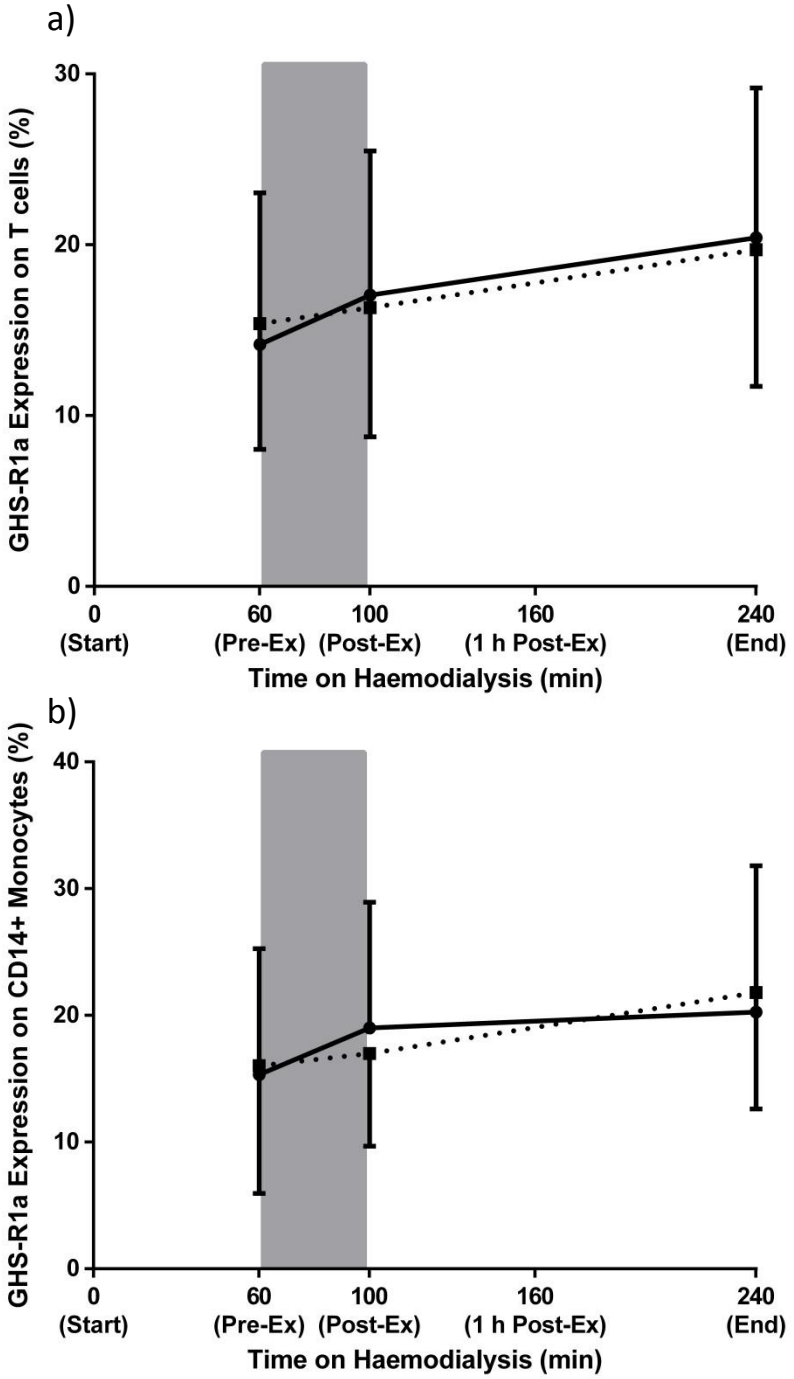

Figure 4.4 - Growth hormone secretagogue expression on **a)** T cells and **b)** CD14+ monocytes during the exercise (●) and the control (■) trial. The grey bar represents the 30 min of exercise completed on the exercise trial. A main effect of time was found on both cell types.
Data is presented as mean ± standard deviation (n = 14).

Neutrophil degranulation

Plasma elastase concentrations showed an effect of time ($F_{(2,22)}$ = 3.476, *P* = 0.049; Figure 4.5a); *post hoc* analysis found plasma elastase concentrations were significantly different between 60 min and 100 min even after adjustment for multiple comparisons (127 ± 94.5 vs. 157 ± 109 $\mu g \cdot L^{-1}$, *P* = 0.018, ES = 0.29). No effect of trial or time*trial interactions were found (*P* = 0.568 and *P* = 0.485).

There were no significant main effects found in elastase concentrations in response to bacterial stimulation (*P* ≥ 0.094). When adjusted to elastase release per neutrophil a significant time*trial interaction was observed ($F_{(2,22)}$ = 4.289, *P* = 0.027; Figure 4.5b). *Post hoc* paired t-tests found no significant differences at each time-point (*P* ≥ 0.231); repeated contrasts suggested a trend toward a difference between trials in the pattern of change in elastase release per neutrophil between 100 min and 240 min (*P* = 0.096).

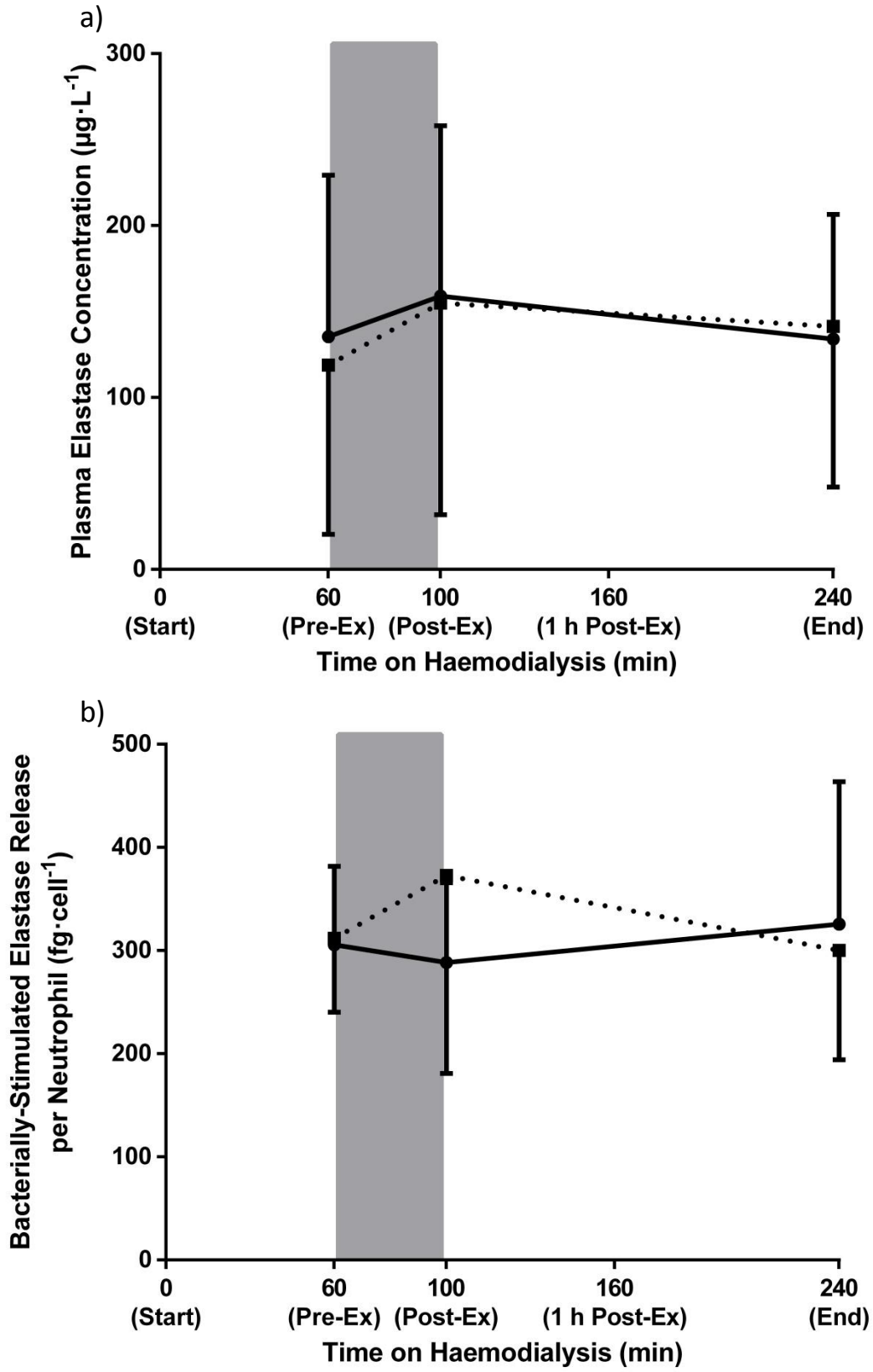

Figure 4.5 - **a)** Plasma elastase concentration and **b)** degranulation of elastase by neutrophils in response to bacterial stimulation throughout the exercise (●) and the control (■) trials. The grey bar represents the time where exercise was completed on the exercise trial.
Data is presented as mean ± standard deviation (n = 12).
Statistical analysis performed on log-transformed data.
A main effect of time was found for plasma elastase (graph a, $P = 0.049$); a time*trial interaction was found in stimulated elastase release per neutrophil (graph b, $P = 0.027$).

4.5 Discussion

The main aim of this study was to analyse the acute effects of exercising during HD on circulating markers of inflammation and immunity to assess the safety of exercise in this vulnerable population. Exercise during HD did not induce any clear alterations to leucocytes or circulating factors associated with systemic inflammation.

Exercise

The exercise completed by the patients was similar to that achieved during a regular intradialytic exercise session. Due to the different equipment used in various aerobic intradialytic training studies (e.g. dialysis chairs or beds, cycle ergometers or steppers) direct comparisons between studies should be made with caution. However, the power output of this cohort are comparable with those that have reported objective measures of exercise intensity in intradialytic cycling and achieved significant improvements in physical function (DePaul *et al*., 2002; Kopple *et al*., 2007; Moore *et al*., 1993a; Storer *et al*., 2005).

Cell counts

Intradialytic exercise had no effect on circulating leucocytes. The two main drivers of the typical exercise-induced leucocytosis seen in healthy populations, shear stress and circulating adrenaline, are reduced in ESRD patients. Cardiac output and blood flow are reduced in elderly populations (Poole *et al*., 2003) and this would be exacerbated by HD treatment, diminished cardiac function (McIntyre, 2009) and cardiac medications common in ESRD (e.g. beta-blockers). Furthermore, adrenaline secretion and the responsiveness of adrenoreceptors after exercise are lower in ESRD (Daul *et al*., 1985). Finally, HD leads to sequestration of leucocytes even when biocompatible dialysers are used (Nockher, Wiemer and Scherberich, 2001; Sester *et al*., 2001). It is clear that ESRD patients have a number of barriers to leucocytosis that the intradialytic exercise in this study did not overcome.

In healthy cohorts the exercise threshold required to elicit leucocytosis is unclear. Very short duration low intensity exercise (such as 16 min cycling at 35% peak power) has been shown to induce significant lymphocytosis (Campbell J. *et al*., 2009). Alternatively, 30 min of moderate-intensity walking had no effect on circulating leucocytes (Markovitch, Tyrrell and Thompson, 2008). Given that the intensity and duration of exercise are highly influential on the magnitude of leucocytosis (Gimenez *et al*., 1986; Shek *et al*., 1995) it is possible a higher intensity or a longer duration of exercise may overcome these barriers and alter circulating leucocytes in HD patients. Importantly, moderate-intensity exercise during HD did not cause abnormal changes in leucocyte counts.

Interleukin-6 and circulating cytokines

Intradialytic exercise had no effect on circulating concentrations of IL-6. In healthy individuals IL-6 is consistently increased after a bout of exercise (typically a 10-fold increase would be expected after 2 h exercise; Pedersen and Febbraio, 2008), although more modest increases are seen after shorter durations of exercise. Indeed, duration of exercise accounts for more than 50% of the variation in post-exercise plasma IL-6, but exercise without sufficient workload or duration does not trigger IL-6 secretion (Fischer, 2006; Markovitch, Tyrrell and Thompson, 2008). As well as exercise mode, duration and intensity, the subject characteristics (e.g. age, gender, fitness, and disease status) are also important factors; low intensity exercise for a short duration has been shown to induce an increase in plasma IL-6 in some cohorts. For example, submaximal cycling at 40 W for a duration of 7 – 20 min gave a significant increase in circulating IL-6 (Δ 0.9 $pg \cdot mL^{-1}$) in COPD patients (van Helvoort *et al*., 2006a). Moreover, in pre-dialysis CKD patients 30 min of submaximal walking stimulated a significant increase in both plasma IL-6 (Δ 1.7 $pg \cdot mL^{-1}$) and IL-10 (Δ 1.1 $pg \cdot mL^{-1}$; Viana *et al*., 2014).

Even a minor increase in IL-6 was not observed in the present study as no difference was found from pre- to post-exercise, even in patients that generated the highest power output. It is likely that the intradialytic exercise completed was not of an intensity or duration sufficient to stimulate myocyte secretion of IL-6. This is similar to findings in low-

intensity exercise in healthy volunteers where 30 min of walking at 50% $\dot{V}O_{2max}$ had no effect on IL-6 (Markovitch, Tyrrell and Thompson, 2008). Whether other forms of exercise in this population (e.g. extra-dialytic, resistance, higher intensity interval training) may stimulate a detectable muscular release of IL-6 into circulation is unknown. A recent systematic review showed a distinct lack of knowledge in the acute effects of exercise on inflammatory markers in chronic inflammatory diseases; indeed this appears to be the first such study in ESRD (Ploeger *et al*., 2009).

The primary source of exercise-induced increases in plasma IL-6 are the contracting muscles themselves and not the circulating leucocytes (Starkie *et al*., 2001; Steensberg *et al*., 2000), therefore immune dysfunction is not likely responsible for the lack of effect observed in HD patients here. Interestingly, at rest IL-6 expression within the muscle is elevated in ESRD patients compared with healthy controls and a release of IL-6 from peripheral tissues at rest, not usually seen in healthy individuals, is associated with higher plasma IL-6 and muscle catabolism (Garibotto *et al*., 2006). Furthermore, HD itself also induces an increase in muscle IL-6 gene expression that may be partly responsible for elevated plasma IL-6 concentrations (Raj *et al*., 2005). A number of possible causes of elevated muscle IL-6 release at rest have been suggested including metabolic acidosis, ROS, muscle glycogen depletion and increases in inflammatory cytokines (IL-1β), all of which are commonly reported in ESRD patients (Garibotto *et al*., 2006). An increased IL-6 secretion from leucocytes during HD (Takahashi *et al*., 2000) also likely contributes to the increase in plasma IL-6 during HD that has been reported elsewhere (Caglar *et al*., 2002; Yamamoto *et al*., 2013); a trend for increased plasma IL-6 during HD was seen here.

Despite the apparent potential for an exacerbated post-exercise cytokine secretion (reported in some inflammatory diseases; e.g. COPD: van Helvoort *et al*., 2006b; cystic fibrosis: Ionescu *et al*., 2006) intradialytic exercise had no effect. This may be because the exercise stimulus was insufficient, or that a minor release was not noticeable as a result of the release of IL-6 from the muscle at rest, or that uraemia *per se* inhibits the pathway for exercise-induced IL-6 secretion in the muscle as indicated by reduced muscle IL-6 mRNA in response to exercise seen in '5/6 nephrectomy' rats (Dünner *et al*., 2011). Another possible explanation is due to the altered metabolism of HD patients. Glycogen depletion is a key stimulus for the release of IL-6 from the muscle (Steensberg *et al.,*

2001). In healthy individuals after regular exercise training the muscle becomes less dependent on glycogen as a fuel source due to an increased enzymatic capacity for β-oxidation of free fatty acids (Phillips *et al*., 1996) and there is a reduced IL-6 secretion after each exercise bout (Pedersen and Febbraio, 2008). In ESRD patients muscle substrate utilisation during and after HD is significantly altered toward diminished carbohydrate oxidation and accelerated lipid and amino acid oxidation (Ikizler *et al*., 2002); hypothetically, this altered metabolism within the muscle may inhibit IL-6 secretion.

It is uncertain whether an exercise-induced increase in IL-6 would be desirable in this vulnerable population. In healthy populations the increase in IL-6 occurs transiently in the absence of other pro-inflammatory factors and is met by an effective anti-inflammatory response that leads to an anti-inflammatory environment post-exercise thought to be beneficial for cardiovascular health (Fischer, 2006). With the dysfunctional immune system prevalent in ESRD patients it is possible an influx of IL-6 may persist for longer and without an adequate anti-inflammatory response; therefore, exacerbating the pro-inflammatory environment. On the other hand, an exercise-induced increase in IL-6 in the absence of other pro-inflammatory cytokines may help reduce elevated basal circulating inflammatory factors.

Given the lack of IL-6 response it is unsurprising that no effect was seen in the anti-inflammatory cytokines measured. IL-1ra and IL-10 are reported to increase in circulation in response to IL-6 infusion (Steensberg *et al*., 2003). It is unfortunate that IL-10 could not be accurately quantified, but as there was no apparent difference in IL-6 it is likely that IL-10 would have followed a similar pattern to IL-6.

In the general population only highly strenuous prolonged exercise may result in a small production of TNF-α (Starkie *et al*., 2001). However, in COPD and heart failure patients short duration exercise has shown to increase circulating concentrations of TNF-α (Kinugawa *et al*., 2003; Rabinovich *et al*., 2003). This study found no increase in plasma TNF-α after exercise. On the contrary a perceptible decrease can be observed post-exercise, although without statistical significance. The relationship between power output achieved and the change in TNF-α suggests exercise may have the potential to decrease plasma TNF-α, especially if the intensity can be increased. Given the large gap in

knowledge in exercise-induced cytokine response in ESRD further studies analysing different exercise protocols would be of interest.

Overall it appears that intradialytic exercise at the intensity and duration that HD patients could manage in this trial did not exacerbate inflammation in the circulating inflammatory factors measured.

Monocytes

In the present study both intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocytes exhibited a trend for an influence of exercise on post-exercise distribution. There was a trend for a greater reduction of intermediate monocytes from the beginning of HD to the end of HD on the exercise trial. The proportion of non-classical monocytes tended to increase post-exercise but reduced to a greater extent than on the control trial by the end of HD. This appears to represent a small-scale normal response to exercise but superimposed onto the large contrasting effects of HD. A larger cohort study is needed to clarify these findings.

In healthy volunteers a preferential mobilisation of $CD16^{+}$ (intermediate and non-classical combined) monocytes (specifically non-classical) is observed after exercise compared with classical ($CD14^{++}CD16^{-}$) monocytes (Simpson *et al*., 2009) with similar results founds in pre-dialysis CKD patients (Van Craenenbroeck *et al*., 2014), this is in agreement with the trends of the present study. Selective mobilisation of $CD16^{+}$ monocytes occur after as little as 1 min of cycling at 400 W (Steppich *et al*., 2000) and 20 min of running at 65-70% $\dot{V}O_{2peak}$ (Hong and Mills, 2008). Mobilisation of monocytes is reduced by beta-blocker administration suggesting exercise related increases in catecholamines are partly responsible (Steppich *et al*., 2000). Frequent beta-blocker use and reduced adrenaline and adrenoreceptor responsiveness in ESRD (Daul *et al*., 1985) would be expected to limit exercise-induced monocyte demargination, in addition to the other factors discussed earlier (cardiac output, blood flow, exercise intensity and duration).

In contrast to exercise, a selective sequestration of $CD16^{+}$ monocytes, probably to the capillary bed of the lung, occurs at the initiation of HD and leaves a profound decrease in circulation even with biocompatible HD (Nockher, Wiemer and Scherberich, 2001;

Rogacev *et al*., 2009; Sester *et al*., 2001). This likely occurs due to an upregulation of surface adhesion molecules on these monocytes as a result of activation in the extracorporeal blood circuit. This process is transient and $CD16^+$ monocytes reappear in circulation after 30 min of HD (Nockher, Wiemer and Scherberich, 2001). Therefore, because no blood samples were taken prior to dialysis it is impossible to accurately comment on the kinetics of monocyte phenotypes due to HD alone in this study. Nevertheless, it is evident that the cell counts seen during HD in this chapter are markedly lower than seen in the pre-HD samples in the previous chapter (Table 3.4). Elsewhere, observational data infers the egression of monocytes out of the circulation represents a normal response to HD. Patients who only exhibit a minor drop in intermediate monocytes at the start of HD had an increased risk of a cardiovascular event (hazard ratio 2.41 compared with a large fall in intermediate monocytes), possibly because this represents an inadequate migratory response to stimulus and therefore dysfunctional monocytes (Rogacev *et al*., 2009).

Growth hormone secretagogue receptor expression

The present findings suggest that PBMCs expressing GHS-R1a are increased in proportion during HD. Definite conclusions about the effect of HD cannot be made with certainty as no pre-HD samples were taken; however, samples during HD were higher than those taken prior to HD in similar patients in the previous chapter (Table 3.4). It is likely that an increase in PBMCs expressing GHS-R1a is due to a greater number of activated leucocytes in circulation resulting from HD. Activation of T cells and monocytes is associated with a reorganisation of GHS-R1a within the cell and expression is significantly increased (Dixit *et al*., 2004).

In young healthy individuals a bout of exercise is associated with an increased number of $GHS\text{-}R1a^+$ PBMCs in circulation (Bishop *et al*., 2013). This was not seen as a result of intradialytic exercise here. The authors cited a preferential influx of activated T cells and monocytes into circulation to explain the increase in GHS-R1a. It therefore may not be surprising that exercise during HD had no significant effect as HD itself appears to lead to

a preferential recruitment of these cells in the absence of exercise; exercise had no clear additive effect.

Neutrophil degranulation

An increase in plasma elastase was seen in both trials, but with no effect of exercise. Both exercise (Blannin *et al*., 1996; Smith *et al*., 1996) and HD (Costa *et al*., 2008b; Pesanti, 2001) have been shown to stimulate spontaneous elastase release. Exercise had no significant effect in this study so it would appear that HD gave a greater stimulus than exercise, although this may be exercise intensity and duration dependant. Given that neutrophils are primed in HD patients and spontaneously produce elastase during HD (Costa *et al*., 2008b; Pesanti, 2001; Sela *et al.,* 2005) it is important that exercise did not exacerbate unstimulated elastase release as this likely has a role in the enhanced inflammatory status of these patients.

Degranulation in response to bacterial stimulant was different on the exercise trial compared with the control trial but this difference had disappeared by the end of HD. It is important to note that despite the apparent difference between trials this was primarily due to an increase and then decrease on the control trial whereas the neutrophil response remained consistent during the exercise trial. Exercise did not suppress neutrophil function significantly lower than pre-exercise levels and the difference between trials was transient.

In a recent study in pre-dialysis CKD patients a similar exercise bout of 30 min of walking at an RPE 12-14 was associated with an enhanced neutrophil responsiveness to bacterial stimulation 1 h after exercise (Viana *et al*., 2014). This is in contrast to a number of studies in healthy volunteers that show a suppressed neutrophil degranulation in response to bacterial stimulation in the recovery period after exercise (Blannin *et al*., 1996; Robson *et al*., 1999b). As seen in the previous chapter, degranulation responsiveness of neutrophils to bacterial stimulant is reduced in HD patients (Figure 3.4b). The improvement in neutrophil responsiveness seen in the pre-dialysis population coincided with neutrophilia, the author therefore speculated that the appearance of ‘fresh’ neutrophils into circulation with improved function compared with dysfunctional

uraemic neutrophils, may have been responsible for the post-exercise protective effect observed (Viana, 2011). This may explain the differing results observed in this study as post-exercise neutrophilia did not occur.

Limitations

The randomised crossover design of the study ensured that comparisons could be made between control and exercise trials that were not confounded by variations in circadian rhythm and inter-individual differences in terms of disease status and HD treatment. However, it is also important to acknowledge the limitations of the study. Only a single mode, duration, intensity of exercise was analysed, other exercise prescriptions are likely to have differing consequences. This exercise mode was chosen as the study was designed to be pragmatic and this exercise can be utilised and sustained in a HD unit without too much supervision.

The patients that were recruited to this study were accustomed to completing intradialytic exercise. The results of this study cannot be extrapolated to patients starting exercise for the first time where the exercise stimulus may be novel and have different outcomes.

Regrettably, no blood sample was collected at the beginning of HD. This does not influence any conclusions made on the impact of exercise but may have added insight into the effect of HD itself as no conclusions can be made on the effect of the HD process without a pre-HD sample.

Implications

Overall, this study has demonstrated that 30 min of intradialytic exercise at an intensity that patients reported to be “somewhat hard” does not have a substantial impact upon circulating markers of inflammation or immune function. From an immunological perspective this exercise can be regarded as safe. Exercise did not exacerbate circulating pro-inflammatory factors. Further research into higher intensity exercise, if tolerated by

patients, could be of interest as TNF-α appeared to decrease most in patients who produced the greatest power output.

Exercise did not stimulate an increase in plasma IL-6, but a long-term anti-inflammatory effect of regular exercise has been observed in low-to-moderate-intensity exercise that is not associated with a release of IL-6 (Fischer, 2006). Whether long-term regular exercise of this nature has beneficial anti-inflammatory properties is unclear.

4.6 Conclusions

In summary, exercise at an intensity that can be routinely carried out on HD appears to have relatively little impact upon circulating markers of inflammation or immune function. Intradialytic exercise did not exacerbate the pro-inflammatory environment associated with HD nor induce abnormal alterations to leucocytes. From an immunological perspective, moderate-intensity cycling exercise during HD is well tolerated and appears safe.

Chapter 5

An Investigation into the Effects of an Acute Bout of Exercise during Haemodialysis on Haemodynamic Parameters and Markers of Cardiac Injury

5.1 Abstract

Patients with ESRD are highly susceptible to cardiovascular mortality and the vast majority present with hypertension. HD treatment has profound haemodynamic effects. An acute pronounced fall in blood pressure during HD, also known as intradialytic hypotension, is highly associated with myocardial ischaemia, subclinical cardiac injury and an increased risk of mortality. In healthy individuals exercise leads to an increase in SBP. Moreover, upon completion of exercise, a drop in blood pressure below resting levels has been observed; this is particularly clear in hypertensive individuals. This chapter aimed to investigate the effects of an acute bout of exercise completed during HD treatment on haemodynamic parameters and markers of cardiac injury.

Fifteen patients completed two trials in a randomised-crossover design. On the control trial the patient rested throughout HD. On the exercise trial patients completed 30 min of cycling exercise during HD, 1 h into the treatment, at a perceived exertion of “somewhat hard”. On both trials blood pressure was measured at the start of HD (0 min) and then with blood samples immediately pre-exercise (60 min), post-exercise (100 min), 1 h post-exercise (160 min) and at the end of HD (240 min). Plasma markers of cardiac injury were measured using a biochip method.

Across both trials SBP decreased upon initiation of HD ($P < 0.001$). A time*trial interaction was observed suggesting exercise significantly influenced SBP ($P < 0.001$). *Post hoc* tests revealed SBP was significantly greater at 100 min (post-exercise) on the exercise trial compared with the control trial (125 ± 18 vs. 112 ± 20 mmHg; $P = 0.025$) but significantly lower at 160 min (1 h post-exercise; 106 ± 22 vs. 117 ± 25 mmHg; $P = 0.040$). Investigation of markers of cardiac injury found no significant effects of exercise. Heart-type fatty acid-binding protein and myoglobin were both elevated compared with non-ESRD healthy ranges but decreased significantly during the HD period ($P < 0.001$).

When ESRD patients exercise during HD it appears a normal cardiovascular response to exercise is superimposed onto the response to HD. The post-exercise hypotension witnessed in this study is pronounced but occurs without any clear suggestion of myocardial injury, both are novel findings. As intradialytic hypotension is associated with cardiac injury and mortality; the data reported here underlines the importance of further studies investigating the effect of intradialytic exercise on the myocardium.

5.2 Background

ESRD patients are highly susceptible to cardiovascular disease and have a substantially increased risk of cardiovascular mortality (Pruthi, Steenkamp and Feest, 2013). The vast majority of patients with CKD present with hypertension as a co-morbidity and prevalence increases as disease severity progresses (Rao *et al*., 2008). The kidneys have many crucial roles in the regulation of blood pressure and therefore damage to the kidneys has systemic consequences on blood pressure control. On the other hand, hypertension is also implicated in the aetiology of CKD in a number of patients.

In ESRD patients, blood pressure management is complicated by interdialytic fluid accumulation and the HD procedure *per se* has profound impacts upon haemodynamics. For most patients the removal of fluid during HD leads to a lower blood pressure at the end of treatment. Intradialytic hypotension is a term used to describe a pronounced fall in blood pressure especially when it is symptomatic, and this is reported to affect around 17.2% of all HD treatments but variation between individual patients and HD units is reported (Sands *et al.,* 2014). Intradialytic hypotension is associated with a number of unpleasant symptoms including nausea, dizziness, cramps and syncope. Intradialytic hypotension is also highly associated with asymptomatic myocardial stunning and HD-induced cardiac injury (Burton *et al*., 2009). Treatments known to reduce the incidence of intradialytic hypotension such as dialysate cooling and biofeedback techniques have shown to maintain left ventricular ejection fraction and abrogate the incidence of regional wall motion abnormalities (Selby *et al*., 2006).

A recent large retrospective cohort study found that modest reductions in SBP during HD were associated with the greatest survival rates compared with large falls or increases in SBP (Park *et al*., 2013). Large decreases in SBP are related to myocardial ischaemia, myocardial stunning and cardiac injury and intradialytic increases in SBP represent fluid overload or cardiovascular dysfunction both of which are associated with poor outcome. It appears desirable to maintain a consistent blood pressure during HD.

Exercise in the general population has dramatic haemodynamic effects. Cardiac output is increased due to an increased demand for oxygen to the working muscles and this is met

by increased heart-rate and stroke volume. Blood from the heart is directed to the working muscles through vasodilation of arteries feeding the active tissues and vasoconstriction to inactive tissues and non-essential processes (e.g. the skin, kidneys). Consequently SBP is usually increased during exercise (MacDonald, 2002).

Upon completion of exercise SBP falls and can decrease to below pre-exercise levels. Research into this post-exercise hypotension was popularised after an anecdotal report reporting lower blood pressures following a bout of jogging (Fitzgerald, 1981). Ensuing research has suggested that exercise of differing modes, even at low intensity (40% $\dot{V}O_{2max}$; Pescatello *et al*., 1991), results in a reduction in blood pressure for 1-2 h after completion (MacDonald, 2002). Furthermore, during free-living conditions using ambulatory blood pressure monitors this reduction may be seen for 12 h or even longer (Pescatello *et al*., 1991; Rondon *et al*., 2002; Wallace *et al*., 1999). These findings led to the ACSM recommending exercise as a cornerstone therapy for primary prevention, treatment and control of hypertension (Pescatello *et al*., 2004).

It is unclear whether the addition of exercise during a HD session has desirable or indeed undesirable consequences in terms of blood pressure. The aim of this chapter is to explore and analyse the immediate haemodynamic response to exercising during HD treatment in patients with ESRD.

5.3 Methods

The results presented in this chapter use the same protocol and patients reported in the previous chapter (Chapter 4). A detailed description of the protocol and participant information can be found earlier, see Figure 4.2 and Table 4.1.

Blood pressure

Blood pressure was taken using an electronic sphygmomanometer built into the HD machines at the start of dialysis (0 min), pre-exercise (60 min), post-exercise (100 min), 1 h post-exercise (160 min) and at the end of dialysis (240 min) and at the equivalent times during the non-exercise trial (see Figure 4.4). Pulse pressure (PP) was calculated as the difference between SBP and DBP; mean arterial pressure (MAP) estimated as DBP + 1/3(SBP – DBP); and rate pressure product (RPP) as heart rate (HR) x SBP.

Markers of cardiac injury

A range of markers of cardiac injury are available, each with varying specificity and sensitivity dependent upon the time passed following cardiac injury.

Troponin I is a regulatory protein that controls calcium mediated interaction between actin and myosin; in contraction calcium binds with the troponin complex leading to translocation of troponin T and tropomyosin, subsequently myosin binds to actin and the cross bridge cycle is initiated. Cardiac troponin I (cTnI) is specific to the myocardium and leaks into the blood upon cardiac damage.

Myoglobin is the oxygen carrying pigment in muscle tissue and delivers oxygen to the myocardium. When cell membranes are damaged (i.e. after an infarction) myoglobin quickly appears in circulation.

Creatine kinase MB (CKMB) facilitates the movement of phosphates into and out of the mitochondria, catalysing the conversion of creatine to phosphocreatine. The majority of CKMB resides in cardiac muscle and increases in circulation represent cardiac damage.

Fatty acid-binding protein (FABP) is present in the cytoplasm of myocytes and is an intracellular transport protein for fatty acids. The heart-type FABP (h-FABP) is specific to the heart and rapidly appears in circulation after damage to the myocardium.

Cardiac array

Blood samples were collected from arterial HD lines into K_3EDTA monovettes at pre-exercise, post-exercise, 1 h post-exercise and at the end of HD on both exercise and control trials. Samples were centrifuged for 10 min at 1500 x *g* and the plasma was frozen at < -20°C for later analysis. Early and late markers of myocardial injury in these plasma samples were assessed using a biochip method. The cardiac array supplied by Randox Ltd (Crumlin, UK) using the Evidence Investigator analyser allows simultaneous detection of early markers myoglobin and h-FABP and late markers CKMB and cTnI.

60 µL of plasma was added to 240 µL of assay diluent in each well. These samples were incubated for 30 min at 37°C at 380 rpm on a thermoshaker (Randox Ltd, Crumlin, UK). Each well underwent a 6-stage wash cycle and a conjugate was added prior to another 30 min incubation and wash cycle. A signal reagent (luminol-EV701 and peroxide) was added to each well and chemiluminescence intensity was read by the Evidence Investigator system after exactly 2 min. A calibration curve was created using 9 standards provided by the kit, a curve fit of $r > 0.95$ was required (CKMB $r = 1.000$; myoglobin $r = 0.995$; h-FABP $r = 0.999$; cTnI $r = 0.995$). CV was calculated on 3 quality control samples to estimate intra-assay variation (CKMB 7.6%; myoglobin 5.5%; h-FABP 7.0%; cTnI 17.8%).

All values were adjusted for changes in plasma volume (Dill and Costill, 1974).

Statistical analysis

Repeated-measures two-factor ANOVA was used to analyse data: trial (exercise vs. control) x time (0 vs. 60 vs. 100 vs. 160 vs. 240 min). Where a significant effect was observed *post hoc* paired t-tests were used to analysis significant differences.

5.4 Results

All 15 patients successfully completed 30 min of exercise at an average power output of 21.5 ± 8.1 W and RPE of 13 ± 1, or "somewhat hard" (Borg, 1973). There were no significant differences in HD treatment variables between trials. For more detailed information about the HD treatment and exercise refer to Chapter 4. No formal measures of symptoms were collected; however, anecdotally no negative outcomes were reported during the exercise trials.

Haemodynamic parameters

There were no significant differences between the exercise and control trial at the start of HD for all haemodynamic parameters (all $P > 0.7$; Table 5.1).

Systolic blood pressure

ANOVA found no effect of trial ($F_{(1,14)} = 0.29$; $P = 0.60$). An effect of time was found ($F_{(4,56)} = 14.7$; $P < 0.001$), *post hoc* analysis revealed a significant difference between SBP prior to starting dialysis (0 min) to all subsequent time-points (all $P \leq 0.007$; ES ≥ 0.84).

A time*trial interaction was found ($F_{(3,37)} = 8.26$; $P < 0.001$). *Post hoc* paired t-tests revealed SBP was significantly higher at 100 min (post-ex) on the exercise trial compared with the same time on control trial ($P = 0.025$; ES = 0.71) and significantly lower at 160 min (1 h post-ex) on the exercise trial compared with control ($P = 0.040$; ES = 0.46); Figure 5.1.

Table 5.1 - Haemodynamic parameters during exercise and control trials

	Exercise Trial					Control Trial				
	Start	Pre-Ex	Post-Ex	1 h post	End	Start	Pre-Ex	Post-Ex	1 h post	End
Time (min)	*0*	*60*	*100*	*160*	*240*	*0*	*60*	*100*	*160*	*240*
SBP [Lg] (mmHg)	137 ± 26	118 ± 16	125 ± 18*	106 ± 22*	117 ± 23	138 ± 28	114 ± 17	112 ± 20	117 ± 25	117 ± 24
DBP (mmHg)	67 ± 14	63 ± 14	67 ± 15	57 ± 15	63 ± 18	67 ± 17	63 ± 12	59 ± 12	60 ± 12	59 ± 17
Heart rate (bpm)	70 ± 17	71 ± 15	82 ± 15*	70 ± 16	74 ± 12	71 ± 16	74 ± 16	71 ± 12	72 ± 11	76 ± 13
PP (mmHg)	69 ± 19	55 ± 13	58 ± 16	49 ± 17	54 ± 17	71 ± 18	52 ± 17	54 ± 16	57 ± 19	58 ± 19
MAP (mmHg)	91 ± 17	81 ± 14	86 ± 15	74 ± 16	81 ± 18	91 ± 19	80 ± 12	77 ± 13	79 ± 15	78 ± 18
RPP [Lg] (mmHg·bpm)	9738 ± 3634	8379 ± 2198	10200 ± 2253*	7427 ± 1948	8538 ± 2033*	9779 ± 3413	8314 ± 1683	7929 ± 1546	8322 ± 2024	8813 ± 2023

SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HR, Heart-rate; bpm, beats per minute; PP, Pulse pressure; MAP, Mean arterial pressure; RPP, Rate pressure product.

Data is presented as mean ± SD. (n = 15)

[Lg] Statistical analysis performed on log-transformed data.

* Significantly different from same time on control trial ($P < 0.05$).

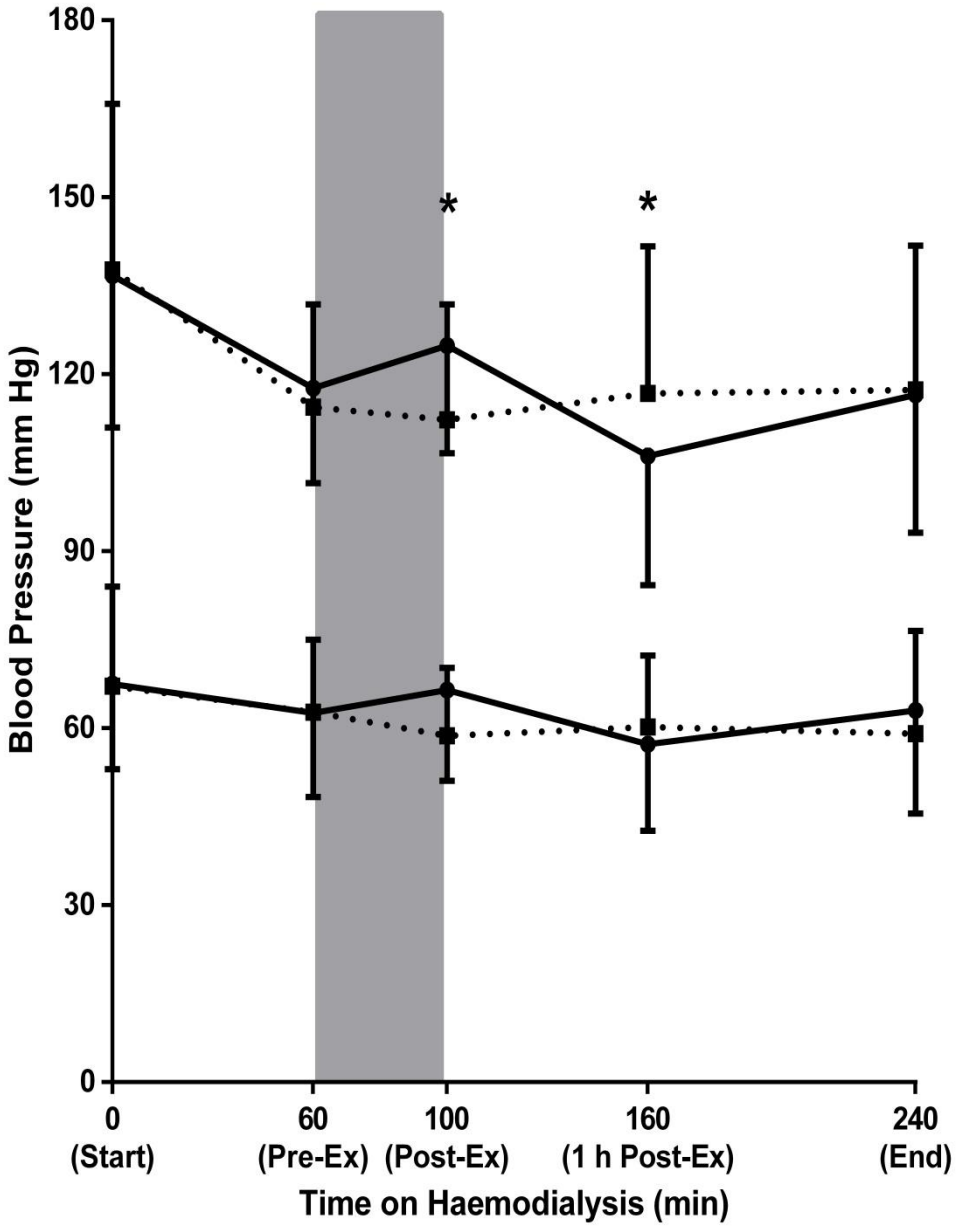

Figure 5.1 - Systolic and diastolic blood pressure throughout haemodialysis on the exercise ( ) and control ( ) trials. The grey bar represents the 30 min of exercise completed on the exercise trial. Data is presented as mean ± standard deviation (n = 15).
A significant main effect of time ($P < 0.001$) and a time*trial interaction ($P < 0.001$) were observed.
* denotes a significant differences between trials at that time.

The statistical results for comparison of systolic, diastolic and mean arterial blood pressure, heart-rate, pulse pressure, and rate pressure product are detailed in Table 5.2.

Table 5.2 - Statistical results from analysis of variance and *post hoc* tests for haemodynamic variables

	Trial effect	**Time effect**	***Post hoc***	**Time*Trial interaction**	***Post hoc* (paired t tests)**
SBP	NS	*P* < 0.001	0 min > all other times	*P* < 0.001	100 min (*P* = 0.025) 160 min (*P* = 0.040)
DBP	NS	*P* = 0.016	0 min > 240 min	NS	
MAP	NS	*P* < 0.001	0 min > 60, 160 & 240 min	*P* = 0.004	NS
HR	NS	NS		*P* = 0.001	100 min (*P* < 0.001)
PP	NS	*P* < 0.001	0 min > all other times	*P* = 0.014	NS
RPP	NS	*P* < 0.001	160 min < 0, 100 & 240 min	*P* < 0.001	100 min (*P* < 0.001) 240 min (*P* = 0.024)

SBP, Systolic blood pressure; DBP, Diastolic blood pressure; PP, Pulse pressure; MAP, Mean arterial pressure; RPP, Rate pressure product; NS, non-significant.

Diastolic blood pressure

DBP followed the same pattern as SBP but was less pronounced (Figure 5.1). The DBP at the end of HD (240 min) was significantly lower than at the beginning of HD (*P* = 0.014; ES = 0.39). Exercise had no statistically significant impact.

Mean arterial pressure

MAP also followed the same trend. MAP across both trials decreased from 0 min (start of HD) to 60, 160 and 240 min (all $P \leq 0.020$; ES ≥ 0.63). A time*trial interaction was found, and a trend for differences in MAP at 100 min (post-ex) and 160 min (1 h post-ex) were found, but these did not maintain significance after adjustments for multiple comparisons (*P* = 0.100, ES = 0.68 and *P* = 0.075, ES = 0.35 respectively).

Heart rate

Heart rate was significantly increased at 100 min (post-ex) on the exercise trial compared with the control trial ($P < 0.001$; ES = 0.78). No other differences were observed.

Pulse pressure

PP was greater at the start of HD than at all subsequent times across both trials ($P \leq 0.027$; ES ≥ 0.80). A time*trial interaction was also observed but no significant differences were observed after *post hoc* paired t-tests between trials.

Rate pressure product

Across both trials a time effect was found, RPP at 160 min (1 h post-ex) differed compared with the 0 (start), 100 (post-ex) and 240 min (end); $P = 0.010$, ES = 0.71, $P = 0.048$, ES = 0.66, and $P = 0.027$, ES = 0.45 respectively.

A time*trial interaction was found ($F_{(3,36)} = 10.8$; $P < 0.001$). Significant differences were seen between trials at 100 min (post-ex; ES = 1.15), and 240 min (end of dialysis; ES = 0.15). RPP at 160 min (1 h post-ex) approached significance but did not remain statistically different after adjustments for multiple comparisons ($P = 0.081$; ES = 0.47).

Markers of cardiac injury

Plasma samples from 12 patients were assessed for an array of markers of cardiac injury (Table 5.3).

Heart-type fatty acid-binding protein

ANOVA found no main effects of trial ($F_{(1,11)}$ = 0.78, P = 0.395). A main effect of time was observed ($F_{(3,33)}$ = 33.9, P < 0.001). *Post hoc* analysis found a significant difference between all time-points (all $P \leq 0.034$, ES range 0.30 (100 vs. 160 min) to 0.95 (60 vs. 240 min)), thus suggesting that h-FABP decreased significantly between each time-point over both trials (Figure 5.2a). No significant time*trial interaction effects were seen ($F_{(3,33)}$ = 1.04, P = 0.388).

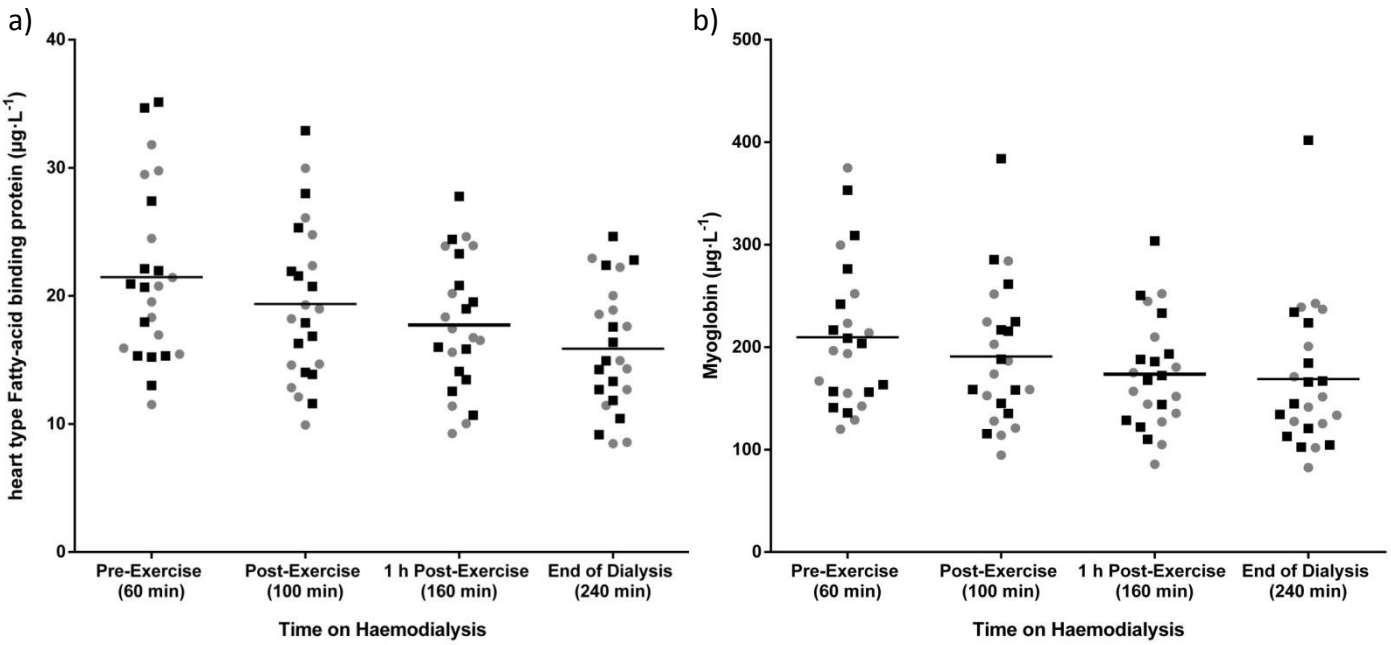

Figure 5.2 – **a)** heart-type fatty acid-binding protein, and **b)** myoglobin, at the different times across both trials. Grey circles (●) represent patients on the exercise trial, black squares (■) represent patients on the control trial. Lines denote the mean for all samples at that time. A main effect of time was observed in both markers (P < 0.001).

Table 5.3 - Markers of cardiac injury during exercise and control trials

	Exercise Trial				Control Trial			
Time (min)	Pre-Ex *60*	Post-Ex *100*	1 h post *160*	End *240*	Pre-Ex *60*	Post-Ex *100*	1 h post *160*	End *240*
h-FABP ($\mu g \cdot L^{-1}$)	21.3 ± 6.4	18.6 ± 6.2	17.3 ± 5.3	15.9 ± 4.9	21.6 ± 7.4	20.1 ± 6.3	18.1 ± 5.2	15.9 ± 5.1
Myoglobin ($\mu g \cdot L^{-1}$)	206 ± 75	174 ± 58	164 ± 52	163 ± 55	213 ± 70	207 ± 76	183 ± 57	175 ± 84
CKMB † ($\mu g \cdot L^{-1}$)	1.39 (1.11–2.68)	1.37 (1.26–1.82)	1.33 (1.20–2.07)	1.39 (1.24–1.65)	1.67 (1.36–2.80)	1.49 (1.08–2.94)	1.69 (1.44–2.42)	1.53 (1.16–1.96)
Myoglobin / h-FABP ratio ($\mu g \cdot L^{-1}$)	9.9 ± 2.8	9.7 ± 2.8	9.9 ± 3.0	10.9 ± 4.2	10.2 ± 2.6	10.6 ± 2.9	10.3 ± 2.4	11.0 ± 3.2

h-FABP, heart-type fatty acid-binding protein; CKMB, Creatine kinase MB.
Data is presented as mean ± SD, (n = 12)
† Data presented as median (interquartile range).

Myoglobin

No main effect of trial was observed ($F_{(1,11)}$ = 3.39, P = 0.093; Figure 5.2b). A main effect of time was seen ($F_{(3,33)}$ = 19.4, P < 0.001), *post hoc* analysis found significant differences between 60 min (pre-ex) and all subsequent times ($P \leq 0.004$, ES = 0.32 (100 min) to ES = 0.71 (240 min)) and between 100 min (post-ex) with 240 min (end); P = 0.021, ES = 0.39. There were no significant time*trial interactions ($F_{(3,33)}$ = 1.37, P = 0.268).

Myoglobin to h-FABP ratio

There were no main effects of trial ($F_{(1,11)}$ = 0.89, P = 0.365), time ($F_{(3,33)}$ = 2.65, P = 0.065) or time*trial interaction ($F_{(3,33)}$ = 0.23, P = 0.877); Table 5.3.

Creatine kinase MB

Ten values were below the limits of detection of the assay. Values below 0.40 $\mu g \cdot L^{-1}$ are reported as 0.40 $\mu g \cdot L^{-1}$ (Figure 5.3a). There were no significant effects of trial ($F_{(1,11)}$ = 1.69, P = 0.220) or interaction ($F_{(3,33)}$ = 0.18, P = 0.909). There was a trend for a main effect of time ($F_{(3,33)}$ = 3.42, P = 0.057). ANOVA run on data excluding values below the limits of detection found a significant effect of trial ($F_{(1,8)}$ = 5.47, P = 0.048) suggesting that CKMB was lower on the exercise trial compared with the control trial. No significant effects of time ($F_{(3,24)}$ = 3.14, P = 0.089) or time*trial interaction ($F_{(2,24)}$ = 0.138, P = 0.936) were found.

Cardiac troponin I

The majority of values were below the limits of detection of the assay (70 of 96; Figure 5.3b). Values below 0.18 $\mu g \cdot L^{-1}$ are presented as 0.18 $\mu g \cdot L^{-1}$. No trends were observed. ANOVA did not find any main or interaction effects (P > 0.40).

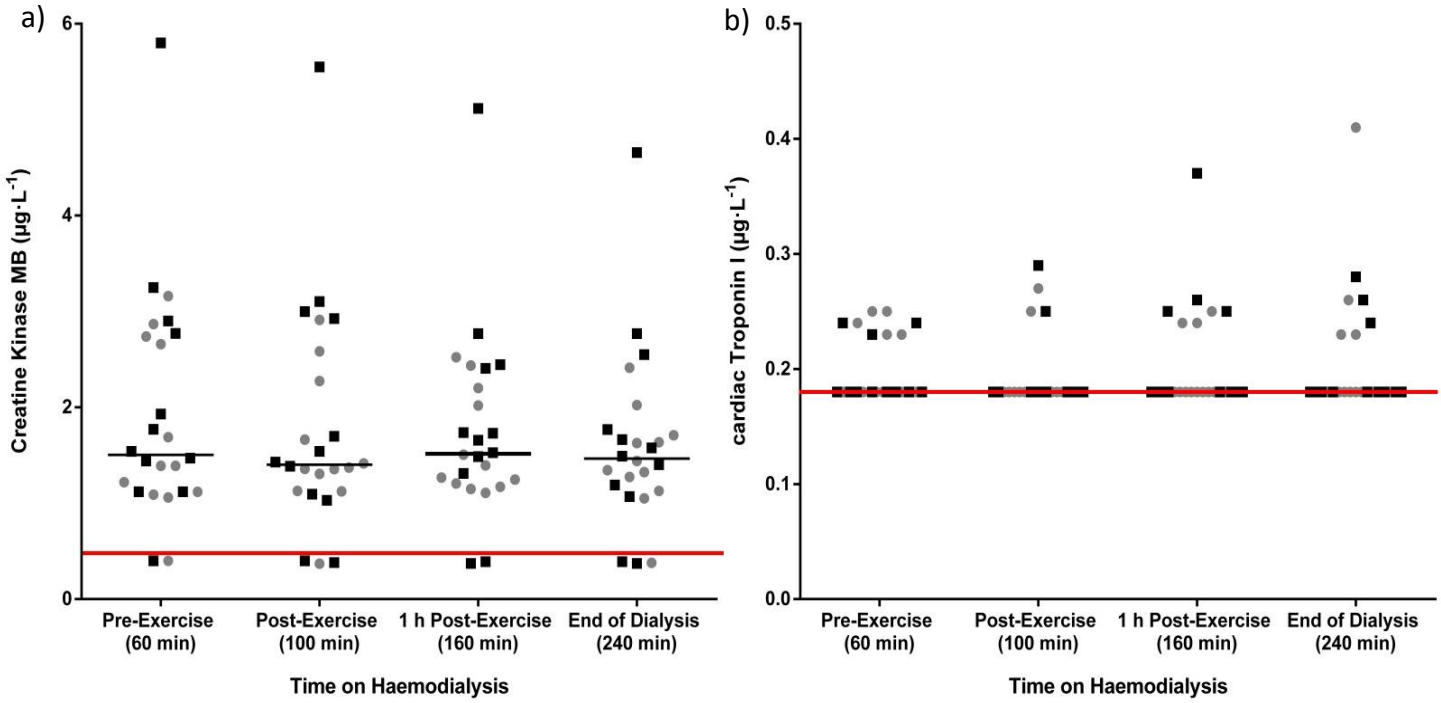

Figure 5.3 – **a)** Creatine kinase MB, and **b)** cardiac troponin I, at the different times across both trials. Grey circles (●) represent patients on the exercise trial, black squares (■) represent patients on the control trial. Black lines denote the median for all samples at that time. The red lines denote the limits of detection of the assays.

5.5 Discussion

Exercise at an intensity that patients found achievable during HD was sufficient to cause transient alterations in SBP. Across both trials SBP, and as a consequence MAP and PP, fell during the HD treatment. The initiation of HD had a profound effect, SBP fell a substantial amount (15.4%) from before starting HD to the measurement 60 min into HD (pre-exercise).

SBP during HD was different between the exercise trial and the control trial. Exercise appeared to cause an increase in blood pressure and was elevated post-exercise compared with the resting trial. This was then followed by a post-exercise hypotension observed 1 h post-exercise and a recovery back to normal SBP at the end of HD.

Unsurprisingly, the heart rate was elevated immediately after exercise compared with the same time on the control trial. The RPP, a function of heart rate and SBP, was therefore elevated post-exercise but then appeared to fall later and was lower at the end of HD.

Haemodialysis and post-exercise hypotension

The increased SBP post-exercise in this study supports other studies that have observed blood pressure during an intradialytic exercise bout (Banerjee, Kong and Farrington, 2004; Barnea *et al*., 1980; Burke *et al*., 1984; Moore *et al*., 1998). Exercise during HD for as little as 10 min increased SBP and reduced relative blood volume; the change in blood volume is a normal response to exercise as fluid moves from the vascular space to the interstitium of the working muscles (Banerjee, Kong and Farrington, 2004). Furthermore, Krause and colleagues found the increase in SBP in response to exercise was reduced after 5 months of regular exercise (Krause et al., 1990). The overwhelming conclusion from these studies is that exercise during HD is met with a stable haemodynamic response and that the cardiovascular response to exercise is superimposed onto the response to the HD process (Moore *et al*., 1998). One caveat is that when ultrafiltration volumes are high, exercise may be contraindicated after 3 h of HD treatment due to a gradual decrease in MAP and cardiac output, suggesting that exercise should be completed during the early stages of HD to avoid these complications (Moore *et al*.,

1998). Unfortunately most of these studies did not continue their measurements of haemodynamic response after the completion of exercise. The one study to continue to report SBP after exercise found a return to pre-exercise levels within 4 min (Burke *et al*., 1984). These results conflict with the post-exercise hypotension observed in our study. This may be explained by the small cohort, lack of a control trial and short duration of the exercise, only 5 min in duration, although during the exercise significant increases in HR, SBP and DBP were reported.

The fall in blood pressure post-exercise observed here appears to support the idea that a normal exercise response is superimposed onto the response to HD. Extensive reviews of 'post-exercise hypotension' have shown that SBP is frequently decreased below pre-exercise levels in the hours following a bout of exercise. The response in healthy individuals is sometimes equivocal but in hypertensive populations it is marked (MacDonald, 2002). The majority of advanced CKD patients have essential or secondary hypertension (> 95% of stage 4 and 5 CKD patients; Rao *et al*., 2008), as is the case for most patients in this study, and therefore it is logical that the post-exercise response in these patients is similar to that seen in hypertensive individuals. The divergent observations in Burke and colleagues study (1984) may also be explained as no participants were on anti-hypertensive medication and therefore may not have been hypertensive; post-exercise hypotension in normotensive individuals is less evident (Kenney and Seals, 1993).

In line with our findings, walking exercise at an intensity of 50-60% $\dot{V}O_{2peak}$ in earlier stage CKD patients (stage 2-4) was associated with an increase in SBP immediately post-exercise. Further, during the hour in the laboratory following exercise SBP and DBP were significantly lower than on the control trial (6.5 mmHg and 2.5 mmHg). However, 24-h ambulatory blood pressure monitoring found no significant differences between trials over the course of a free-living day (Headley *et al*., 2008). Therefore, patients with less severe CKD than in the present study have a similar haemodynamic response to exercise.

The decrease in SBP witnessed in this study (11 mmHg lower than at the same time on the control trial) is more than that observed in pre-dialysis patients in the above study, but less than some results reported in non-CKD hypertensive studies (15 mmHg; Pescatello *et al*., 2004). This may be due to the HD procedure itself causing a decrease in

SBP in both exercise and control trials thereby reducing the potential effect size. Furthermore, it has been suggested that the greatest declines in blood pressure are seen in individuals with the highest baseline levels (Pescatello and Kulikowich, 2001); the patients in this study had higher baseline SBP than in the pre-dialysis population in the earlier study, but are well controlled and lower than many non-CKD studies.

In studies attempting to delineate the mechanisms for post-exercise hypotension patients have previously been asked to cease anti-hypertensive medications or were excluded. Patients continued with their regular medication in this study and so data may better reflect a real-world scenario. In the present study blood pressure had returned to normal values at the end of HD (just over 2 h post-exercise), it may be that longer duration or greater intensity exercise may increase the duration of this response (MacDonald, MacDougall and Hogben, 2000); the impact of anti-hypertensive medications in this group may also influence the duration of post-exercise hypotension. Future studies could monitor SBP during free-living conditions post-HD to see whether this post-exercise hypotension persists for the following day under normal living conditions, as seen in hypertensive individuals in the absence of CKD (Pescatello *et al*., 1991; Rondon *et al*., 2002; Wallace *et al*., 1999).

The mechanisms underpinning post-exercise hypotension are still somewhat undefined. Aside from one early study (Hagberg, Montain and Martin, 1987) the majority of the literature suggests post-exercise hypotension is due to reduced total peripheral resistance (Pescatello *et al*., 2004). Coats and colleagues present strong evidence for this; they found persistent vasodilation in arm limbs that were not directly involved in the exercise in the hour after leg exercise suggesting a centrally acting mechanism that reduced peripheral resistance was the cause (Coats *et al*., 1989). In the present study there was no evidence of tachycardia or bradycardia suggesting that heart-rate was not influential in the genesis of the drop in blood pressure, consistent with findings in pre-dialysis patients (Headley *et al.,* 2008).

Three of the fifteen patients in the present study did not exhibit substantial post-exercise hypotension, in contrast to the other patients who showed profound alterations in SBP. These ‘non-responders’ to post-exercise alterations in haemodynamics have also been noted in pre-dialysis patients (Headley *et al*., 2008) and in otherwise healthy individuals

(Pescatello *et al*., 2004). Of note, two patients in the present study had a clear increase in SBP during HD on the control trial, a phenomenon known as 'intradialytic hypertension'[3] that affects a minority of ESRD patients. Both of these patients were non-responsive to post-exercise alterations in SBP. The mechanisms responsible for intradialytic hypertension and post-exercise hypotension centre on total peripheral resistance; from our data it appears likely that patients are not susceptible to both conditions as the underlying cause of one may inhibit the other.

Intradialytic exercise and risk of subclinical myocardial injury

It appears from the literature that maintenance of blood pressure during HD is crucial and significant intradialytic hypotension greater than normal is detrimental to health. Exercise may place an extra demand upon the heart (as seen by the RPP) at a time when it appears to be at an increased susceptibility to demand ischaemia that may increase the risk of myocardial stunning and permanent left ventricular dysfunction. The post-exercise hypotension witnessed would appear to suggest increased risk of cardiac dysfunction. In this regard, further analysis was performed to assess markers of cardiac injury.

Early markers of cardiac injury h-FABP and myoglobin showed no difference between trials, nor did later markers of cardiac injury CKMB and cTnI. These markers have shown to be useful in detecting cardiac injury in the immediate time following a potential event (McCann *et al*., 2008; McMahon *et al*., 2012). In ESRD patients some concern has been raised at using circulating markers to detect a myocardial event from a single sample because normal values are abnormally high due to reduced renal clearance or repeated subclinical myocardial events (Górski *et al*., 1997); but with numerous samples collected in this study a change should have been detectable. Reports from healthy participants in other studies suggest that the h-FABP and myoglobin results reported here are elevated (Ghani *et al*., 2000; McMahon *et al*., 2012; Wunderlich *et al*., 2005); this has been described in renal failure patients (Górski *et al*., 1997). The significant decreases seen in

[3] Intradialytic hypertension is a condition where blood pressure persistently rises during HD, this is counter-intuitive as blood volume is reduced (Levin, 1993). The phenomenon is thought to effect around 10% of patients and the mechanisms that cause it are somewhat unclear. These patients may be fluid overloaded or have inappropriate levels of endothelin or nitric oxide / endothelin balance that cause exaggerated vasoconstriction and increased blood pressure (Chou *et al*., 2006).

myoglobin and h-FABP across both trials suggest a clearance from circulation by HD and follows similar results previously reported (Furuhashi *et al*., 2004). Importantly, there is no observable trend for increases in any of the markers of cardiac injury and exercise appears to have no effect.

The only cTnI value of concern for a single patient occurs at the end of HD on the exercise trial (see figure 5.5). It is possible that this result may represent sub-clinical myocardial damage; others (in non-CKD patients) have used 0.37 $\mu g \cdot L^{-1}$ as the cut-off for detection of cardiac injury (McMahon *et al*., 2012). Interestingly, this patient had the highest myoglobin and CKMB values at pre-exercise compared with the other patients suggesting that any possible cardiac injury may have occurred before exercise took place.

In this study no clear evidence of cardiac injury was found and this suggests that exercise is not clearly exacerbating myocardial injury and should be considered safe. However, it is important to note that the final blood sample was taken approximately 2.5 h post-exercise and at this time after a cardiac event sensitivity is low. The best sensitivity to a cardiac event 0-3 h after an event was found by considering both cTnI and h-FABP and produced a sensitivity of 71.4% and specificity of 81.2% (McMahon *et al*., 2012). Greater sensitivity and specificity are found 12 h or later after an event. It is not possible with the present protocol to completely rule out exercise induced cardiac injury and a specifically designed protocol to determine the effect of intradialytic exercise on the myocardium is required.

Overall, despite the negative associations of intradialytic hypotension, at present there is not sufficient evidence, from this or other studies, to suggest exercise causes any subclinical cardiac injury. Elsewhere, not a single serious adverse event has been reported after around 28,000 h of intradialytic exercise (Smart and Steele, 2011). Moreover, ESRD patients who regularly exercise during HD improve heart rate variability, left ventricular ejection fraction and risk rating of sudden cardiac death (Kouidi, Grekas and Deligiannis, 2009; Kouidi *et al*., 2010; Reboredo *et al.,* 2010) and improve baroreflex sensitivity and effectiveness (Petraki *et al*., 2008). In non-CKD individuals, recent evidence suggests a single bout of exercise decreases the risk of myocardial stunning, size of subsequent infarctions, reduces arrhythmia and pre-conditions the heart to ischaemia-reperfusion injury (Frasier, Moore and Brown, 2011); whether these findings can be applied to HD

patients is unclear. Further research is required that delineates exactly what is happening to the heart during intradialytic exercise and what the long-term impact is of regular training.

Limitations

A limitation of this data is that blood pressure was not the primary outcome measure and therefore more frequent measurements were not taken. The sphygmomanometers used were automated and built into the HD machinery and were not validated by the research team, although they were used in clinical care. The randomised-crossover design allows direct comparisons to be made between trials. Early research in post-exercise hypotension did not account for circadian rhythm or ensure adequate rest prior to the pre-exercise blood pressure reading (Kaufman, Hughson and Schaman, 1987); the present design reduced the influence of those potential confounders. However, further investigations examining post-exercise hypotension in HD patients are required, especially to see if a single bout of exercise may have prolonged effects on blood pressure outside of HD time as this may have implications for timing and prescription of anti-hypertensive medications and whether different exercise types have different effects.

5.6 Conclusions

Post-intradialytic-exercise hypotension likely represents a normal response to exercise that occurs in the majority of individuals with high blood pressure and is possibly related to a reduction in total peripheral resistance. Post-exercise hypotension appears to be superimposed onto the normal decrease in blood pressure observed during HD. However, the impact exercise has on the myocardial health of CKD patients that undergo HD is clearly an extremely complicated issue that requires a more detailed investigation.

On the one hand exercise during HD could be argued as exacerbating the oxygen demand of the myocardium at a time when it is most susceptible to demand ischaemia therefore increasing the risk of myocardial stunning and permanent deterioration of left ventricular function, a process that could be made worse by the post-exercise hypotension witnessed in this study. However, this study found no evidence of cardiac injury in the 2.5 h after exercise.

On the other hand, there is a large amount of evidence in healthy and other disease populations showing that regular exercise training has a multitude of beneficial adaptations for the heart and cardiovascular system. Exercise decreases the risk of myocardial stunning, reduces the size of infarctions and appears to pre-condition the heart to ischaemia-reperfusion injury. Research has also shown that patients who regularly exercise during HD improve many aspects of various cardiac parameters. The data reported here highlights the importance of further research in the area.

Chapter 6

The Effect of a 6-month Intradialytic Exercise Programme on Circulating Markers and Leucocyte Phenotypes associated with Inflammation

6.1 Abstract

HD patients have a dysfunctional and chronically activated immune system that manifests itself with elevated levels of circulating markers of inflammation, altered leucocyte populations and phenotypes, and anergy to antigen challenge. Evidence from observational studies and other populations suggest that regular exercise may have beneficial anti-inflammatory effects, but this is unclear in HD patients. This study aimed to assess the effect of 6 months of regular intradialytic exercise on circulating markers of inflammation, pro-inflammatory and anti-inflammatory leucocyte phenotypes and aspects of quality of life.

Patients were recruited from 2 HD units, 22 from a unit where exercise was offered during HD and 16 from a non-exercising control HD unit. Exercising patients were encouraged to exercise during HD up to 3x per week and to aim for 30 min of cycling at an RPE of "somewhat hard". Blood samples were taken prior to HD at baseline and after 3 and 6 months of regular exercise. Plasma CRP, IL-6 and TNF-α were measured via ELISA, the numbers and proportions of monocyte phenotypes and Tregs were measured via flow cytometry. Physical function was assessed at baseline and after 6 months using the STS 60 test. Aspects of quality of life were assessed at baseline, and after 3 and 6 months using the DASI, LUSS and HADS questionnaires.

Sixteen exercise (57.0 ± 10.5 y) and 15 control (70.2 ± 13.7 y) patients completed the 6-month study period. 6 months of regular intradialytic exercise significantly improved STS 60 score in the exercise group (13 ± 10 to 24 ± 6 reps; $P < 0.001$) with no change in the control group. Training had no significant effect on circulating CRP, IL-6 or TNF-α ($P \geq 0.411$). The exercise group had a significant decline in the proportion of monocytes that were the pro-inflammatory intermediate phenotype ($CD14^{++}CD16^{+}$) compared with the usual care group (7.72 ± 1.70% to 6.61 ± 1.74% vs. 6.90 ± 1.56% to 7.82 ± 1.81%; $P = 0.020$). Further, the number of the anti-inflammatory Tregs was enhanced after 6 months in the exercise group compared with usual care (28.1 ± 17.1 to 36.2 ± 17.3 cells·μL^{-1} vs. 28.7 ± 13.2 to 22.4 ± 10.7 cells·μL^{-1}; interaction: $P = 0.009$); however, this may be explained by a greater number of total $CD4^{+}$ lymphocytes compared with control ($P = 0.001$).

Exercise was associated with an improvement in perceived functional capacity (DASI: + 4 (-2 – +7) vs. – 5 (-8 – 0); $P = 0.004$) and anxiety score (-2 ± 2 vs. +1 ± 2; $P = 0.007$). No adverse or abnormal responses to regular exercise were observed.

These findings suggest that regular exercise has an anti-inflammatory effect at a circulating cellular level and therefore may be protective against the increased risk of CVD and mortality that is associated with chronic inflammation and elevated numbers of intermediate monocytes.

Exercise was also shown to enhance physical function and patient reported outcomes such as anxiety and perceived functional capacity. These improvements can have a profound positive impact on the patient's quality of life.

6.2 Background

HD patients have a dysfunctional and chronically activated immune system that manifests itself with elevated circulating markers of inflammation, altered leucocyte populations and phenotypes, and anergy to antigen challenge (Chapter 3). Chronic systemic inflammation is highly predictive of mortality and CVD in this population and represents an area of therapeutic potential (Kalantar-Zadeh *et al.,* 2003; Stenvinkel, 2001).

HD patients are generally very sedentary and inactivity is exacerbated on days when they receive HD treatment. Intradialytic exercise can be considered safe, has been shown to improve compliance and drop-out rates compared with extradialytic exercise and has a significant impact on physical parameters, aspects of quality of life and cardiovascular health (Heiwe and Jacobson, 2014; Kouidi *et al.*, 2004; Smart and Steele, 2011). In observational studies, an inflammatory environment may be associated with physical inactivity in ESRD patients (Anand *et al.,* 2011; Mafra *et al.,* 2011), or with decreased physical function (Chapter 3); findings that are common with healthy and other chronic disease populations (Beavers *et al.,* 2010).

In the previous study exercise during HD did not have significant acute effects on leucocytes or markers of inflammation (Chapter 4). However, the impact of regular exercise in HD patients on CRP and IL-6 appears mixed and remains unclear (Dungey *et al.,* 2013). Further, there appear to be no studies in ESRD patients that have analysed the effect of exercise training on pro-inflammatory or anti-inflammatory leucocyte phenotypes, factors that appear to be improved in physically active healthy cohorts (Gleeson *et al.,* 2011).

The aim of this study was to develop a pragmatic exercise programme and then assess the effects of 6 months of regular intradialytic exercise on circulating markers of inflammation, pro-inflammatory and anti-inflammatory leucocyte phenotypes and aspects of quality of life.

6.3 Methods

Ethics statement

The protocols and study documents utilised throughout this training study were given full ethical approval by the NHS trust R&D office (ref. UHL 11045) and the NHS Research Ethical committee (ref. 11/EM/0149).

Recruitment

Patients were recruited from two separate HD units, one that offered an exercise rehabilitation programme as part of routine clinical care and one that did not. All patients who were deemed eligible (Table 2.1) to take part in the exercise programme were approached to take part in the research project (Figure 6.1); no patients had recently participated in an exercise programme.

To avoid a clear sampling bias and contamination of the non-exercising control patients, the controls were recruited from a separate HD unit within the UHL trust that did not run an exercise programme and an independent Consultant Nephrologist was asked to recommend patients who would be eligible to exercise if a programme was available and these patients were approached for recruitment. The patient characteristics of the two HD units were distinct (in line with locality demographics) and made accurate age- and ethnicity-matching impossible. The HD machinery, nursing practices and general treatment procedures were similar between the centres.

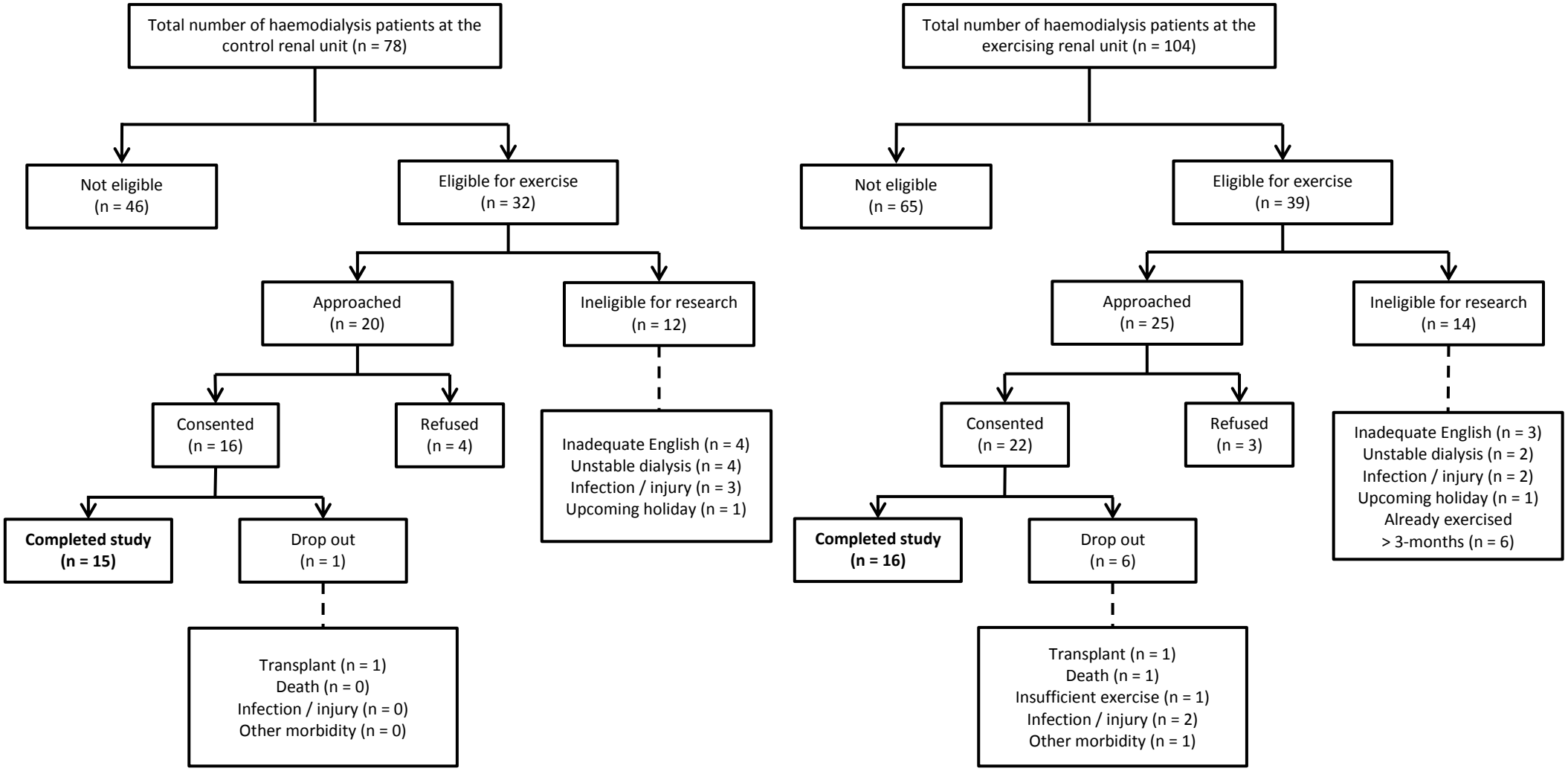

Figure 6.1 - Flow diagram for eligibility, recruitment and completion of participants in the exercise and control groups.

Protocol

The exercise group participated in an intradialytic exercise programme for 6 months; the control group continued their HD treatment as per their usual care. Blood samples were taken from the patients at baseline (month 0), at 3 months and at 6 months. Patients completed questionnaires during HD on the same days as the blood samples. The STS 60 physical function test was completed twice, at the beginning and end of the study, on a separate day as close as possible to the blood sample. Medical notes were accessed and kept updated on a monthly basis along with a record of all exercise sessions completed. The schematic below details a timeline of outcome measures (Figure 6.2).

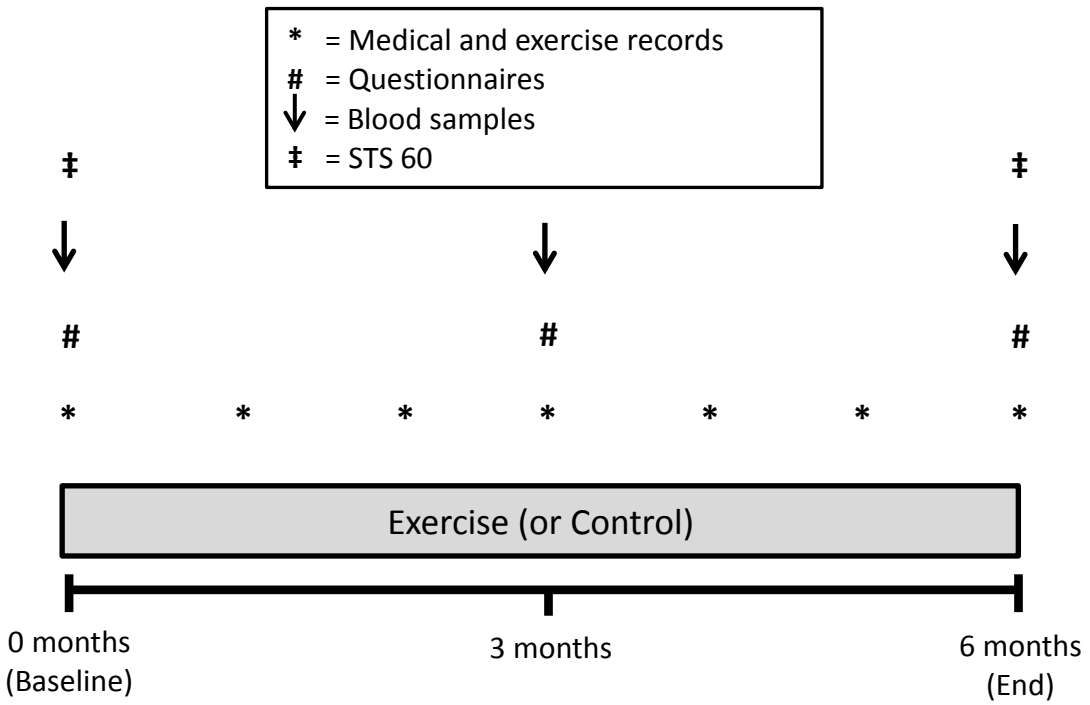

Figure 6.2 – Trial schematic.
STS 60, Sit-to-Stand 60 test**.**

Exercise

This study was designed with a pragmatic approach; the exercise was not at a set intensity or volume for the purpose of research. Rather, the aim of the study was to analyse the effectiveness of intradialytic exercise in general rather than a specific volume

of exercise. The range of abilities and morbidities in the patients meant that there was a range of exercise duration and power output; however, all patients were asked to cycle at a perceived exertion of "somewhat hard" (RPE: 12-14; Borg, 1973). For further details on the exercise programme the reader is asked to refer to Chapter 2.

Blood samples

A resting 30 mL sample was collected at the beginning of HD, prior to the patient being connected to the HD machine, this ensured samples were not affected by the HD process. Blood samples were drawn directly off the HD line using a dry syringe and immediately aliquot into heparin and K_3EDTA pre-treated monovettes.

Outcome measures

Plasma concentrations of CRP (intra-assay CV: 6.6%, inter-assay CV: 9.9%), IL-6 (10.7% and 10.5%) and TNF-alpha (8.2% and 8.5%) were analysed undiluted using ELISA as described earlier (Chapter 2). Heparinised whole blood was examined using flow cytometry for the presence of Tregs ($CD4^{+}CD25^{+}CD127^{low/-}$) and the distribution of monocyte phenotypes (classical: $CD14^{++}CD16^{-}$, intermediate: $CD14^{++}CD16^{+}$, non-classical: $CD14^{+}CD16^{++}$).

Aspects of quality of life were analysed using questionnaires. The HADS was used to assess anxiety and depression and give a total combined score. Perceived physical function was measured using the DASI questionnaire. The LUSS questionnaire was used to assess symptom burden, the frequency and intrusiveness of each symptom and a composite score; an overall score of all symptoms combined was used.

Omitted samples

One participant from the exercise group and 2 from the control group were omitted from analysis of inflammatory markers due to the immunosuppressant medication they were taking; all were previous recipients of a renal transplant. One exercising patient was

omitted from blood analysis as a blood sample was missed (patient choice) and an additional control patient omitted due to a blood transfusion. Four exercisers were not included in the analysis of monocyte phenotypes due to an error in staining samples and 1 control patient was excluded as an outlier due to being over 2 orders of SD from the mean.

In the quality of life outcomes 2 exercisers were removed from analysis (1 due to diagnosed memory loss and 1 due to a planned transplant closely following the final study date that noticeably affected quality of life scores – particularly anxiety).

Participant information

The baseline characteristics of the exercise and control groups are presented in Table 6.1. Sixteen exercisers and 15 control participants completed 6 months of the study. There was a significantly different distribution of age (ES = 1.08) and ethnicity between the exercise and control groups, this was due to the differing demographics of the HD units where recruitment took place. There were no differences between the groups in the distribution of gender, HD access, primary renal disease, HD vintage, or the number of co-morbidities and medications.

Table 6.1 – Participant characteristics

	Exercise group (n = 16)	**Control group (n = 15)**	***P* value**
Age (y)	57.0 ± 10.5	70.2 ± 13.7	0.005*
Gender ‡			
Male	8 (50%)	10 (67%)	0.347
Ethnicity ‡			0.018*
White British	9 (56%)	14 (93%)	
Indian	7 (44%)	1 (7%)	
Haemodialysis vintage (y) †	1.53 (1.04-2.90)	2.02 (1.43-5.16)	0.252
Access ‡			0.570
AVF	14 (88%)	12 (80%)	
Catheter	2 (13%)	3 (20%)	
Aetiology ‡#			0.347
Glomerulonephritis	4 (25%)	2 (13%)	
Cystic / Poly	3 (19%)	2 (13%)	
Renal vascular disease	0	3 (20%)	
Pyelonephritis	1 (6%)	4 (27%)	
Interstitial	1 (6%)	0	
Diabetes	1 (6%)	1 (7%)	
Uncertain	3 (19%)	2 (13%)	
Other	3 (19%)	1 (7%)	
Number of co-morbidities	4 ± 2	4 ± 2	0.573
Number of medications	10 ± 4	11 ± 3	0.203
Immunosuppressive therapy ‡[a]	1 (6%)	2 (13%)	

AVF, Arteriovenous fistula.

Data presented as mean ± SD, † Data presented as median (interquartile range),

‡ Data presented as n (%).

* Significant differences between groups.

based on UK Renal Registry classification (UK Renal Registry, 2010).

[a] excluded from analysis of markers of inflammation.

Co-morbidities

All patients had one or more significant non-renal co-morbidity reported in their medical history. Both groups of patients had a large proportion diagnosed with hypertension

(exercise group: 88%, control group: 87%). Other frequent morbidities included dyslipidaemia (13% and 27%), type II diabetes mellitus (19% and 40%) and diagnosed incidences of ischaemic heart disease, transient ischaemic attack, atrial fibrillation or left ventricular hypertrophy (38% and 33%).

Medications

Prescribed medications were not dissimilar between the groups; the same clinicians were involved in the care of both groups. As would be expected, all patients were prescribed an anticoagulant for HD. The majority of patients had intravenous erythropoietin therapy (88% and 87%), a form of anti-hypertensive (75% and 53%) and vitamin D supplementation (88% and 87%). Similar to the previous studies, other common medications included phosphate binders, analgesics, proton-pump inhibitors and statins.

Statistical analysis

Baseline comparisons between groups were completed using independent t-tests or the non-parametric Mann-Whitney test where applicable. Chi square was used to assess differences in gender, ethnicity and aetiology between groups. Two-factor mixed-measures ANOVA was used to analyse data: group (exercise vs. control) x time (baseline vs. 3 months vs. 6 months). Where a significant time*group interaction was found *post hoc* analysis explored the differences using one-way repeated measures ANOVA for each group, independent t-tests between groups at each time point and the between group differences in change in the outcome measure at 6 months from baseline (i.e. 6 months – baseline).

6.4 Results

Exercise

The exercise group participated in 38 ± 12 exercise sessions over 6 months. The average exercise session was 34.7 (29.6-41.7) min at 63 ± 8 rpm and was perceived to be "somewhat hard" (RPE: 12 (11-13); Borg, 1973). The average power output given by the ergometer was 15.6 ± 7.0 W, and the estimated distance per session was 12.5 (10.0-13.3) km and energy expenditure of 69.2 (53.5-90.0) kcal.

Sit-to-stand 60 test

At baseline, STS 60 scores were not dissimilar between groups (P = 0.880). ANOVA revealed a time*group interaction ($F_{(1,21)}$ = 28.091, P < 0.001; Figure 6.3). *Post hoc* analysis revealed a significant increase in the number of STS 60 reps completed in the exercise group (P < 0.001, ES = 1.47) compared with no change in the control group (P = 0.208). Further, the change in the exercise group was significantly different from the change in the control group (P < 0.001, ES = 1.65).

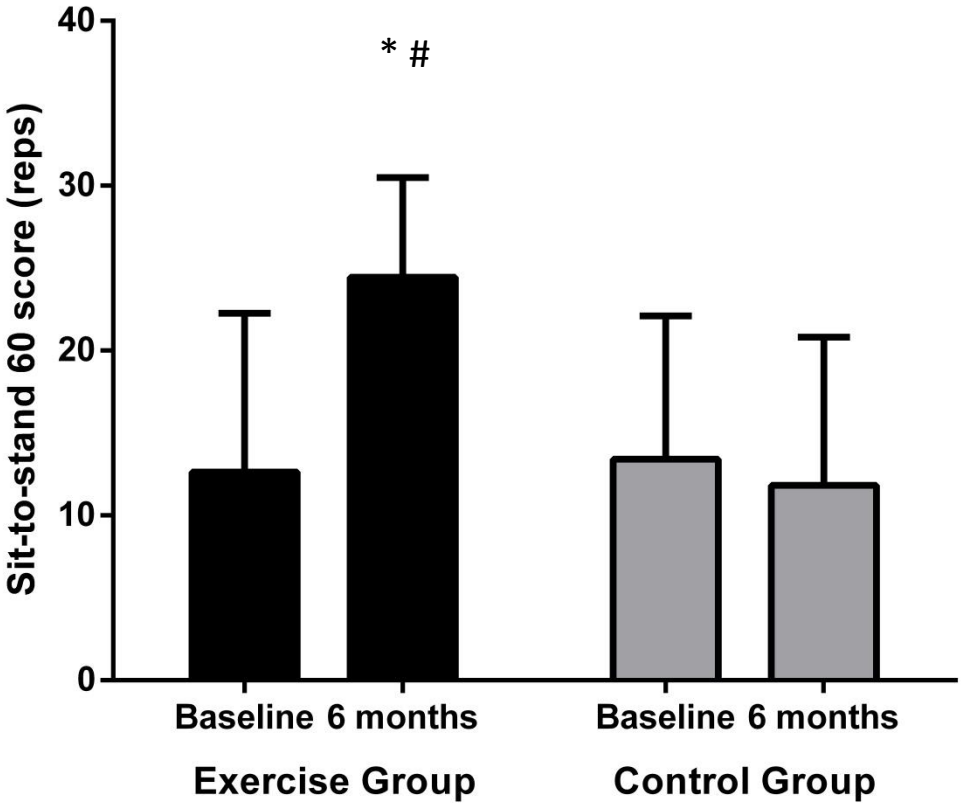

Figure 6.3 - Sit-to-stand 60 score at baseline and 6 months in the exercise and control group.
Significant time*group interaction ($P < 0.001$),
* Significant difference from baseline ($P < 0.001$),
# Significant difference from the control group ($P = 0.001$).
Data is presented as mean ± standard deviation. Exercise group n = 11, Control group n = 12.

Body composition

There were no statistically significant differences in post-HD weight (P = 0.227) or BMI (P = 0.292) at baseline. The exercise intervention did not affect either parameter (P > 0.05; Table 6.2).

Blood pressure

At baseline SBP was not different between groups (P = 0.680) but DBP was significantly higher in the control group (P = 0.010, ES = 0.99). The exercise intervention had no effect on either outcome (P > 0.05; Table 6.2).

Biochemistry

The exercise intervention had no effect on the measures of biochemistry extracted from the medical records. There were no changes in blood phosphate, creatinine, bicarbonate, albumin, potassium, sodium or urea (P > 0.05, data not shown).

Medications

During the 6 months within the exercise group there were a total of 14 new prescribed medications, 5 increases and 4 decreases in prescription, and 17 prescriptions were stopped. In the control cohort there were a total of 13 new prescriptions, 5 increases and 4 decreases in prescription and 9 medications were stopped. These results are purely descriptive; there were no statistical differences between groups ($P \geq 0.611$) and no patterns for changes in any particular type of medication (i.e. anti-hypertensives).

Table 6.2 - Body composition and blood pressure at baseline and after 3 months and 6 months in the exercise and control groups

	Exercise Group (n = 16)			**Control Group (n = 15)**		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Body Composition						
Post HD weight (kg)	71.0 ± 24.9	71.8 ± 24.9	72.2 ± 24.9	81.2 ± 20.6	81.8 ± 20.5	81.8 ± 20.2
BMI ($kg \cdot m^{-2}$)	25.9 ± 6.7	26.2 ± 6.7	26.4 ± 6.7	28.4 ± 6.1	28.6 ± 5.9	28.6 ± 5.6
Blood Pressure						
SBP (mmHg)	135 ± 24	137 ± 25	130 ± 18	138 ± 19	136 ± 18	138 ± 23
DBP (mmHg)	65 ± 13*	66 ± 15*	67 ± 18	78 ± 14	76 ± 11	74 ± 13

BMI, body mass index; DBP, diastolic blood pressure; HD, haemodialysis; SBP, systolic blood pressure.

* Significantly different from control group ($P < 0.05$).

Data is presented as mean ± SD

Circulating markers of inflammation

At baseline there were no significant differences in IL-6 (P = 0.909), TNF-α (P = 0.560), or CRP (P = 0.212) between the exercise and the control group (Table 6.3).

For CRP, ANOVA revealed a significant main effect of group ($F_{(1,24)}$ = 6.435, P = 0.018; Table 6.3). Circulating concentrations of CRP were greater in the control group over the 6-month period compared with the exercise group. However, the exercise intervention had no significant effect as no time*group interaction was found ($F_{(2,48)}$ = 0.906, P = 0.411), nor was there an effect of time ($F_{(2,48)}$ = 1.432, P = 0.249).

For IL-6, there were no main effects of time ($F_{(2,46)}$ = 1.223, P = 0.304; Table 6.3) or group ($F_{(1,23)}$ = 0.093, P = 0.763). There was no time*group interaction ($F_{(2,46)}$ = 0.390, P = 0.679); circulating concentrations of IL-6 did not change in either group.

Similarly for TNF-α, ANOVA found no main effect of time ($F_{(2,48)}$ = 0.418, P = 0.661; Table 6.3) or group ($F_{(1,24)}$ = 1.162, P = 0.292) in circulating concentrations of TNF-α and there was no time*group interaction ($F_{(2,48)}$ = 0.027, P = 0.973).

Table 6.3 - Circulating markers of inflammation at baseline and after 3 months and 6 months in the exercise and control groups

	Exercise Group (n = 14)			**Control Group (n = 12)**		
	Baseline	3 months	6 months	Baseline	3 months	6 months
CRP ($mg \cdot L^{-1}$)	4.75 ± 3.38	3.63 ± 2.36	4.69 ± 3.42	6.93 ± 3.73	7.19 ± 3.48	7.96 ± 4.00
IL-6 [n1] ($pg \cdot mL^{-1}$)	5.34 ± 3.09	5.67 ± 2.85	5.90 ± 3.44	5.21 ± 2.71	5.71 ± 3.81	7.01 ± 5.08
TNF-α [Lg] ($pg \cdot mL^{-1}$)	3.39 ± 1.17	3.50 ± 0.92	3.39 ± 1.16	4.55 ± 3.27	4.27 ± 1.95	4.21 ± 2.20

CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumour necrosis factor – alpha.
Data presented as mean ± SD.
[Lg] Statistical analysis performed on log-transformed data.
[n1] n = 13 for exercise group.

Haematology

At baseline there were no significant differences in the haematocrit (P = 0.962), haemoglobin (P = 0.766) or red blood cells (P = 0.280) between groups. Total blood leucocytes (P = 0.253), neutrophils (P = 0.247) and lymphocytes (P = 0.255) were not significantly different at baseline. Monocyte counts were higher in the control group at baseline compared with the exercise group (P = 0.006, ES = 0.92). The exercise intervention had no significant effects on haematology or circulating leucocyte counts (P > 0.05; Table 6.4).

Table 6.4 – Haematology at baseline and after 3 months and 6 months in the exercise and control groups

	Exercise Group (n = 14)			**Control Group (n = 12)**		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Haematocrit [n1] **(%)**	36.3 ± 3.8	36.8 ± 4.4	36.6 ± 4.5	36.4 ± 3.9	34.0 ± 2.6	34.9 ± 2.4
Haemoglobin [n1] **($g \cdot dL^{-1}$)**	11.8 ± 1.1	12.1 ± 1.4	12.1 ± 1.5	11.7 ± 1.3	11.0 ± 0.8	11.4 ± 0.8
Red blood cells [n1] **($x10^{12} \cdot L^{-1}$)**	3.75 ± 0.56	3.73 ± 0.5	3.76 ± 0.51	3.60 ± 0.29	3.42 ± 0.32	3.43 ± 0.37
Leucocytes ($x10^{9} \cdot L^{-1}$)	7.04 ± 2.34	6.71 ± 1.95	6.59 ± 1.82	7.41 ± 2.20	6.83 ± 2.06	7.40 ± 2.48
Neutrophils ($x10^{9} \cdot L^{-1}$)	4.31 ± 1.98	3.80 ± 1.54	3.56 ± 1.57	4.58 ± 1.97	3.99 ± 1.51	4.60 ± 2.15
Lymphocytes [Lg] **($x10^{9} \cdot L^{-1}$)**	1.70 ± 0.60	1.81 ± 0.63	2.02 ± 0.86	1.77 ± 0.65	1.67 ± 0.52	1.66 ± 0.54
Monocytes [Lg] **($x10^{9} \cdot L^{-1}$)**	0.61 ± 0.12*	0.59 ± 0.12*	0.60 ± 0.19*	0.73 ± 0.14	0.73 ± 0.21	0.73 ± 0.24

[Lg] Statistical analysis performed on log transformed data.
[n1] Exercise group n = 16, Control group n = 15.
Data is presented as mean ± SD.
* Significantly different from control group (P < 0.05).

Monocytes

Classical monocytes

At baseline there were no significant differences in the percentage of classical monocytes (P = 0.946) or the number of classical monocytes (P = 0.114) between the exercise and control groups. The exercise intervention did not have a significant effect on either the percentage ($F_{(2,38)}$ = 0.500, P = 0.611; Figure 6.5a) or the number of classical monocytes ($F_{(2,38)}$ = 0.284, P = 0.754; Table 6.5).

Intermediate monocytes

No differences were seen at baseline in the percentage (P = 0.267) or number (P = 0.905) of intermediate monocytes. A time*group interaction was observed in the percentage of intermediate monocytes ($F_{(2,38)}$ = 4.815, P = 0.014; Figure 6.4). *Post hoc* analysis revealed a significant difference in the change from baseline to 6 months between the two groups (P = 0.020, ES = 1.14), suggesting a decrease in the exercise group compared with an increase in the control group. No significant interaction was found in the number of intermediate monocytes ($F_{(2,38)}$ = 2.262, P = 0.118; Table 6.5).

Non-classical monocytes

No differences were found at baseline in the percentage or the number of non-classical monocytes (P = 0.642 and P = 0.271). Furthermore, no significant time*group interactions were found suggesting the exercise intervention had no effect on the percentage ($F_{(2,38)}$ = 0.921, P = 0.407; Figure 6.5b), or the number ($F_{(2,38)}$ = 0.203, P = 0.817; Table 6.5) of non-classical monocytes.

Table 6.5 - Number of each monocyte phenotype at baseline and after 3 months and 6 months in the exercise and control groups

	Exercise Group (n = 10)			Control Group (n = 11)		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Classical monocytes						
Count (cells·µL^{-1})	485 ± 108	461 ± 84	490 ± 155	556 ± 119	583 ± 182	585 ± 190
Intermediate monocytes [Lg]						
Count (cells·µL^{-1})	47.9 ± 14.5	40.8 ± 10.6	42.5 ± 24.9	48.7 ± 14.6	48.4 ± 16.5	55.9 ± 18.4
Non-classical monocytes [Lg]						
Count (cells·µL^{-1})	86.9 ± 36.6	78.1 ± 20.4	87.2 ± 52.9	104.5 ± 34.7	87.3 ± 28.5	95.6 ± 55.0

[Lg] Statistical analysis performed on log-transformed data.
Data presented as mean ± SD.

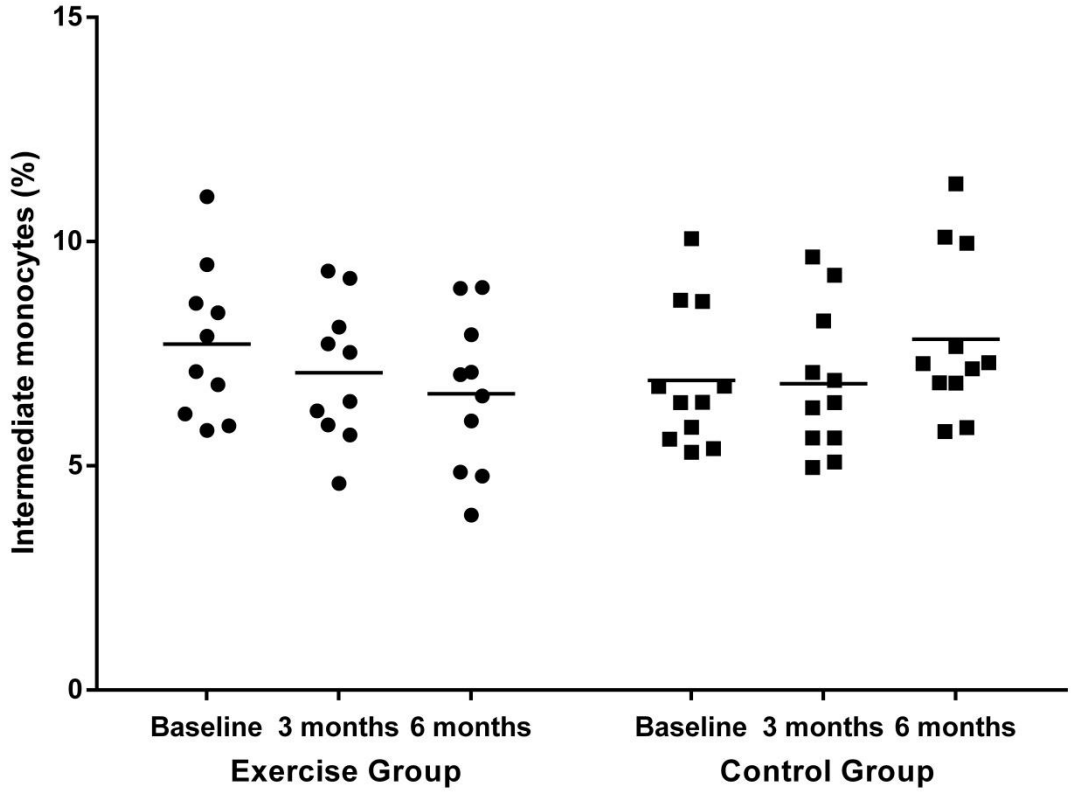

Figure 6.4 - Proportion of intermediate monocytes at baseline, 3 months and 6 months in the exercise and control groups.
Data is presented as individual values and the line represents the mean.
A significant time*group interaction: *P* = 0.014.
Exercise group n = 10, Control group n = 11.

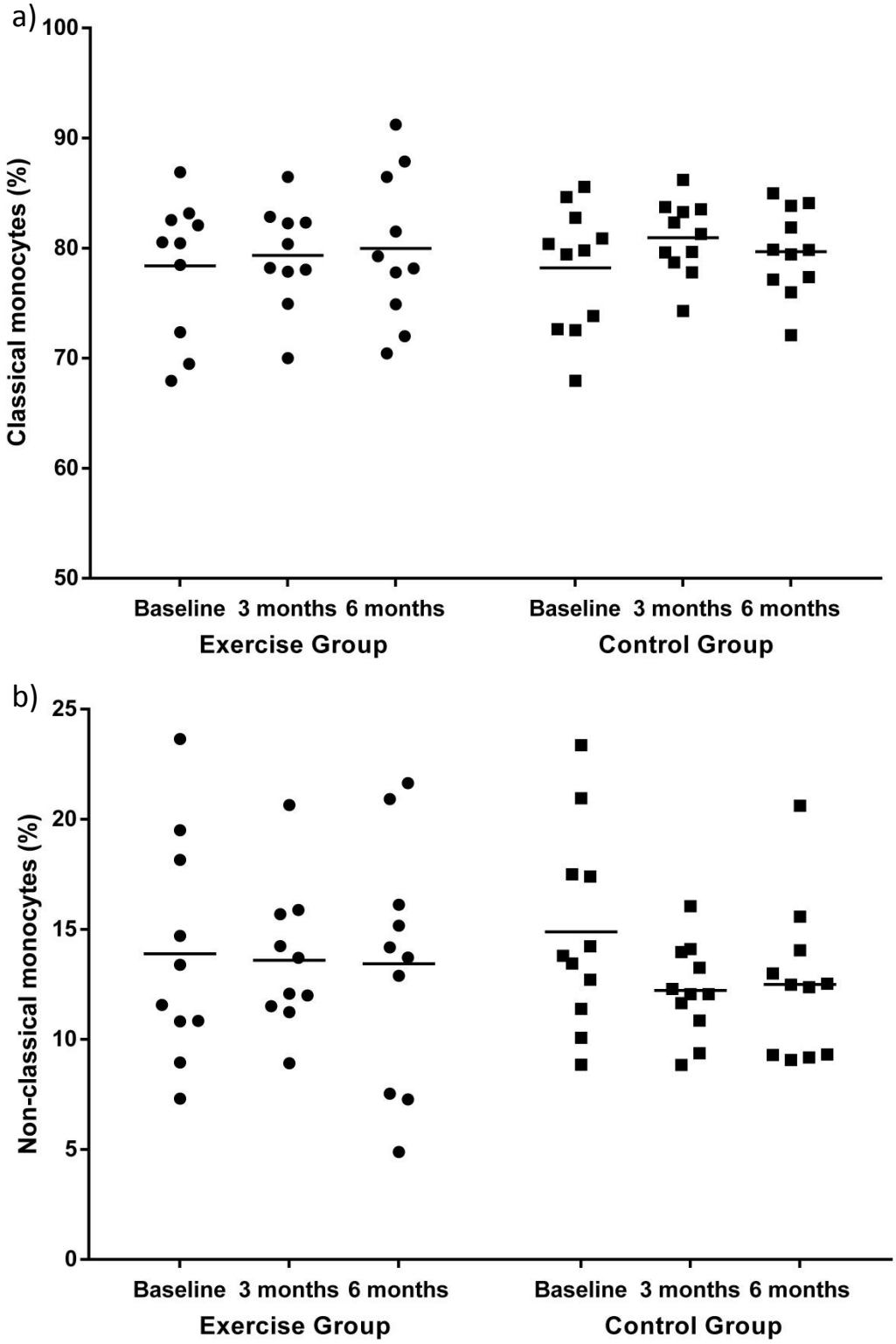

Figure 6.5 - Proportion of **a)** Classical and **b)** Non-classical monocytes at baseline, 3 months and 6 months in the exercise and control groups.
Data is presented as individual values and the line represents the mean.
Exercise group n = 10, Control group n = 11.
Statistical analysis performed on log-transformed data for non-classical monocytes.

Regulatory T cells

At baseline there were no significant differences in the number or the proportion of $CD4^+$ lymphocytes ($P = 0.374$ and $P = 0.419$), or Tregs ($P = 0.494$ and $P = 0.563$).

A time*group interaction was found for the proportion of lymphocytes that were $CD4^+$ ($F_{(2,48)} = 7.869$, $P = 0.001$; Table 6.6). *Post hoc* analysis found a significant difference in the change over time between the exercise and control group ($P = 0.001$, ES = 1.09). One-way ANOVA showed a significant decrease in the proportion of $CD4^+$ lymphocytes in the control group (baseline to 6 months: $P = 0.009$, ES = 0.73), whereas there were no significant changes in the exercise group ($P = 0.178$).

Similarly, a time*group interaction for the number of $CD4^+$ lymphocytes was found ($F_{(2,48)} = 8.696$, $P = 0.001$; Figure 6.6a). *Post hoc* one-way ANOVA revealed a decrease in the control group ($P = 0.004$) and a trend for an increase in the exercise group ($P = 0.075$).

There were no significant effects in the proportion of $CD4^+$ lymphocytes that were Tregs (time*group interaction: $F_{(2,48)} = 0.145$, $P = 0.865$; Table 6.6). There was a time*group interaction for the number of Tregs ($F_{(2,48)} = 6.768$, $P = 0.003$; Figure 6.6b). *Post hoc* analysis found a significant difference in the change over time (baseline to 6 months) between the exercise and control group ($P = 0.009$, ES = 1.01). One-way ANOVA revealed a decrease in the control group (baseline to 6 months: $P = 0.018$, ES = 0.52) and a trend for an increase in the exercise group ($P = 0.113$, ES = 0.47).

Table 6.6 - Proportion of $CD4^+$ lymphocytes and regulatory T cells at baseline and after 3 months and 6 months in the exercise and control groups

	Exercise Group (n = 14)			**Control Group (n = 12)**		
	Baseline	3 months	6 months	Baseline	3 months	6 months
$CD4^+$ lymphocytes						
Proportion (%)	23.2 ± 7.8	24.1 ± 7.3	26.0 ± 5.7	25.6 ± 6.7	23.3 ± 6.4	20.8 ± 6.4*
Regulatory T cells						
Proportion (%)	6.96 ± 1.83	6.87 ± 1.69	7.09 ± 2.19	6.59 ± 1.28	6.55 ± 1.22	6.61 ± 1.35

Regulatory T cells refers to the proportion of $CD4^+$ lymphocytes that express $CD25^+CD127^{low/-}$.
Data presented as mean ± SD.
* Significantly different from baseline ($P < 0.05$).

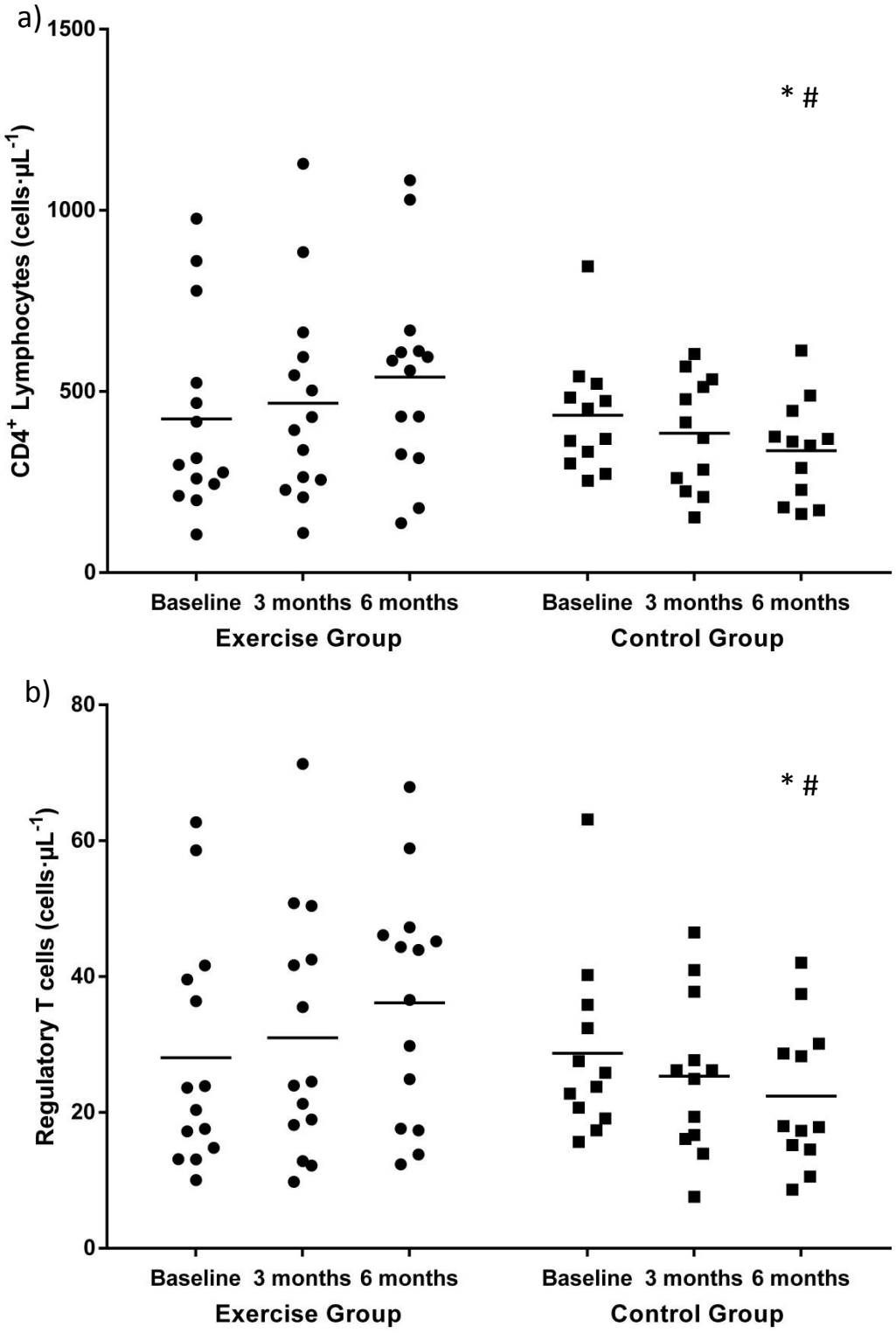

Figure 6.6 - The number of **a)** CD4+ lymphocytes and **b)** Regulatory T cells at baseline, 3 months and 6 months in the exercise and control groups.
Data is presented as individual values and the line represents the mean.
Significant time*group interactions: $P = 0.001$ (a) and $P = 0.003$ (b) respectively. * Significantly different from baseline ($P < 0.05$); # Significantly different from Exercise group ($P < 0.05$).
Statistical analysis performed on log-transformed data. Exercise group n=14, Control group n=12.

Quality of life parameters

The changes in quality of life parameters are presented in Table 6.7.

Duke activity status index

At baseline there was no significant differences between the groups (P = 0.256). An interaction was found between group and time ($F_{(2,50)}$ = 4.332, P = 0.018). *Post hoc* analysis found a significant difference in the change in DASI score from baseline to 6 months in the exercise group compared with the control group (P = 0.004, ES = 1.22; Table 6.7).

Table 6.7 - Changes in quality of life questionnaires from baseline to 6 months in the exercise and control groups.

	Exercise Group (n = 14)	**Control Group (n = 13)**	***P* value**
Duke Activity Status Index			
Δ DASI score †	4 (-2 – 7)	-5 (-8 – 0)	0.004*
Hospital Anxiety and Depression Scale			
Δ HADS total †	-2 (-4 – -1)	0 (-2 – 1)	0.017*
Δ Anxiety	-2 ± 2	1 ± 2	0.007*
Δ Depression †	-1 (-1 – 0)	-1 (-1 – 0)	0.905
Leicester Uraemic Symptom Score			
Δ LUSS total	-3 ± 11	3 ± 11	0.205
Δ LUSS symptoms	0 ± 2	1 ± 2	0.346
Δ LUSS frequency	-2 ± 5	0 ± 5	0.272
Δ LUSS intrusiveness	-1 ± 5	2 ± 7	0.283

DASI, Duke activity status index; HADS, Hospital anxiety and depression scale; LUSS, Leicester uraemic symptom score.
Δ = 6 months – baseline.
Data presented as mean ± SD,
† Data presented as median (interquartile range).
* Significant difference between groups.

Hospital anxiety and depression scale

At baseline the exercise group had a higher total HADS score (10 ± 6 vs. 6 ± 4, *P* = 0.048, ES = 0.81) than the control group as a result of a higher anxiety score (6 ± 4 vs. 2 ± 2, *P* = 0.005, ES = 1.19). No significant difference was seen at baseline in the depression aspect (*P* = 0.644).

ANOVA of the total HADS score revealed a trend toward a time*group interaction ($F_{(2,50)}$ = 2.722, *P* = 0.075) and a different change was found from baseline to 6 months between groups (Table 6.7). Inspection of the depression aspect found no time*group interaction ($F_{(2,50)}$ = 0.598, *P* = 0.554). A significant time*group interaction was found in the anxiety element ($F_{(2,50)}$ = 6.295, *P* = 0.004). *Post hoc* analysis found a significant reduction in anxiety score in the exercise group (baseline to 6 months, 6 ± 3 to 4 ± 3, *P* = 0.046, ES = 0.56) and this was significantly different from the change in the control group (Table 6.7).

Leicester uraemic symptom score

At baseline there were no differences between the exercise and control group in the total LUSS score (*P* = 0.863), symptoms (*P* = 0.867), symptom frequency (*P* = 0.482) or symptom intrusiveness (*P* = 0.788). The exercise intervention had no effect; there were no statistically significant time*group interaction in the total composite LUSS score ($F_{(2,50)}$ = 0.901, *P* = 0.413), the number of symptoms reported ($F_{(2,50)}$ = 0.427, *P* = 0.655), or the frequency or intrusiveness of the symptoms (*P* > 0.05; Table 6.7). On exploring the individual symptoms a significant time*group interaction was found for the poor concentration composite score ($F_{(2,50)}$ = 4.411, *P* = 0.017). *Post hoc* analysis found a significant reduction in the exercise group (baseline to 6 months: Δ -1 ± 1, *P* = 0.045, ES = 0.47) with no change in the control group (*P* = 0.508). No significant effects were observed in any of the other individual symptom scores.

6.5 Discussion

The main aim of this study was to assess the effects of regular exercise on circulating markers of inflammation, pro-inflammatory and anti-inflammatory leucocyte subsets and aspects of quality of life. Regular exercise increased lower-limb functional ability but had no significant impact on circulating pro-inflammatory cytokines and CRP. However, regular intradialytic exercise induced a decrease in the intermediate monocyte population and an increase in the number of Tregs in circulation. Aspects of quality of life were improved in the exercise group in comparison with the routine care group.

Exercise and physical function

The power output of the exercise completed was slightly lower than in the previous acute study (Chapter 4), many patients preferred to cycle for a longer duration rather than increase the intensity as shown by the median exercise duration of 35 min and RPE of 12.

Due to the different equipment used in various aerobic intradialytic training studies (e.g. dialysis chairs or beds, design of cycle ergometers) direct comparisons between studies should be made with caution. The power output of this cohort are comparable with those that have previously reported objective measures of exercise intensity in intradialytic cycling (DePaul *et al*., 2002; Kopple *et al*., 2007; Moore *et al*., 1993a; Storer *et al*., 2005), although the power output is slightly lower than some; a likely reason is the recumbent position of the bike and the older age of the participants in the present study.

The study was pragmatic in design so as to ascertain a realistic response to an intradialytic exercise programme *per se* rather than a specific amount of exercise that may not represent the normal variation in exercise capability in these patients. There was therefore a large variation in exercise achieved in this study but the subjective intensity remained quite consistent (RPE interquartile range: 11-13).

A significant increase in physical function, as measured by the STS 60 test, was achieved in the exercise group after 6 months. The extent of improvement (almost 85%) was much larger than observed in most other studies (≈20%; Giannaki *et al*., 2013; Koufaki, Mercer

and Naish, 2002; Liu *et al*., 2014; Wu *et al*., 2014). The increase in STS 60 score was likely exacerbated by the particularly low baseline values (13 ± 10 vs ≈23 reps in the above studies), likely due to the older age or disease condition of the patients herein. Therefore, some patients that started with particularly low scores had a greater potential for substantial improvements (e.g. a patient improved from 1 rep at baseline to 16 reps after 6 months). Nevertheless, the post-training average was still lower than that reported in the above studies and lower than the healthy similar-aged controls in Chapter 3 (24 ± 6 vs. ≈27 vs. 28 ± 13 reps respectively).

The STS 60 is a good marker of physical function and has been shown to reliably characterise lower extremity muscle performance and to be a consistent determinant of gait performance in renal transplant candidates (Bohannon *et al.,* 1995). The STS 60 is a strong correlate with the 6 min walk test but produces less haemodynamic stress (shown in COPD patients; Ozalevli *et al*., 2007), and takes less time, space and equipment to complete.

An improvement in STS 60 score represents an increase in physical function, an important factor in a patient's ability to live independently. Consequently, an improvement in physical function can be expected to have a positive impact upon quality of life. It is perhaps unsurprising that low physical function is associated with higher hospitalisation and mortality rates (Knight *et al*., 2003; Mapes *et al*., 2003); thus, improvements in physical function are clinically relevant.

Numerous randomised controlled trials have found that regular aerobic exercise improves physical function and exercise capacity, including those conducted during HD (Giannaki *et al*., 2013; Koufaki, Mercer and Naish, 2002; Liu *et al*., 2014; Wu *et al*., 2014). This has been shown in STS 60 scores, 6-min walk-test distance and $\dot{V}O_{2peak}$. Indeed, muscle strength has also been reported to be improved by intradialytic cycling (Storer *et al*., 2005). Strength adaptation would not normally be expected from aerobic exercise, but the authors propose the marked muscle weakness at baseline (as appears to be seen in the present study) may mean that endurance exercise provides an adequate resistance to cause strength adaptation.

There are some important caveats. An improvement in STS 60 score may not represent an improvement in other dimensions of physical function. Cycling exercise that increased STS 60 score and quickened the time to complete 5 STS reps did not improve the ability to complete a walk-test, stair climb or stair descent (Koufaki, Mercer and Naish, 2002); therefore, cycling exercise may give specific benefits to particular physical functions but not necessarily translate well to other tasks. Others have also found inconsistencies in the improvement of STS scores and walking tests (Bohm *et al*., 2014). In addition, the benefits of exercise are short-lived if the patient ceases exercise. MacDonald and colleagues (2005) observed an increase in STS 30 score after 12 wk of training that returned toward pre-training values after a 12-wk period of detraining; thus, exercise must be regular in order to achieve functional improvements.

Circulating markers of inflammation

The findings of the present study suggest that exercise training had no significant impact on circulating concentrations of CRP, IL-6 or TNF-α, markers that are associated with systemic inflammation, atherosclerosis and mortality (Stenvinkel *et al*., 2005).

On careful examination of the literature the lack of change is in agreement with the results of other studies. Of the research analysing various exercise modalities and prescriptions, no study design has found a decrease in IL-6 after training in ESRD patients; furthermore, TNF-α has not reduced after training (please refer to Table 1.8). The findings for CRP are mixed with 6 studies reporting improvements (i.e. reductions) and 5 reporting no change (Table 1.8; for a review: Dungey *et al.,* 2013).

It is noteworthy that many of the positive studies had no control group (Moraes *et al*., 2014; Nindl *et al*., 2004; Załuska *et al*., 2002), making it hard to draw meaningful conclusions from the findings. For example, in an uncontrolled study by Nindl and colleagues (2004) CRP fell from 12.4 ± 3.0 to 10.4 ± 2.7 $mg \cdot L^{-1}$ during a 6-wk control period prior to training commencing and then fell to 7.6 ± 1.6 and 6.1 ± 1.1 $mg \cdot L^{-1}$ after 6- and 12 wk of progressive resistance training, this decrease may represent regression toward the mean rather than an adaptation to training. Additionally, two studies from a single research group describe surprisingly large reductions in CRP (> 80%) after only 8 wk

of short duration exercise (Afshar *et al*., 2010; Afshar *et al*., 2011), since other studies have not found such dramatic results in longer studies of patients training at a greater intensity, it is advisable to interpret these results with caution. The only other controlled trial to report a decrease in CRP in ESRD patients (after 12 wk of intradialytic resistance training; Cheema *et al*., 2007) reported a decrease in log CRP of 0.08 in the exercising patients compared with an increase in log CRP of 0.24 in the control group. This suggests that training may have attenuated increasing CRP rather than decreasing it significantly. Well-controlled trials have found no significant changes in CRP after 3-4 months of intradialytic cycling or extradialytic resistance training (Kopple *et al*., 2007; Toussaint, Polkinghorne and Kerr, 2008; Wilund *et al*., 2010). Therefore, exercise training in HD patients appears to have no significant impact on these circulating markers associated with inflammation.

These findings are in fact similar to many studies reported in healthy individuals. There is clear evidence that regular exercise has anti-inflammatory effects, firstly through numerous large-cohort observational studies (Ford *et al*., 2002; Katja *et al*., 2006), and secondly through alterations of a number of underlying mechanisms (Gleeson *et al*., 2011). However, exercise training interventions (of a greater training load than usually achieved in ESRD patients) have been somewhat unsuccessful at detecting decreases in circulating concentrations of CRP, IL-6 and TNF-α; outcomes are mixed with some groups reporting decreases (Campbell P. *et al*., 2009; Kohut *et al*., 2006) and others finding no change (Campbell *et al*., 2008; Hammett *et al*., 2004).

In a review of the literature Beavers and colleagues (2010) concluded that exercise interventions in participants with elevated inflammatory markers (such a chronic disease populations) or where a loss of adiposity was achieved were successful in reducing levels of inflammatory biomarkers. But increasing physical activity in otherwise healthy individuals had a small (and often undetectable) effect. The authors proposed that the publication of underpowered studies, variances in calorie expenditure therefore leading to differences in adiposity, differences in the type of exercise, differences in the baseline inflammatory characteristics of the patients, and the lack of appropriate control groups were influential in the negative findings (Beavers *et al*., 2010). A large cohort (n = 4,289), 10-y follow-up study did find a significantly lower CRP and IL-6 in the most active

individuals and this decreased in individuals that increased physical activity levels (Hamer *et al.,* 2012). This appears to suggest that regular exercise needs to be continued for a prolonged period, or measured in a large cohort, before the benefits of exercise are observed.

Regular exercise in chronic disease patients with elevated inflammatory markers has shown to ameliorate systemic inflammation. Recently, in pre-dialysis CKD patients 6 months of walking exercise decreased the ratio of IL-6 to IL-10 and down-regulated T-lymphocyte and monocyte activation suggesting regular walking as an anti-inflammatory therapy (Viana *et al.,* 2014).

In ESRD patients the large inter- and intra-individual variations in these factors mean that that any effect of exercise may be overshadowed. This can be seen in Chapter 4 (Figure 4.3d) where the intra-individual variation of circulating CRP in two samples separated by 7 days was almost 30%. With such large variability it would be very difficult to accurately detect a modest decline over time; a very large sample size may be required. There are many potential sources of day-to-day variation in HD patients including recurrent infections (that may or may not be overt), the HD procedure and the immune response associated with it, and oedema (fluid overload).

Large cohort observational and epidemiological studies have reported increased cardiovascular and all-cause mortality risk in ESRD patients with higher plasma CRP and IL-6 (Rao *et al*., 2005; Yeun *et al*., 2000) and inverse relationships between markers of inflammation and physical activity levels (Anand *et al*., 2011; Mafra *et al*., 2011). However, the day-to-day variation in this population may limit the use of these circulating markers in detecting long-term change, particularly in small groups and in those receiving HD treatment. It is important to acknowledge the limitations of these markers of inflammation and to not use them in isolation.

Circulating cellular inflammation

Monocytes

The exercise group had a significant decline in the proportion of monocytes of the intermediate phenotype after training when compared with the usual care control group.

To the author's knowledge this is the first study in any population to show that exercise training selectively diminishes the proportion of monocytes that are the intermediate subset. In an inactive, elderly, but healthy group, 12 wk of exercise training (including 20 min endurance and 20 min resistance exercise repeated thrice weekly) significantly reduced the percentage of $CD14^{+}CD16^{+}$ monocytes and decreased the TNF-α response to LPS stimulation (Timmerman *et al.,* 2008). This was recently supported by Markofski and colleagues (2014) in an overweight middle-aged group who completed 12 wk of resistance training. However neither group analysed the $CD16^{+}$ monocytes separately as intermediate and non-classical subsets.

In the only other apparent study to analyse the effect of exercise training on the trio of monocyte subsets there were no changes in classical, intermediate or non-classical populations in young overweight men who completed 2 wk of high-intensity interval training (Oliveira-Child, Leggate and Gleeson, 2013). The cohort demographics, differing exercise mode and duration and the short length of the study may explain the absence of adaptation, a longer intervention may be required.

Intermediate monocytes are associated with secretion of pro-inflammatory cytokines in response to LPS-stimulation, such as TNF-α, IL-1β and IL-6, higher expression of TLR4 and apparent pro-angiogenesis properties including vascular endothelial growth factor (VEGF) and angiopoietin receptor (Tie2) expression (Cros *et al*., 2010; Rossol *et al.*, 2012; Shantsila *et al.*, 2011). Furthermore, intermediate monocytes have an increased expression of adhesion molecules (CCR5 and CX_3CR1) and exhibit spontaneous ROS production. Thiobarbituric acid reactive substances (TBARS), a marker of oxidative stress, have been shown to decrease 38% after 4 months of intradialytic cycling (Wilund *et al.*, 2010). Speculatively, this may have been as a consequence of reduced intermediate monocyte ROS production. Equally, the decreased TNF-α response to LPS described in the

healthy study earlier could be attributed to a diminished intermediate subset (Timmerman *et al.,* 2008).

Intermediate monocytes increase in circulation with worsening renal function and predict adverse outcomes in pre-dialysis and HD patients (Heine *et al*., 2008; Rogacev *et al.*, 2011). This prompted a recent review to declare intermediate monocytes as a promising therapeutic target for CVD in patients with CKD (Heine *et al.*, 2012). The reduction in intermediate monocytes here is encouraging and may be a mechanism by which exercise has anti-inflammatory and anti-atherosclerotic effects in HD patients.

Regulatory T cells

In the exercise group the number of Tregs was higher after 6 months than in the control group. Tregs suppress the immune response of T lymphocytes; the increase in the number of Tregs in this study would therefore be expected to have anti-inflammatory effects. Consequently, exercise training may help correct the lower number of Tregs found in ESRD patients (Hendrikx *et al.*, 2009; Topal *et al.*, 2013).

Regular physical activity is associated with a higher proportion of Tregs. A young sedentary group had a significantly lower proportion of $CD4^+$ lymphocytes that expressed $CD25^+CD127^{low/-}$ than recreationally active or endurance and sprint trained groups (Handzlik *et al.,* 2013). The proportion of Tregs was also associated with IL-10 production to antigen challenge and the authors concluded that this was a mechanism by which regular exercise has an anti-inflammatory effect and may be partly responsible for the suppressed immunity of athletes with high training loads.

Due to the important regulating capacity of Tregs either a surplus or a deficiency in activity is deleterious. It should be acknowledged that while a deficiency in Tregs can lead to autoimmunity, inflammation and allergy; an over-activation of Tregs is associated with increased risk of chronic infections and tumour growth (Shalev *et al*., 2011). Therefore, an excessive increase in Tregs could increase the risk of infection. However, it is known that CKD patients have a reduced number and suppressive capacity of Tregs (Hendrikx *et al.,* 2009).

A more in-depth examination of the results shows an increase in total $CD4^+$ lymphocytes in both number and proportion in the exercise group compared with the control group. As the proportion of $CD4^+$ lymphocytes that are Tregs did not change it would appear that the expansion of Tregs is due to an increase in the whole $CD4^+$ lymphocyte population. The reasons for this increase are unclear but this does raise doubt on whether this represents a true increase in Tregs. It is not known whether Treg numbers follow or reflect an increase in the general $CD4^+$ lymphocyte population.

Arguably, an increase in the number of Tregs could be more important than a change in the proportion of Tregs as an increased number of anti-inflammatory cells would be expected to have an enhanced anti-inflammatory capacity. On the other hand, the ratio of anti-inflammatory to other cells may be more relevant as an increase in anti-inflammatory capacity will not have any additional benefit if the pro-inflammatory stimulus has also increased proportionally.

Quality of life

The exercising group achieved an increase in perceived functional capacity (DASI questionnaire) compared with the control group; replicating the increase in physical function observed in the STS 60 test. Given that the patients' perception of what they can do appeared to be an important determining factor in physical activity (Chapter 3) this improvement is noteworthy. Regular exercise likely improves the patient's confidence to be active (self-efficacy) that may consequently increase the likelihood of more regular exercise (social cognitive theory; Bandura, 2001) and potentially greater physiological benefits. Others have used the physical component of the Short Form-36 Health Status Questionnaire (SF-36; Ware, 1993) and found improved self-reported physical function after an exercise intervention (Chen *et al*., 2010; Dobsak *et al*., 2012; Painter *et al*., 2000).

Many studies have reported a decrease in depression after exercise training in ESRD patients as described in a recent review of the literature (Mitrou *et al*., 2013). Our findings suggest a decrease in overall anxiety and depression score after 6 months of regular exercise due to an improvement in the anxiety component. Kouidi *et al*. (2010) also reported a similar fall in total HADS score from 11 to 8 after 1 y of intradialytic

exercise training, but they did not assess anxiety and depression elements independently. Elsewhere, anxiety specifically has been shown to decrease after training in ESRD patients (Carney *et al*., 1983; Moug *et al*., 2004; Suh *et al*., 2002). There is a high prevalence of anxiety and depression in HD patients and an association with complications and hospitalisation rates; improvements in psychosocial health would be highly beneficial for patient quality of life (Kimmel, 2001; Lopes *et al.,* 2014).

In terms of the symptoms patients reported and the impact that these symptoms had on the patients, there were no changes over the 6 months of training. Overall, there may have been a trend for reduced symptom burden but this was not close to statistical significance. Other controlled studies examining general quality of life have found this can be improved (Matsumoto *et al*., 2007; Ouzouni *et al*., 2009; Sakkas *et al*., 2008; Wu *et al*., 2014), although others have report no change (Bohm *et al*., 2014; Parsons *et al*., 2004). All studies have used quantitative questionnaires and the differing styles may explain the divergent results (e.g. SF-36, Kidney Disease Quality of Life, Health-Related Quality of Life questionnaires etc.). Analysis of quality of life probably requires a more qualitative approach to fully understand the impact regular exercise has on quality of life; however, this was outside the scope of this study.

Limitations

This study was designed to be as pragmatic as possible but there are limitations that should be acknowledged. The exercise used was only one mode of exercise; it is possible that resistance training or exercise carried out outside of HD may have differing outcomes. Also, this study did not prescribe a set amount of exercise and therefore there was variation within the patients in terms of intensity, duration and frequency. However, this is a representation of an achievable exercise programme carried out with patient involvement.

There was no direct randomisation to the exercise or control group, rather exercise was offered to patients at one HD unit and not at the control HD unit. This design prevented a clear sampling bias of comparing those that want to exercise with those that do not and also prevented contamination of control patients who may be influenced by exercising

counterparts who were responding positively. However, it is unfortunate that the demographics of the exercise and control group were different. The greater number of younger Indian patients in the exercise group was distinct from the predominantly older white British control group. There were no significant differences between Indian patients and white British patients in the response to training (although these subgroups were small). The different demographics could not be avoided in this area with this study design as this represented the local population of the two HD units and therefore, a multi-centre study would have been required to incorporate more patients of differing demographics.

Due to the unique physiology of HD patients caution should be observed when comparing the results of this study with healthy or other chronic disease patients. HD patients are highly differentiated from healthy individuals of a similar age (Chapter 3) and therefore adaptations to training may also be different.

Finally, the sample size may limit the statistical power and the transferability of these findings to other cohorts. Nonetheless, the study was able to distinguish different changes in the exercise and control groups and provide invaluable practical experience and information useful for the design of future larger cohort studies.

Implications

At a circulating cellular level, exercise appears to have an anti-inflammatory effect. The shift away from the pro-inflammatory intermediate monocyte is encouraging as higher proportions of this cell type are associated with CVD and mortality in CKD and ESRD. Furthermore, the increase in the absolute number of Tregs may represent a shift toward an anti-inflammatory environment. Both findings should be confirmed in larger cohorts.

It is also important to note that regular exercise did not have any adverse effects on the inflammatory markers, haematology or biochemistry measured; this adds to the literature indicating the safety of exercise on HD. Furthermore, the improvements in physical function, perceived functional capacity and anxiety confirm the results of others and show that exercise in this population has the potential to have huge benefits to a patient's quality of life.

6.6 Conclusions

In a pragmatic intradialytic training study, 6 months of regular exercise was associated with a decreased proportion of the pro-inflammatory intermediate monocyte phenotype and an increased number of anti-inflammatory Tregs in circulation in comparison to a usual care control group. These findings suggest that regular exercise has an anti-inflammatory effect at a cellular level and therefore may be protective against the increased risk of CVD and mortality that is associated with chronic inflammation and elevated numbers of intermediate monocytes.

Exercise was also shown to enhance physical function and patient reported outcomes such as anxiety and perceived functional capacity. These improvements can have a profound positive impact on the patient's quality of life.

Chapter 7

General Discussion

7.1 Summary of the main findings

The purpose of this thesis was to explore the impact of habitual physical activity, an acute exercise bout and a long-term exercise training programme on markers and mechanisms of systemic inflammation in HD patients. The primary aims were to ascertain the safety of exercise in this cohort from a novel immunological perspective and determine whether exercise can have beneficial effects for the cardiovascular and immunological health and general wellbeing of these patients. Throughout this thesis the safety of exercise was verified although areas for further research were identified. In addition, a number of anti-inflammatory adaptations to training were established that are likely to have favourable consequences for cardiovascular health in these patients.

In Chapter 3 the HD patients were characterised in terms of their physical activity levels, markers and mechanisms of circulating inflammation and aspects of their quality of life. The HD patients were found to be highly sedentary and inactivity was exacerbated on days when they had HD treatment. The patients had higher circulating levels of CRP, IL-6 and TNF-α than their healthy age-matched counterparts. The physical activity levels in the healthy group correlated with circulating markers of inflammation, but not in the HD cohort. However, physical function, assessed by STS 60, was the greatest predictor of IL-6 and CRP in the HD patients. Immune dysfunction in the HD patients was evidenced by a blunted neutrophil degranulation response to bacterial stimulant and a greater distribution of the pro-inflammatory and pro-atherosclerotic intermediate and non-classical monocytes and a lower proportion of the anti-inflammatory Tregs. Thus, this data supports the literature suggesting extensive immune dysfunction in HD patients illustrated by both immunosuppression and over-activation, and great potential for improvements in physical activity levels.

In Chapters 4 and 5, the acute effect of exercising during HD was analysed. Firstly it was demonstrated that moderate-intensity intradialytic exercise did not elicit leucocytosis or an abnormal response to exercise. Further, exercise at this intensity did not stimulate an additional secretion of IL-6 into circulation in comparison to normal HD treatment and no changes were observed in IL-1ra or TNF-α. Neutrophil response to bacterial challenge appeared suppressed immediately post-exercise but this effect was non-significant, transient and minor (ES = 0.22). Exercise did have a considerable impact on blood

pressure. Patients' SBP increased during exercise but dropped significantly 60 min post-exercise. Due to the negative consequences of intradialytic hypotension and associations with myocardial ischaemia markers of cardiac damage were assessed and no abnormalities were found. The haemodynamic response to exercise appears to be normal but superimposed onto the response to HD.

Finally, Chapter 6 demonstrated that regular exercise during HD was achievable with no clear adverse reactions. Although 6 months of training had no impact on circulating CRP, IL-6 or TNF-α, the exercise group had significant changes in the distribution of monocyte phenotypes away from the pro-inflammatory intermediate subset and an increase in the number of the anti-inflammatory Tregs. Therefore, at a circulating cellular level, regular exercise appears to reduce inflammation in HD patients. The training group also attained significant improvements in physical function and patient-reported outcomes such as anxiety score and perceived functional capacity.

Despite a large amount of interest in systemic inflammation in HD patients and within the field of exercise science this represents the first study to explore the acute effects of exercise in ESRD patients on markers of inflammation and the first study to explore the impact of regular bouts of exercise on monocyte phenotypes and Tregs. It is also surprising that no other studies have examined blood pressure beyond the cessation of exercise in HD patients.

7.2 Inflammation

It is reassuring that moderate-intensity exercise does not exacerbate markers of inflammation. Although acute increases in IL-6 after exercise may stimulate an anti-inflammatory mechanism in healthy individuals this may not be the case in HD patients due to reduced clearance of IL-6 and a dysfunctional immune system that may not have the same anti-inflammatory response seen in healthy people. Elevations in circulating IL-6 are associated with pro-atherogenic and pro-catabolic consequences in HD patients (Table 1.6); it remains unclear whether post-exercise increases in IL-6 are desirable in HD patients and what threshold of exercise is required to stimulate this response.

In the acute study the patients had experience of intradialytic exercise; it would therefore be relevant to determine if patients have a similar response when exercise is completely novel. In healthy men it has been shown that when exercise is novel a greater IL-6 response is seen than exercise completed after a training programme (Fischer *et al*., 2004). Further, seeing as exercise duration and intensity are important determinants in muscle contraction release of IL-6 (Fischer, 2006) an investigation into different exercise durations and intensities would help define the safety of different exercise prescriptions in this cohort.

The shift in distribution of monocytes away from the pro-inflammatory intermediate monocyte and increase in the anti-inflammatory Tregs after regular exercise are promising findings as they represents an improvement in the otherwise pro-inflammatory environment exhibited by HD patients. The mechanisms underlying the reduced intermediate monocytes are unclear. In the acute trial a trend was observed for a greater decrease in intermediate monocytes after exercise; it is possible the result of repeated bouts of exercise result in chronic reductions in intermediate cells. Furthermore, it is unclear why adaptations occurred specifically in the intermediate but not the non-classical phenotype.

Intermediate monocytes are associated with CVD risk in non-CKD, CKD and HD patients (Heine *et al*., 2008; Rogacev *et al*., 2011; Rogacev *et al*., 2012). In addition to the secretion of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6), intermediate monocytes have high expression of CCR5 (not seen on other phenotypes) and CX_3CR1 that mediate monocyte recruitment into the vessel walls (Table 1.7). Interestingly, HD patients who exhibit a deletion variant of the CCR5 gene (CCR5Δ32) have protection against inflammation-associated-mortality, confirmed in two independent cohorts of 603 and 302 incident HD patients (Muntinghe *et al*., 2009). Furthermore, CCL2, CX_3CR1 and CCR5 inhibition abolishes atherosclerosis in a mouse model of hypercholesterolaemia (Combadière *et al*., 2008). Thus, intermediate monocytes appear to directly influence CVD risk in CKD patients.

The increase in Tregs observed would be expected to have anti-inflammatory and anti-atherosclerotic effects as well but this has yet to be confirmed in CKD. ESRD combined with reduced Tregs is associated with immune dysfunction, plaque development and

accelerated atherosclerosis (Meier, Meier and Blanc, 2008). The functional balance of Th-17 cells to Tregs is reportedly shifted toward Th-17 cell predominance in HD patients and this is associated with cardiovascular events, calcification and inflammation (Chen *et al*., 2012; Zhang *et al*., 2010). The balance between the pro-inflammatory Th-17 cells and anti-inflammatory Tregs may be a better measure than either cell type in isolation; unfortunately Th-17 was not measured herein. Whether exercise training influences Th-17 or the balance between Th-17 and Tregs warrants further investigation.

Tregs also have an important role in allograft tolerance (Afzali *et al*., 2013; Kang, Tang and Bluestone, 2007). Greater Treg infiltration is associated with reduced graft rejection and inflammation (Taflin *et al*., 2010) and therefore an enhancement in Treg numbers and function could be beneficial for HD patients awaiting transplantation.

The acute effect of exercise on Tregs was not assessed here, and remains unknown in HD patients. However, previously Tregs have shown no change in healthy groups in response to exercise despite significant lymphocytosis (Handzlik *et al*., 2013). It would appear unlikely that in the absence of lymphocytosis exercise would elicit an acute change.

It is important to recognise that, in addition to anti-inflammatory effects, decreases in intermediate monocytes and increased Treg numbers may increase susceptibility to infection by exacerbating immunosuppression. On the other hand, monocyte pre-activation is associated with non-responsiveness to vaccination in HD patients (Girndt *et al*., 1995), suggesting an overall immunocompromised state in these patients. It is unclear what effect improving one aspect (i.e. reducing over-activation) would have on the other (i.e. immunosuppression). Given the high degree of over-activation in HD patients that is highly associated with CVD risk, even if there is a small increased infection risk this may be a small price to pay for anti-inflammatory adaptations.

Preliminary findings on the GHS-R1a in HD patients were reported in this thesis. There appeared to be greater expression of GHS-R1a on monocytes in HD patients compared with healthy controls but this may have merely represented a difference in monocyte phenotypes. Exercise had no immediate effect although the HD treatment itself appeared to upregulate cells expressing GHS-R1a.

Ghrelin administration improves appetite in malnourished HD patients (Ashby *et al*., 2009), may aid in treatment of cachexia and has anti-inflammatory properties (DeBoer *et al*., 2008). The action of ghrelin is dependent upon activation of GHS-R1a, thus the wide distribution of GHS-R1a indicates potential pleiotropic effects. GHS-R1a activation on PBMCs inhibits pro-inflammatory cytokine secretion and inhibits the action of leptin (Dixit *et al*., 2004). At present, GHS-R1a expression on leucocytes remains wholly un-researched in CKD. These findings may have therapeutic implications as this may represent an opportune time (end of HD) to administer ghrelin to inhibit secretion of pro-inflammatory cytokines in addition to its orexigenic properties.

7.3 Immune function

On the whole, immune function is suppressed in HD patients (Betjes, 2013; Girndt *et al*., 1999; Kato *et al*., 2008). In this thesis only the neutrophil degranulation response to bacterial stimulant was measured; it should be acknowledged that this is only one feature of neutrophil function alone, and may not represent other aspects of immune function (i.e. APCs, natural killer cells, acquired immunity).

The neutrophil degranulation response was suppressed in HD patients compared with the healthy group and an apparent minor transient suppression was observed immediately post-exercise compared with the control trial. This observation is similar to research in healthy populations (Walsh *et al*., 2011) but in contrast to a recent study in pre-dialysis CKD patients (Viana *et al*., 2014).

The effect of regular exercise on neutrophil degranulation was not assessed herein. Little is known about adaptations in neutrophil function to regular exercise. Intensified training has the capacity to reduce neutrophil degranulation compared with normal exercise training (Robson *et al*., 1999a), but it is unlikely that HD patients would be able to take part in such intensified training. In pre-dialysis patients, no change was observed after 6 months of walking exercises (Viana *et al*., 2014).

Daniilidis and colleagues (2004) appear to be the only group to analyse exercise and immune function *per se* in HD patients. After 6 months of a renal rehabilitation programme they found a non-significant increase in serum IgM (3%) and IgE (14%) and a

significant decrease in serum IgA (17.5%) but no changes in IgG subclasses, IL-2, IL-4 or IL-6, complement (C3 and C4), or T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD9^+$, $CD16^+$). However, during the study the non-exercising control group reported more common upper respiratory tract infections (URTI; 58%) than the exercising group (31%). Mucosal immunity has an important protective role against URTI. Salivary IgA can decrease after exercise and training and this is associated with greater incidence of URTI (Walsh *et al*., 2011). This represents an area for future research in HD patients, although standard measures of saliva collection may need to be modified due to common xerostomia, which itself may be a complication for mucosal immunity.

A large degree of immune dysfunction in HD patients is attributed to monocytes and APCs and the interaction between innate and acquired immunity (Girndt *et al*., 1999). Given the adaptation observed in this thesis to the proportion of monocyte subsets it is possible that regular exercise may have a significant impact upon monocyte function and antigen presentation; further research into the effect of regular exercise on monocyte functions would be interesting. Likewise, functional assays, cell cultures and delayed type hypersensitivity tests may give a better insight into *in vivo* immune function than measurement of circulating factors.

7.4 Blood pressure

The post-exercise hypotension observed in the acute study did not translate into a decrease in resting blood pressure after taking part in regular exercise for 6 months. This is in contrast to some studies, including many of the early reports, that suggest exercise training decreased resting blood pressure and/or the amount of anti-hypertensive medications the patients required (Goldberg *et al*., 1983; Hagberg *et al*., 1983; Painter *et al*., 1986), although two more recent studies found no improvement (Koh *et al*., 2010; Miller *et al*., 2002). It is notable that the patients in this study had relatively well-controlled blood pressure as demonstrated by similarities with the healthy group in Chapter 3. Advances in medicine, increased awareness of CVD risk, better fluid management, and different clinical practice have led to better blood pressure control in HD patients; consequently, exercise may have less potential for a beneficial adaptation.

Decreased blood pressure after training may partly be the result of repeated acute bouts of exercise rather than a true chronic adaptation. All blood pressure readings taken for the training study herein were 48 h after the last intradialytic exercise bout and would not include a post-exercise decrease. Week-long ambulatory blood pressure monitoring may give a true reflection of daily blood pressure and further research would be interesting. This could also ascertain the acute effect of exercise on blood pressure after HD treatment is completed and whether this is observable for a prolonged period during daily living as has been seen in hypertensive individuals (Pescatello and Kulikowich, 2001; Rondon *et al*., 2002). These results may also aid in advising the best time to take anti-hypertensive medications for patients who are exercising.

7.5 Exercise in haemodialysis patients

Research in the area of exercise for CKD patients has developed over the last 3 decades; however, progress in the provision of exercise has been slow. Unlike other chronic diseases (e.g. COPD, cardiac, cancer) rehabilitation is not commonly available in CKD. The reasons are likely twofold: a) funding, money is not given to support rehabilitation in CKD patients; b) reluctance of clinicians to prescribe or support exercise programmes. Both of these barriers require research to overcome. Firstly, to irrefutably demonstrate that exercise is beneficial to patients to validate the use as part of routine care, and secondly to reassure clinicians and patients alike that exercise is safe. Two highly-funded large cohort NIHR studies with primary outcomes of cardiac health and quality of life are in the developmental stage, and will hopefully provide conclusive answers in the near future.

It is currently unclear how to achieve maximal benefits for HD patients. A couple of research groups have published recommendations for exercise programmes (Greenwood *et al*., 2014; Smart *et al*., 2013). There is difficulty in quantifying exercise; relative $\dot{V}O_{2peak}$ is unlikely to be accurate without conducting peak tests during HD (for intradialytic exercise) and heart rate is altered by medications frequently prescribed to these patients (i.e. beta blockers). Anecdotally, the patients in this study could probably have achieved a higher intensity exercise. Consequently, a ramp protocol to assess intradialytic exercise capacity has been trialled for use in future studies to ensure appropriate progression of

exercise. However, enforcing a strict exercise intensity may lead to lower compliance or greater drop-out, it is important to balance the needs of each patient and therefore individualised exercise programmes are recommended.

The exercise programme in this study was designed to be pragmatic and allow patient involvement; this thesis represents what can be realistically achieved in this population. However, many other exercise modes, durations and intensities have been trialled in HD patients: home exercise programmes, group rehabilitation classes, resistance exercise and electrical-stimulated exercise all present potential avenues for increasing physical activity and involving more patients (Farese *et al*., 2008; Greenwood *et al*., 2012; Kirkman *et al*., 2014; Malagoni *et al*., 2008). Exercise counselling to educate and bring about behaviour change is required to improve overall physical activity including outside of HD; logically this would be expected to bring about the greatest improvements for these patients. For some patients intradialytic exercise may turn out to be a crucial first step in improving physical function and confidence to then increase overall habitual activity levels and attain the concomitant improvements in health.

7.6 Practical implications

This thesis has shown that intradialytic exercise of moderate-intensity does not exacerbate inflammation or have any abnormal effects on leucocytes or neutrophil degranulation; therefore, exercise can be considered safe from an immunological perspective. It is reassuring that exercise can be completed without inducing excessive immunoactivation or suppression that could be deleterious to health.

The post-exercise hypotension discovered, although asymptomatic and in the absence of markers of cardiac damage, requires further investigation. These findings helped support a successful NIHR grant to investigate the effects of intradialytic exercise on the myocardium.

Practically, it would appear advisable to continue to complete exercise in the first half of HD treatment to avoid hypotensive events near the end of HD. Hypotension is associated with nausea, dizziness, cramps and syncope which are unpleasant for patients and may reduce HD compliance and, if related to exercise, exercise compliance. A European Best

Practice Guideline (EBPG) was published in 2007 on how best to avoid and treat intradialytic hypotension (Kooman *et al.,* 2007). The guidelines require careful co-operation of all members of the MDT. First line recommendations include dietary counselling (reduce sodium and UF), refraining from food during HD, reassessment of dry weight, using bicarbonate as dialysis buffer, cooling dialysate to 36.5°C (down to a minimum of 35°C) and checking the dose and timing of anti-hypertensive medication. A slow cool down after exercise combined with careful management of the above factors could minimise intradialytic hypotension. Dialysate cooling has already shown to be beneficial in preserving mean arterial pressure and cardiac function during HD (Selby *et al.,* 2006) and at maintaining arterial blood temperature during intradialytic exercise (Rosales *et al*., 1998). The impact of intradialytic exercise combined with dialysate cooling warrants further investigation.

As well as the functional benefits and improvements in aspects of quality of life that have been well documented (Heiwe and Jacobson, 2014; Smart, McFarlane and Cornelissen, 2013), this thesis demonstrates that regular exercise has an anti-inflammatory impact that may improve cardiovascular health. Regular exercise should be encouraged in this patient cohort as it appears to have clear physiological benefit.

This thesis has laid the foundations for further research in a number of areas. Larger cohort studies are required to confirm the anti-inflammatory adaptation to regular exercise observed here. In addition, what effect repeated bouts of exercise have on cardiac health, particularly in the presence of post-intradialytic exercise hypotension detected herein. Unanswered questions remain, for example the impact of different types of exercise or whether higher intensity exercise is plausible and has different immunological consequences, and the impact of regular exercise on different aspects of immune function that are known to be compromised in ESRD (e.g. APCs, acquired immunity).

7.7 Conclusions

To summarise, this thesis characterised HD patients as highly sedentary with a vastly dysfunctional immune system that is pro-inflammatory and non-responsive to antigen. Next, these studies provide evidence that exercise is safe during HD from an immunological perspective and lays the foundations for further research into the cardiac and cardiovascular implications of intradialytic exercise. Furthermore, this work demonstrates that regular intradialytic exercise, in patients that are otherwise sedentary, can have significant anti-inflammatory effects that may be beneficial in reducing cardiovascular risk in these patients. It is hoped that the findings in this thesis help advance knowledge in the area of exercise in HD patients and enhance future research in the area.

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Appendices

The DASI Patient Questionnaire (to be completed by the patient)

DUKE ACTIVITY STATUS INDEX

Name: _____ Date: _____

Can you: (please circle yes or no)

1. Take care of yourself, that is, eat, dress, bathe or use the toilet?	Yes/No
2. Walk indoors, such as around your house?	Yes/No
3. Walk a block or two on level ground?	Yes/No
4. Climb a flight of stairs or walk up a hill?	Yes/No
5. Run a short distance?	Yes/No
6. Do light work around the house like dusting or washing dishes	Yes/No
7. Do moderate work around the house like vacuuming, sweeping floors or carrying groceries?	Yes/No
8. Do heavy work around the house like scrubbing floors or lifting or moving heavy furniture?	Yes/No
9. Do garden work like raking leaves, weeding or pushing a lawn mower?	Yes/No
10. Have sexual relations?	Yes/No
11. Participate in moderate recreational activities like golf, bowling, dancing, doubles tennis or throwing a ball?	Yes/No
12. Participate in strenuous sports like swimming, singles tennis, football, basketball or skiing?	Yes/No

(To be completed by staff) Duke Activity Status Index (DASI) = sum of "Yes" replies

General Practice Physical Activity Questionnaire

Date……

Name……..

1. Please tell us the type and amount of physical activity involved in your work.

		Please mark one box only
a	I am not in employment (e.g. retired, retired for health reasons, unemployed, full-time carer etc.)	
b	I spend most of my time at work sitting (such as in an office)	
c	I spend most of my time at work standing or walking. However, my work does not require much intense physical effort (e.g. shop assistant, hairdresser, security guard, childminder, etc.)	
d	My work involves definite physical effort including handling of heavy objects and use of tools (e.g. plumber, electrician, carpenter, cleaner, hospital nurse, gardener, postal delivery workers etc.)	
e	My work involves vigorous physical activity including handling of very heavy objects (e.g. scaffolder, construction worker, refuse collector, etc.)	

2. During the *last week*, how many hours did you spend on each of the following activities? *Please answer whether you are in employment or not*

Please mark one box only on each row

		None	Some but less than 1 hour	1 hour but less than 3 hours	3 hours or more
a	Physical exercise such as swimming, jogging, aerobics, football, tennis, gym workout etc.				
b	Cycling, including cycling to work and during leisure time				
c	Walking, including walking to work, shopping, for pleasure etc.				
d	Housework/Childcare				
e	Gardening/DIY				

3. How would you describe your usual walking pace? Please mark one box only.

Slow pace (i.e. less than 3 mph)		Steady average pace	
Brisk pace		Fast pace (i.e. over 4mph)	

Hospital Anxiety and Depression Scale (HADS)

Please choose one response from the four given for each of the 14 questions. Give an immediate response without thinking too long about your answer. Choose the answer that currently describes your feelings.

1	**I feel tense or 'wound up'**		**8**	**I still enjoy the things I used to enjoy:**	
	Most of the time			Hardly at all	
	A lot of the time			Only a little	
	From time to time, occasionally			Not quite so much	
	Not at all			Definitely as much	
2	**I get a sort of frightened feeling as if something awful is about to happen**		**9**	**I can laugh and see the funny side of things**	
	Very definitely and quite badly			Not at all	
	Yes, but not too badly			Definitely not so much now	
	A little, but it doesn't worry me			Not quite so much now	
	Not at all			As much as I always could	
3	**Worrying thoughts go through my mind**		**10**	**I feel cheerful**	
	A great deal of the time			Not at all	
	A lot of the time			Not often	
	From time to time, but not too often			Sometimes	
	Only occasionally			Most of the time	
4	**I can sit at ease and feel relaxed**		**11**	**I feel as if I am slowed down**	
	Not at all			Nearly all the time	
	Not Often			Very often	
	Usually			Sometimes	
	Definitely			Not at all	
5	**I get a sort of frightened feeling like 'butterflies' in the stomach:**		**12**	**I have lost interest in my appearance**	
	Very Often			Definitely	
	Quite Often			I don't take as much care as I should	
	Occasionally			I may not take quite as much care	
	Not at all			I take just as much care as ever	
6	**I feel restless as I have to be on the move**		**13**	**I look forward with enjoyment to things**	
	Very much indeed			Hardly at all	
	Quite a lot			Definitely less than I used to	
	Not very much			Rather less than I used to	
	Not at all			As much as I ever did	
7	**I get sudden feelings of panic**		**14**	**I can enjoy a good book or radio or TV program**	
	Very often indeed			Very seldom	
	Quite often			Not often	
	Not very often			Sometimes	
	Not at all			Often	

Participant Code :
Date :

LUSS: Leicester Uraemic Symptom Score Tool

Part 1

The following is a list of 11 symptoms commonly associated with kidney problems. Please tick the box which best describes how frequently you experience each symptom.

SYMPTOM	**Never**	**Less than once per week**	**1-2 times per week**	**Several times per week**	**Every day**
Itching					
Sleep disturbance					
Loss of appetite					
Excessive tiredness					
Pain in bones/ joints					
Poor concentration/ mental alertness					
Impotence/ lack of sex drive					
Loss of muscle strength/ power					
Shortness of breath					
Muscle spasm/stiffness					
Restless legs					

continued…

Participant Code :
Date :

…continued

Part 2

Some of the symptoms that you ticked overleaf may be more intrusive than others. Please tick the boxes below which best describe how intrusive you find each symptom.

If you do not experience a symptom, please tick the box marked N/A (not applicable).

SYMPTOM	N/A	Not at all intrusive	Slightly intrusive	Quite intrusive	Very intrusive	Extremely intrusive
Itching						
Sleep disturbance						
Loss of appetite						
Excessive tiredness						
Pain in bones/ joints						
Poor concentration/ mental alertness						
Impotence/ lack of sex drive						
Loss of muscle strength/ power						
Shortness of breath						
Muscle spasm/ stiffness						
Restless legs						

SCORE TABLE	
No. of uraemic symptoms identified	
Part 1 Total Score	
Part 2 Total Score	

Thank you for completing this questionnaire.