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# Exercise in Chronic Kidney Disease: Impact on Immunity and Inflammation

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A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

September 2011

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### Abstract

Chronic kidney disease (CKD) is associated with a complex state of immune dysfunction characterised by immune depression, which predisposes CKD patients to infections, and by immune activation resulting in inflammation, which is associated with cardiovascular disease among these patients. It has been suggested that regular moderate exercise may enhance immune function and exert anti-inflammatory effects. However, such effects are still unclear in CKD. Therefore, we investigated the effects of acute and regular (1-month and 6-months) moderate intensity aerobic exercise (walking) on measures of immunity and inflammation in pre-dialysis CKD patients.

A single bout of walking exercise induced an overall immune and inflammatory response that was comparable to that observed in healthy individuals, with no indication of harmful effects to patients' underlying state of immune dysfunction. Acute exercise induced a normal pattern of mobilisation of immune cells. Concerning immune cell function, acute exercise had no effect on T lymphocyte and monocyte activation, while it actually improved neutrophil responsiveness to a bacterial challenge in the recovery period. In addition, acute exercise induced a systemic anti-inflammatory environment, evidenced by the marked elevation in plasma IL-10 levels after exercise, which was most likely mediated by the observed increase in plasma IL-6 levels.

Regular walking exercise exerted anti-inflammatory effects, with no apparent detrimental effects to patients' immune and inflammatory status. Regular exercise led to improvements in the systemic inflammatory status (ratio of pro-inflammatory IL-6 to anti-inflammatory IL-10 cytokine levels) that were accompanied, and most likely mediated, by the observed down-regulation of T lymphocyte (only evident at 6-months) and monocyte activation. In addition, a reduction in IL-6 production in PBMC and whole blood cultures was also observed (only assessed at 1-month). Regular exercise had no effect on circulating immune cell numbers and neutrophil degranulation responses.

These findings provide compelling evidence that walking exercise is safe from an immune and inflammatory perspective and has the potential to be an effective antiinflammatory therapy in pre-dialysis CKD patients.

**Keywords:** chronic kidney disease; exercise; walking; immunity; inflammation; cytokines; T lymphocyte; monocyte; neutrophil

## Acknowledgments

It has been a long journey and although a PhD can be a lonely task I would never have been able to get to this stage without the support of many people, to whom I would like to express my sincere gratitude.

Firstly I would like to thank Lettie for her constant assistance, support, and guidance. These words also extend to Alice who, although not officially, acted as a true supervisor. To both of you I thank for the constant encouragement and for allowing me to gain invaluable experience during the long course of my PhD. Beyond the academic roles, you were true friends and I do not want to say that it was a pleasure to work with you, not because it wasn't, but because I hope this was just the beginning...

Thank you Emma and George, partners in the fieldwork, for being so enthusiastic about this project as I was. It was a long but great experience! I would also like to thank Professor John Feehally for making this entire project possible.

I am very grateful to all the patients and healthy volunteers that participated in the studies of this thesis and without whom there would simple be no thesis!

Thank you to everyone in the Renal Research Group at the University of Leicester for always making me feel so welcomed and for all the help around the lab. Special thanks to Tricia for all her support and help with lab analyses.

Thanks to all the members of our research group over the years, as well as to other staff and students within the wider Exercise Physiology group at Loughborough University for their help, support and moments shared. Special thanks to John for his constant support during my time at Loughborough and for being a great friend!

Finally I must thank my own people. To my family and closest friends, whom despite the distance, where always there to share the highs and lows of this experience with me. Special thanks to Berto for his constant support and shared thoughts! To my brother for his constant support and encouragement. To my parents for constantly supporting my dreams and for coping with all my mood swings. I hope that this achievement to some extent compensates my absence! Last but not the least, to Duda, who has literally lived this long journey with me. I thank you for all your love, patience and encouragement and for keeping me sane until the last minute! I hope I can make it up for the time I have stolen us in the future...

# Funding

The author was supported by a doctoral grant from the Portuguese Foundation for Science and Technology (FCT)

SFRH/BD/27838/2006

# Publications

Publications arisen from the studies reported in this thesis:

### Journal articles

- Kosmadakis G, Stephen JG, Clapp EL, Smith AC, Bevington A, Bishop NC, Viana JL, Owen PJ, McIntyre CW and Feehally J (2011). Benefits of regular walking exercise in advanced predialysis chronic kidney disease. *Nephrology Dialysis Transplantation*; Epub ahead of print DOI: 10.1093/ndt/gfr364.
- Kosmadakis G, Bevington A, Smith AC, Clapp EL, **Viana JL**, Bishop NC and Feehally J (2010). Physical exercise in patients with severe kidney disease. *Nephron Clinical Practice*, 115: c7-c16.

#### Scientific meeting proceedings

- Viana JL, Smith AC, Kosmadakis G, Bevington A, Clapp EL, Feehally J, Bishop NC (2011). "Antigen-stimulated monocyte and T-lymphocyte activation and systemic inflammatory cytokine concentration in chronic kidney disease: effect of regular moderate intensity aerobic exercise." Abstracts from the 10<sup>th</sup> International Society of Exercise Immunology (ISEI) Symposium, Oxford, England, UK (p.22).
- Viana JL, Bishop NC, Kosmadakis G, Bevington A, Clapp EL, Feehally J, Smith AC (2011). "Inflammatory cell activation and cytokine levels in chronic kidney disease: effects of regular exercise." Nephrology Dialysis Transplantation Plus, 4 (Supplement 2): Abstracts from the XLVIII European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) Congress, Prague, Czech Republic.
- Clapp E, Kosmadakis G, Smith A, Viana J, Shirreffs S, Maughan R, Feehally J, Bevington A (2011). "Collapse of intramuscular free amino acid pools in patients with CKD4-5 after 6 months of walking exercise is prevented by alkali supplements." Nephrology Dialysis Transplantation Plus, 4 (Supplement 2): Abstracts from the XLVIII European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) Congress, Prague, Czech Republic.
- Viana J, Bishop N, Kosmadakis G, Bevington A, Clapp E, Feehally J, Smith A (2011). "Inflammatory activation in chronic kidney disease: effect of regular aerobic exercise." Abstracts from the British Renal Society/Renal Association (BRS/RA) Annual Conference, Birmingham, England, UK.
- Viana JL, Bishop NC, Kosmadakis G, Bevington A, Clapp E, Feehally J, Smith AC (2010). "Systemic inflammation in chronic kidney disease: effect of acute and regular moderate intensity aerobic exercise." Abstracts from the XV International Congress on Nutrition and Metabolism in Renal Disease, Lausanne, Switzerland (p.81).
- Viana JL, Bishop NC, Kosmadakis G, Bevington A, Clapp E, Feehally J, Smith AC (2010). "Neutrophil function in chronic kidney disease: effect of acute and regular moderate intensity aerobic exercise." Abstracts from the XV International Congress on Nutrition and Metabolism in Renal Disease, Lausanne, Switzerland (p.82).

- Smith AC, Kosmadakis G, John S, Owen P, Clapp E, Viana JL, Bevington A, McIntyre C and Feehally J (2010). "A six month study of regular walking exercise in advanced pre-dialysis chronic kidney disease." Abstracts from the XV International Congress on Nutrition and Metabolism in Renal Disease, Lausanne, Switzerland (p.111).
- Clapp E, Kosmadakis G, Smith AC, Viana JL, Shirreffs S, Maughan R, Feehally J, Bevington A (2010). "Collapse of intramuscular free amino acid pools in patients with CKD4-5 after 6 months of walking exercise is prevented by alkali supplements." Abstracts from the XV International Congress on Nutrition and Metabolism in Renal Disease, Lausanne, Switzerland (p.47).
- Viana J, Bishop N, Kosmadakis G, Bevington A, Clapp E, Feehally J, Smith A (2010). "Systemic inflammation in chronic kidney disease: effect of acute and regular moderate intensity aerobic exercise." Abstracts from the British Renal Society/Renal Association (BRS/RA) Annual Conference, Manchester, England, UK.
- Bevington A, Clapp E, Kosmadakis G, Smith A, Viana J, Shirreffs S, Maughan R, Owen P, John S, McIntyre C, and Feehally J (2010). "Combined walking exercise and alkali therapy in patients with CKD4-5 generates anabolic signals but no net suppression of muscle protein catabolism." Abstracts from the British Renal Society/Renal Association (BRS/RA) Annual Conference, Manchester, England, UK.
- Viana JL, Smith AC, Kosmadakis G, Clapp EL, Feehally J, Bishop NC (2009). "Effect of acute and regular exercise on systemic inflammation in chronic kidney disease patients." Abstracts from the 9<sup>th</sup> International Society of Exercise Immunology (ISEI) Symposium, Tübingen, Germany (p.32).
- Viana JL, Smith AC, Kosmadakis G, Clapp EL, Feehally J, Bishop NC (2009). "Effect of acute and regular exercise on CD4+ lymphocyte activation in chronic kidney disease patients." Medicine & Science in Sports and Exercise, 41 (5 Supplement): Abstracts from the 56<sup>th</sup> American College of Sports Medicine (ACSM) Annual Meeting, Seattle, WA, USA (p.S269).
- Viana JL, Bishop NC, Kosmadakis G, Clapp EL, Bevington A, Feehally J, Smith AC (2009).
  "Exercise in chronic kidney disease: effect of a one month exercise programme on neutrophil function and systemic inflammation." Nephrology Dialysis Transplantation Plus, 2 (Supplement 2): Abstracts from the World Congress of Nephrology (XLVI ERA-EDTA Congress and XX ISN Congress), Milan, Italy (p.ii882).
- Viana JL, Bishop NC, Kosmadakis G, Clapp EL, Bevington A, Feehally J, Smith AC (2009). "Exercise in chronic kidney disease: acute effects on neutrophil and T cell function." Nephrology Dialysis Transplantation Plus, 2 (Supplement 2): Abstracts from the World Congress of Nephrology (XLVI ERA-EDTA Congress and XX ISN Congress), Milan, Italy (p.ii882).
- Kosmadakis G, Bevington A, Smith AC, Viana JL, Clapp EL, Bishop NC, Feehally J (2009). "Effects of a six month exercise programme on uraemic symptoms and functional parameters of patients with chronic kidney disease." Nephrology Dialysis Transplantation Plus, 2 (Supplement 2): Abstracts from the World Congress of Nephrology (XLVI ERA-EDTA Congress and XX ISN Congress), Milan, Italy (p.ii1677).
- Viana JL, Bishop NC, Kosmadakis G, Clapp EL, Bevington A, Feehally J, Smith AC (2009). "Neutrophil function and systemic inflammation in chronic kidney disease: effect of a onemonth programme of moderate intensity exercise." Abstracts from the Renal Association/British Transplantation Society (RA/BTS) Conference, Liverpool, England, UK (p.365).

- Viana JL, Bishop NC, Kosmadakis G, Clapp EL, Bevington A, Feehally J, Smith AC (2009). "Neutrophil and T cell function in chronic kidney disease: acute effect of a bout of moderate intensity exercise." Abstracts from the Renal Association/British Transplantation Society (RA/BTS) Conference, Liverpool, England, UK (p.366).
- Kosmadakis G, Bevington A, Smith AC, Viana JL, Clapp EL, Bishop NC, Feehally J (2009). "Effects of a six month exercise programme on uraemic symptoms and functional parameters of patients with chronic kidney disease." Abstracts from the Renal Association/British Transplantation Society (RA/BTS) Conference, Liverpool, England, UK (p.313).
- Viana J, Smith A, Kosmadakis G, Clapp E, Feehally J, Bishop N (2008). "Systemic inflammatory status in chronic kidney disease: effect of one month of moderate aerobic exercise." Archivos de medicina del deporte, XXV (Número 128): Abstracts from the XXX Fédération Internationale de Médecine du Sport (FIMS) World Congress of Sports Medicine, Barcelona, Spain (pp.473-474).
- Viana JL, Smith AC, Kosmadakis G, Clapp EC, Feehally J, Bishop NC (2008). "No effect of one month of moderate aerobic exercise on bacterially-stimulated neutrophil degranulation responses in chronic kidney disease patients." Journal of Sports Sciences, 26 (S2): Abstracts from the British Association of Sport and Exercise Sciences (BASES) Annual Conference, Brunel University, London, England, UK. (p.S30).
- Viana JL, Smith AC, Kosmadakis G, Clapp EC, Feehally J, Bishop NC (2008). "The effect of acute moderate aerobic exercise on bacterially-stimulated neutrophil degranulation responses in patients with chronic kidney disease." Abstracts from the 13<sup>th</sup> Annual Congress of the European College of Sports Science (ECSS), Estoril, Portugal (p.149).
- Kosmadakis G, Bevington A, Smith A, Clapp E, Viana J, Feehally J (2008). "Effects of an exercise programme on uraemic symptoms and functional parameters of patients with renal failure." Journal of Renal Nutrition, 18 (3 Supplement): Abstracts from the XIV International Congress on Nutrition and Metabolism in Renal Disease, Marseilles, France (p.S19).
- Kosmadakis G, Bevington A, Smith A, Clapp E, **Viana J**, Bishop N, Feehally J (2008). "Effects of an exercise programme on uraemic symptoms and functional parameters of patients with chronic kidney disease." Abstracts from the British Renal Society/Renal Association (BRS/RA) Conference, Glasgow, Scotland, UK.
- Kosmadakis G, Bevington A, Smith A, Clapp E, Viana J, Feehally J (2008). "Effects of an exercise programme on uraemic symptoms and functional parameters of patients with renal failure." Nephrology Dialysis Transplantation Plus, 1 (Supplement 2): Abstracts from the XLV European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) Congress, Stockholm, Sweden (p. ii280).
- Viana J, Clapp E, Smith AC, Bishop NC (2007). "The effect of moderate exercise and ex vivo extracellular acidosis on T lymphocyte and monocyte activation." Abstracts from the 8<sup>th</sup> International Society of Exercise Immunology (ISEI) Symposium, Sendai, Japan (p.49).

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Chapter 1

**General Introduction** 

### 1.1. Rationale and structure of the thesis

Chronic kidney disease (CKD) is associated with profound alterations of both innate and adaptive immunity, resulting in a complex and still not entirely understood state of immune dysfunction, wherein signs of immune depression and activation coexist. While immune depression contributes to the high incidence of infectious complications in patients with CKD, persistent immune activation leads to a state of chronic inflammation, which is associated with the increased risk of cardiovascular disease among these patients. Importantly, infection and cardiovascular disease are major causes of morbidity and mortality in CKD. Hence, the immune dysfunction that accompanies CKD represents a major target for therapeutic interventions aiming to improve outcome in CKD. Exercise has the potential to be one of such therapies. It has been suggested that regular engagement in moderate intensity exercise may enhance certain aspects of the immune function and may exert antiinflammatory effects, which is believed to contribute, at least in part, to the lower risk of infection and cardiovascular disease that is observed in physically active individuals in comparison with their sedentary counterparts. However, the impact of exercise on immune and inflammatory parameters has been poorly investigated in CKD. Apart from the potential benefits, given that exercise is being advocated into the routine clinical care of patients with CKD, it is also paramount to determine if exercise is safe to their underlying compromised immunity and inflammatory status. Therefore, this thesis focuses on the effects of acute and regular moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients.

In the present chapter, the relevant background information that supports this research is presented. Chapter 2 outlines the research design and describes the methods that were common to the main experimental research. Chapter 3 presents a preliminary study conducted in healthy volunteers. The following 3 chapters include the main research studies of this thesis. In Chapter 4, the effects of acute exercise are presented. Chapters 5 and 6 address the effects of regular exercise, reporting the findings from a 1-month uncontrolled trial and a 6-months controlled trial, respectively. Finally, Chapter 7 provides a summary of the main findings of this thesis as well as their possible implications.

# 1.2. Background

### Chronic kidney disease: definition and classification

CKD is a general term for heterogeneous disorders affecting the structure and function of the kidney. The variation in disease expression is related partially to cause and pathology, severity, and rate of progression (Levey & Coresh, 2011). CKD is defined as either kidney damage (proteinuria, haematuria or anatomical abnormality) or glomerular filtration rate  $(GFR)^1 < 60 \text{ ml/min/1.73m}^2$  present on at least 2 occasions for  $\geq$  3 months and it is classified into 5 stages as shown in Table 1.1 (National Collaborating Centre for Chronic Conditions, 2008). Stages 3 to 5 may be defined solely by GFR, while stages 1 and 2 also require the presence of persistent proteinuria, albuminuria, haematuria or structural abnormalities. Established renal failure (stage 5), often called end-stage renal disease (ESRD), is an irreversible, long-term condition that may require renal replacement therapy (RRT), such as regular dialysis treatment or kidney transplantation, to maintain life.

<sup>&</sup>lt;sup>1</sup> GFR is the best overall index of kidney function. Normal values, which are related to age, gender, and body size, are approximately 130 ml/min/1.73 m<sup>2</sup> in young men and 120 ml/min/1.73 m<sup>2</sup> in young women. In the clinical setting, GFR is generally estimated (eGFR) based on serum creatinine and demographic data (age, gender and ethnic origin) with the Cockcroft-Gault or MDRD (modification of diet in renal disease) equations (Stevens *et al.*, 2006). The use of the abbreviated MDRD equation (Levey *et al.*, 2000) is currently recommended in the UK to estimate GFR (National Collaborating Centre for Chronic Conditions, 2008).

Stage*	<b>GFR</b> ml/min/1.73 m <sup>2</sup>	Description		
1	≥ 90	Normal or increased GFR, with other evidence of kidney damage		
2	60 - 89	Slight decrease in GFR, with other evidence of kidney damage		
3A	45 - 59	Moderate decrease in GFR,		
3B	30 - 44	with or without other evidence of kidney damage		
4	15 - 29	Severe decrease in GFR, with or without other evidence of kidney damage		
5	< 15 (or dialysis)	Established renal failure		

**Table 1.1. Stages of chronic kidney disease.** Adapted from National Collaborating Centre for Chronic Conditions (2008)<sup>2</sup>.

\*Use the suffix (p) to denote the presence of proteinuria<sup>3</sup> when staging CKD

<sup>&</sup>lt;sup>2</sup> The 5-stage classification system for CKD was initially proposed by the US National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines (National Kidney Foundation, 2002; Levey et al., 2003). This was adopted internationally (Levey et al., 2005), including in the UK (Burden et al., 2005; Department of Health, 2005). However, the current UK National Institute for Health and Clinical Excellence (NICE) guidelines (National Collaborating Centre for Chronic Conditions, 2008) introduced some relevant modifications (subdivision of stage 3 CKD into 3A and 3B; adoption of a "(p)" suffix to denote proteinuria at all stages of CKD). In fact, the definition and classification for CKD has been subject of extensive debate over the last decade. Recently, Kidney disease: Improving Global Outcomes (KDIGO) hosted a conference on the topic and, based on data from collaborative meta-analysis examining the relationship between eGFR and albuminuria with mortality (all-cause and cardiovascular) and kidney outcomes (ESRD, acute kidney injury and progressive CKD) in diverse populations, it was recommended to retain the current definition and to modify the NFK-KDOQI classification by adding albuminuria stage, subdivision of stage 3, and emphasizing clinical diagnosis (Levey et al., 2011). The CKD staging system is therefore expected to evolve in the very near future.

<sup>&</sup>lt;sup>3</sup> Urinary albumin:creatinine ratio  $\geq$  30 mg/mmol or protein:creatinine ratio  $\geq$  50 mg/mmol.

#### Chronic kidney disease: prevalence

Many countries have surveillance systems to monitor ESRD treated by RRT. Incidence and prevalence vary because of differences in underlying disease rates and treatment availability. In 2008, incidence was as high as 200 per million population (pmp) in many countries, and it was nearing 400 pmp in the US, Taiwan and some regions of Mexico. Of note, diabetes was the most common cause of ESRD is most countries, accounting for more than 40% of incident ESRD patients in several countries (US Renal Data System, 2010). In the UK, the number of adult patients starting RRT in 2008 was 108 pmp, with 24% of these patients having diabetes as the primary renal diagnosis (Byrne et al., 2010a). Prevalence has been increasing worldwide. By the end of 2008, Taiwan and Japan continued to report the greatest rates, at 2,311 and 2,126 pmp, respectively, followed by the US, at 1,752 pmp (US Renal Data System, 2010). In the UK, there were 47,525 adult patients receiving RRT at the end of 2008, equating to a prevalence of 774 pmp. This represents an annual increase in prevalence of approximately 4.4%, which has been fairly consistent over the last 10-15 years (Byrne et al., 2010b). Of note, over 2% of the total UK National Health Service (NHS) budget is spent on RRT, with costs of around £30,000 per year for each patient (Feehally et al., 2008).

Whilst these numbers clearly show the importance of early identification of CKD and, where possible, the prevention of progression to ESRD, they do not illustrate the total burden of CKD because patients with CKD are more likely to die than to progress to ESRD. For example, Keith *et al.* (2004) followed a representative population of 27,998 patients who had eGFRs < 90 ml/min/1.73 m<sup>2</sup>, and found that over a 5-year observation period, 1.1%, 1.3%, and 19.9% of, respectively, stage 2, 3, and 4 CKD patients progressed to RRT, while 19.5%, 24.3%, and 45.7% died. It is also important to highlight that data from the number of people undergoing RRT cannot even been seen as a surrogate of the number of people with stage 5 CKD, which can be illustrated by the fact that the mean eGFR at initiation of RRT in 2008 in the UK was 8.6 ml/min/1.73 m<sup>2</sup> (Byrne *et al.*, 2010*a*).

Estimates of the prevalence of early CKD have only started to emerge over the last decade. A systematic review of 26 population-based studies across Europe, Asia, North America, and Australia showed a median prevalence of CKD (defined as eGFR < 60 ml/min/1.73 m<sup>2</sup>) of 7.2% in patients older than 30 years and a prevalence ranging from 23.4% to 35.8% in those older than 64 vears (Zhang & Rothenbacher, 2008). In the UK, a large primary care study suggested an age-standardised prevalence of stages 3-5 CKD (i.e., eGFR < 60 ml/min/1.73 m<sup>2</sup>) of 8.5% (10.6% in females and 5.8% in males). In these patients, the age- and gender-adjusted odds ratio for hypertension, diabetes and cardiovascular disease were respectively, 2.1, 1.33, and 1.69. The prevalence of CKD rose dramatically with age (Stevens et al., 2007). It is noteworthy that these studies do not account for stages 1 and 2 CKD. Data from the National Health and Nutrition Examination Survey (NHANES) in the US not only provide estimates of the overall population prevalence of CKD (based on persistent albuminuria and decreased eGFR), but also indicate that the prevalence is increasing. Comparison of the prevalence of stages 1-4 CKD in NHANES 1998-1994 with NHANES 1999-2004 showed an increase in the population prevalence from 10.0 to 13.1%; the ageing population and increased prevalence of diabetes, hypertension, and obesity seem to account partially for this increase (Coresh et al., 2007). Although the estimates of the prevalence of CKD might be limited by selection bias, the criteria used to define CKD, and the methods used to estimate GFR and define kidney damage, they certainly raise much concern. CKD is now increasingly recognised as global public health problem (Levey et al., 2007).

#### Chronic kidney disease: causes and consequences

CKD has many potential pathologic causes, which can be grouped into diabetic and non-diabetic diseases. The latter can be further subdivided into vascular, glomerular, tubulointerstitial and cystic diseases. Some selected examples of causes of CKD are provided in Figure 1.1. A variety of kidney injuries may eventually progress to CKD. Disease may start in the tubules and interstitium (tubulointerstitial diseases), in the glomeruli (glomerular diseases) or in the renal vascular tree (vascular diseases), as a consequence of (i) systemic diseases such as diabetes and hypertension, (ii) autoimmune reactions and renal transplant rejection, (iii) the action of drugs, toxins and metals, (iv) infections, (v) mechanical damage, (vi) ischemia, (vii) obstruction of the urinary tract, (viii) primary genetic alterations, and (ix) undetermined causes. Yet, a number of conditions, like genetic cystic diseases, affect renal structures and function through mostly unspecific mechanisms, progressing into CKD for undetermined reasons. Whether started as glomerular, tubular or vascular damage, chronic progression eventually converges into common renal histological and functional alterations affecting most renal structures, which lead to progressive and generalized fibrosis and glomerulosclerosis. Once initiated, kidney injury gradually aggravates even in the absence of the triggering insult. Consistently with a common chronic phenotype, CKD can be diagnosed independently from the knowledge of its cause.

Diabetes and hypertension are the most common causes of CKD, and therefore major risk factors for the development and progression of CKD. According to the current UK NICE clinical guidelines, other risk factors for the development of CKD include: cardiovascular disease (ischaemic heart failure, peripheral vascular disease and cerebral vascular disease); structural renal tract disease, renal calculi or prostatic hypertrophy; multisystem diseases with potential kidney involvement (e.g. systemic lupus erythematosus); family history of stage 5 CKD or hereditary kidney disease. It is noteworthy that although age, gender, and ethnicity (South Asian and African Caribbean) might be associated with increased risk of CKD, their use as risk markers to test people for CKD is not recommended in the absence of the above risk factors. Likewise, in the absence of metabolic syndrome, diabetes or hypertension, obesity alone is not

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recommended as a risk marker to test people for CKD (National Collaborating Centre for Chronic Conditions, 2008).



**Figure 1.1. Classification and selected examples of causes of chronic kidney disease.** Reproduced from James *et al.* (2010).

Although ESRD is traditionally considered the most serious outcome of CKD, complications of CKD can occur at any stage and often lead to death with no progression to ESRD. Epidemiological studies have consistently shown that people with diagnosed CKD have a far greater likelihood of cardiovascular death than progression to ESRD (Drey *et al.*, 2003; Keith *et al.*, 2004; John *et al.*, 2004; Go *et al.*, 2004; Foley *et al.*, 2005). Hence, cardiovascular disease is regarded as the most frequent complication of CKD. Nevertheless, progressive deterioration of the excretory and endocrine functions of the kidney leads to complex disorders, which collectively constitute the uraemic syndrome. The clinical manifestations of uraemia are rather non-specific and may involve any system of the body. The principal clinical features of uraemia are summarised in Table 1.2.

Central nervous system	Diurnal somnolence, night insomnia, disorders of the memory and the concentration, asthenia, headache, confusion	
Peripheral nervous system	Polyneuritis, restless legs, cramps	
Gastrointestinal	Anorexia, nausea, gastroparesia, parotiditis, stomatitis	
Hematologic	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-microglobulin amyloidosis	
Nutrition	Malnutrition, weight loss, muscular catabolism	
Immunity	Low response to vaccination, increased sensitivity to infectious diseases	
Biochemical	Metabolic acidosis, hyperphosphatemia, hyperkaliemia	

 Table 1.2. Principal clinical features of uraemia.
 Adapted from Almeras & Argilés (2009).

### Immune dysfunction in chronic kidney disease

CKD is associated with profound alterations in both branches of the immune system, innate and adaptive immunity, resulting in a complex state of immune dysfunction (Cohen *et al.*, 1997; Girndt *et al.*, 2001*a*; Pesanti, 2001; Amore & Coppo, 2002; Descamps-Latscha *et al.*, 2002; Stenvinkel *et al.*, 2005; Chonchol, 2006; Eleftheriadis *et al.*, 2007; Hauser *et al.*, 2008; Kato *et al.*, 2008). Research in this area is vast and the available data suggest that, in general, no aspect of the immune system is unaffected. An in-depth review of the literature on the immunological disturbances associated with CKD is beyond the scope of this thesis. Overall, the most striking observation is that patients with CKD paradoxically present signs of immune depression and immune activation. The aim of this section is to illustrate this, providing examples that are related to the measurements made in this thesis. In addition, the potential role of the immune dysfunction that accompanies CKD as an underlying cause of morbidity and mortality in these patients in emphasised.

It should be acknowledged that a great part of the research in this area was conducted in ESRD, particularly haemodialysis, patients. Nevertheless, crosssectional studies that have included both patient groups have commonly reported intermediate disturbances in pre-dialysis patients in comparison with healthy subjects and haemodialysis patients. Moreover, the available correlational evidence between immunological alterations and kidney function further support this. Accordingly, it is well accepted that the immune alterations observed in CKD are multifactorial in origin and causes may include uraemia and its consequences and treatment, altered renal metabolism of immunologically active proteins, as well as specific effects of RRT, such as dialysis procedures.

Historically, the observation that uraemic patients showed extended survival of skin grafts (Dammin *et al.*, 1957) is often quoted as the first evidence of impaired host defences in CKD. Subsequently, several clinical manifestations, including increased susceptibility for infections (discussed below), cutaneous anergy in delayed-type hypersensitivity reactions to a broad panel of antigens and poor responses to vaccination, have established that CKD is clearly

associated with an immunodeficient state (Cohen *et al.*, 1997; Girndt *et al.*, 2001*a*; Pesanti, 2001; Eleftheriadis *et al.*, 2007).

Given the central role of the neutrophils in the host defence against infections, their defective function has been well characterised in the context of CKD, and neutrophil chemotaxis, adhesion, migration, phagocytosis and bactericidal activities (including degranulation), have all been shown to be impaired (Cohen *et al.*, 1997; Pesanti, 2001). On the other hand, neutrophils from CKD patients show clear signs of activation (e.g. elevated plasma elastase levels) and are know to exist in a primed state, which is associated with the chronic state of inflammation and oxidative stress that accompanies the disease (Sela *et al.*, 2005; Costa *et al.*, 2008; Caimi *et al.*, 2009; Pereira *et al.*, 2010; Polańska *et al.*, 2010).

T lymphocyte functional defects have also been widely reported in patients with CKD. These include reduced proliferation, reduced production of interleukin (IL)-2 and interferon- $\gamma$  and altered type 1/type 2 T helper cell balance (Girndt *et al.*, 2001*a*; Stenvinkel *et al.*, 2005; Eleftheriadis *et al.*, 2007). On the other hand T lymphocytes from CKD patients show increased expression of early activation markers (e.g. CD69 and CD25) and heightened apoptotic turnover (Stachowski *et al.*, 1991; Meier *et al.*, 2000; Ankersmit *et al.*, 2001; Meier *et al.*, 2002; Moser *et al.*, 2003; Meier *et al.*, 2005; Litjens *et al.*, 2006; Meier *et al.*, 2007; 2008).

It is currently recognised that the altered T lymphocyte function observed in CKD can be attributed to the defective interaction between the antigenpresenting cell (APC), such as the monocyte, and the T lymphocyte (Girndt *et al.*, 2001*a*; Eleftheriadis *et al.*, 2007; Kato *et al.*, 2008), because T cell activation is normal in the presence of APCs from healthy donors (Meuer *et al.*, 1987; Girndt *et al.*, 1993). T cell activation requires at least two signals. The interaction between major histocompatibility complex (MHC)-antigen peptide complex on the APC with the T cell receptor provides the first signal, while the second signal occurs through the interaction between co-stimulatory cell surface molecules (CD80/86) on the APC and CD28 on the T cell membrane. In the absence of co-stimulatory signalling, T cells fail to respond effectively and are rendered anergic (Sharpe & Freeman, 2002). The finding that the addition of heterologous APCs or stimulating antibodies against CD28 led to a normalised proliferative function of T lymphocytes from haemodialysis patients indicated that a disturbance in the co-stimulatory signalling between CD80/CD86 and CD28 could be responsible for the impaired T lymphocyte responses in CKD patients (Girndt et al., 1993). Latter, flow cytometry revealed reduced monocyte CD86 expression in haemodialysis patients, which was associated with the clinical immune defect (lower levels in non-responders than responders to hepatitis B vaccination), as well as with the *in vitro* lymphocyte proliferative response. Importantly, monocyte CD86 expression was not reduced in pre-dialysis patients (Girndt et al., 2001b). On the other hand, monocytes from CKD patients are "pre-activated" and overproduce proinflammatory cytokines, such as tumour necrosis factor (TNF)- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-8 (Girndt et al., 1995; Higuchi et al., 1997; Morita et al., 1997; Girndt et al., 1998; Sardenberg et al., 2004), which contributes to further impairment of the immune response (Girndt et al., 1995) but also to the chronic inflammation state that accompanies CKD (Stenvinkel et al., 2005; Carrero et al., 2008).

At the systemic level, markers of a chronically activated immune system, such as pro-inflammatory cytokines and acute-phase proteins (e.g. C-reactive protein (CRP)), are a constant finding in patients with CKD and it is now well established that CKD is associated with persistent inflammation (Carrero & Stenvinkel, 2010). Several causes may contribute to the chronic inflammatory state of patients with CKD but the increased production of cytokines (immune activation) coupled with their reduced renal clearance (the kidney is the major site for elimination of many cytokines) are likely to play a prominent role. In addition, recurrent infections (reflecting immune depression) further activate the immune system and induce inflammation. Importantly, this chronic inflammation state is associated with cardiovascular disease and other uraemic complications, such as protein energy wasting (Stenvinkel *et al.*, 1999; 2005; Carrero *et al.*, 2009; Carrero & Stenvinkel, 2009).

Cardiovascular disease is extremely prevalent and is the leading cause of death in CKD (Foley *et al.*, 1998; Parfrey & Foley, 1999; Vanholder *et al.*, 2005; Tonelli *et al.*, 2006; Saran & DuBose, 2008; Stenvinkel, 2010). In fact, CKD is a risk factor for cardiovascular disease (Sarnak *et al.*, 2003) and the presence of CKD worsens outcomes of cardiovascular disease (Weiner et al., 2004; Herzog et al., 2011). The risk of cardiovascular events is already elevated at early stages of CKD and it increases continuously as kidney function deteriorates (Go et al., 2004). The involvement of immunity and inflammation in cardiovascular disease is well documented (Libby, 2002; Hansson, 2005; Libby et al., 2009; Hansson & Hermansson, 2011; Libby et al., 2011). In CKD, this association has also been subject of great interest. It is noteworthy that the cardiovascular disease risk factor profile appears to be different in CKD compared with the general population. While "traditional" risk factors only partially explain the increased cardiovascular disease risk, the "novel" risk factors, which include inflammation markers, are not only highly prevalent in CKD but also more strongly associated with cardiovascular disease in these patients than in the general population (Stenvinkel et al., 2008). Of interest, it has been recently shown that plasma IL-6 concentration independently predicted overall and cardiovascular mortality in a cohort of patients at different CKD stages with greater prediction power than CRP, TNF- $\alpha$ , and albumin (Barreto *et al.*, 2010). This confirms the view that IL-6 is a better prognostic tool compared with other commonly measured markers of inflammation and oxidative stress previously reported in haemodialysis patients (Tripepi et al., 2005; Honda et al., 2006; Zoccali et al., 2006; Pachaly et al., 2008; Wetmore et al., 2008).

Although having received considerably less attention than cardiovascular disease, infectious complications are also a major cause of morbidity and mortality in CKD (Naqvi & Collins, 2006; Levey *et al.*, 2007; Foley, 2007; 2008; Dalrymple & Go, 2008). Infection has consistently ranked second to cardiovascular disease in causes of death in ESRD patients treated by dialysis. In the UK, infection and cardiovascular disease accounted, respectively, for 17% and 29% of deaths in the prevalent dialysis population in 2008 (Ansell *et al.*, 2010). Mortality rates secondary to sepsis and pneumonia are markedly higher in dialysis patients compared with the general population (Sarnak & Jaber, 2000; 2001). The epidemiology of infections at earlier stages of CKD has been less investigated but there is also evidence that pre-dialysis CKD are at increased risk for infections. Data from the US Renal Data System indicates that patients with diagnosed CKD have substantially higher rates of

hospitalisation for pneumonia, bacteraemia/sepsis and urinary tract infections, compared with patients without diagnosed CKD. In addition, patients with CKD have longer lengths of hospital stay during infection-related admissions compared with patients without CKD (Nagvi & Collins, 2006; Dalrymple & Go, 2008). Recent studies have confirmed the higher risk of infection-related hospitalisation and mortality among pre-dialysis CKD patients, and as expected it appears that the risk increases as kidney function decreases (James et al., 2008; 2009; Wang et al., 2011). Various factors may predispose patients with CKD to infectious complications (Dalrymple & Go, 2008) but the alterations in the immune system (discussed above) are the obvious major culprit. On the other hand, infections are typical inflammatory states, and accumulating evidence indicates that they are a common antecedent of new cardiovascular events in dialysis patients (Stenvinkel et al., 2002a; Zoccali et al., 2003; Ishani et al., 2005; Foley, 2006; Schiavoni et al., 2010; Dalrymple et al., 2011). The association between infection and cardiovascular disease is also documented in the general population (Kiechl et al., 2001; Nieto, 2002; Smeeth et al., 2004).

In summary, CKD is associated with a complex state of immune dysfunction. While immune depression contributes to the high risk of infection, immune activation leads to a state of chronic inflammation that contributes to the high risk of cardiovascular disease. In addition, recurrent infections also contribute to cardiovascular disease (probably through inducing chronic inflammation). On the other hand, persistent immune activation further worsens the already compromised immune response. Remarkably, infection and cardiovascular disease are major causes of morbidity and mortality in CKD. In this context it is worth mentioning a unique study in haemodialysis patients that, although not addressing the specific mortality causes, demonstrated that higher levels of circulating pro-inflammatory cytokines were associated with mortality, while immune parameters reflecting improved T cell function were associated with survival, independent of other medical risk factors (Kimmel *et al.*, 1998). Clearly, the immune dysfunction that accompanies CKD represents a major target for therapeutic interventions aiming to improve outcome in CKD.

### Can regular exercise help?

Physically active individuals are at lower risk of infection, particularly of the upper respiratory tract, in comparison with their sedentary counterparts (Matthews et al., 2002; Kostka & Praczko, 2007; Kostka et al., 2008; Nieman, 2011; Fondell et al., 2011). It should be acknowledged, however, that most of these studies rely on subject self-reported infection symptoms and only focus on upper respiratory tract infections. Evidence from studies of diagnosed and more severe infection is less compelling but also suggests that physical activity might confer some protection (Baik et al., 2000; Leveille et al., 2000; Neuman et al., 2010). Although a direct link still remains to be determined, the lower risk of infection in physically active individuals has been attributed, at least in part, to the effects of regular exercise on the immune system because several crosssectional studies (physically active vs. sedentary) and some limited evidence from longitudinal studies (exercise intervention) support the idea that regular moderate intensity exercise may enhance certain aspects of immune function (Woods et al., 2002; Kohut & Senchina, 2004; Senchina & Kohut, 2007; Haaland et al., 2008; Martin et al., 2009; Nieman, 2011).

It is well established that physical activity or physical fitness are associated with reduced cardiovascular disease risk (Berlin & Colditz, 1990; Erikssen, 2001; Wannamethee & Shaper, 2001; Blair *et al.*, 2001; Tanasescu *et al.*, 2002; Batty & Lee, 2004; Blair & Morris, 2009). Large cohort studies consistently show an inverse association between markers of systemic inflammation and physical activity or fitness, and data from several exercise intervention studies support that regular moderate intensity exercise reduces inflammation (Kasapis & Thompson, 2005; Bruunsgaard, 2005; Plaisance & Grandjean, 2006; Nicklas & Brinkley, 2009; Beavers *et al.*, 2010). Thus, it is now widely accepted that regular moderate intensity exercise reduces the risk of cardiovascular disease, in part because exercise exerts anti-inflammatory effects (Petersen & Pedersen, 2005; Wilund, 2007; Handschin & Spiegelman, 2008; Gleeson *et al.*, 2011).

Hence, regular exercise has the potential to counteract the immune dysfunction state that accompanies CKD and in this way contribute to a reduced risk of infection and cardiovascular disease.

#### Has this already been investigated in chronic kidney disease?

Although the potential anti-inflammatory effects of regular exercise in CKD patients have been recognised, research in this area is currently very limited, particularly in pre-dialysis CKD patients (Bronas, 2009). Published exercise intervention studies reporting effects of regular exercise on markers of systemic inflammation in patients with CKD are listed in Table 1.3. Only 2 studies have focused on pre-dialysis patients (Castaneda et al., 2004; Leehey et al., 2009). Castaneda et al. (2004) reported that 12-weeks of resistance exercise training reduced CRP and IL-6 levels, while Leehey et al. (2009) reported that 24-weeks of aerobic exercise training had no effect on CRP levels. Studies on haemodialysis patients have also yielded conflicting results. Exercise training reduced CRP levels in 5 studies (Załuska et al., 2002; Nindl et al., 2004; Cheema et al., 2007a; Afshar et al., 2010; 2011), while the other 3 reports have found no effect (Kopple et al., 2007; Toussaint et al., 2008; Wilund et al., 2010). Besides the lack of effect on CRP levels, Kopple et al. (2007) reported no effect of approximately 21-weeks of aerobic (intradialytic) or resistance (before dialysis) training or both on IL-6 and TNF- $\alpha$  levels, whilst Wilund *et al.* (2010) also reported no effect of 4-months of intradialytic aerobic exercise on IL-6 levels. However, in the study of Kopple et al. (2007) a trend for a reduction in CRP and IL-6 levels was apparent following aerobic exercise training but not after resistance exercise training or a combination of both regimens. Intriguingly, Cheema et al. (2011) have recently reported that 12-weeks of intradialytic progressive resistance training had no effect on TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-10 and IL-12 levels, while a reduction in CRP levels had been previously reported (Cheema et al., 2007a). Differences between the studies described above, including the length of the intervention, and the exercise regimen used make comparisons difficult, but is important to highlight that most of these studies had small sample sizes and systemic inflammatory markers have been secondary study outcomes. Apart from inflammatory markers, there is only a single report addressing the impact of regular exercise on immune parameters in haemodialysis patients (Daniilidis et al., 2004). These authors investigated the effects of a 6-months, mostly aerobic, exercise programme on some serum immunoglobulins (Igs), complement factors and ILs, as well as T

lymphocyte subsets (exercise group n=18 vs. control group n=14). Although the authors noted a few non-significant differences, the only significant difference reported in immune parameters was a reduction in serum IgA in the exercise group. In addition, it was found that patients in the exercise group had fewer upper respiratory tract infections than patients in the control group over the study period (6 out 18 patients in the exercise group vs. 8 out 14 patients in the control group, P < 0.05). The authors attributed the reduction in serum IgA to this low incidence of upper respiratory tract infection. Data from this study is obviously very limited.

Clearly, the impact of regular exercise on immune and inflammatory parameters in patients with CKD still remains uncertain.

Study details		Outcome
Pre-dialysis		
(Castaneda <i>et al.</i> , 2004)		
12-weeks resistance training (n=14) or control (n=12)		↓ CRP ↓ IL-6
(Leehey <i>et al.</i> , 2009)		
24-weeks aerobic training (n=7) or control (n=4)		NS CRP
Haemodialysis		
(Załuska <i>et al.</i> , 2002)		
6-months intradialytic cycling (n=10)		↓ CRP
(Nindl <i>et al.</i> , 2004)		
12-weeks resistance training (n=10)		↓ CRP
(Cheema <i>et al.</i> , 2007 <i>a</i> )*		
12-weeks intradialytic resistance training (n=24) or cont	rol (n=25)	↓ CRP
(Cheema <i>et al.</i> , 2011)*		
12-weeks intradialytic resistance training (n=13) or cont	rol (n=12)	NS INF-α NS IL-1β NS IL-6 NS IL-8, NS IL-10 NS IL-12
(Kopple <i>et al.</i> , 2007)		
21-weeks intradialytic cycling (n=10) or resistance train intradialytic cycling + resistance training (n=12) or contr	NS CRP NS TNF-α NS IL-6	
(Toussaint <i>et al.</i> , 2008)		
3-months intradialytic cycling + 3-months control (cross	NS CRP	
(Wilund <i>et al.</i> , 2010)		
4-months intradialytic cycling (n=8) or control (n=9)		NS CRP NS IL-6
(Afshar <i>et al.</i> , 2010)		
8-weeks intradialytic cycling (n=7) or intradialytic resis (n=7) or control (n=7)	↓ CRP	
(Afshar <i>et al.</i> , 2011)		
8-weeks intradialytic cycling (n=14) or control (n=14)		↓ CRP
*Same study but different number of samples assayed CRP: C-reactive protein IL: interleukin TNF: tumour necrosis fa		

Table 1.3. Exercise intervention studies reporting effects of regular exercise on markers of systemic inflammation in pre-dialysis and haemodialysis patients.

Chapter 2

**General Methods** 

### 2.1. Research design

The studies reported in this thesis were part of a collaborative research project between Loughborough University and Leicester General Hospital entitled "the effect of acidosis correction and exercise on tissue wasting and immune function in renal patients", which received prior approval by the UK National Health Service Research Ethics Committee. Originally this project was divided in three parts as follows: 1) pilot study of 1-month duration in patients receiving renal replacement therapy (RRT) by peritoneal dialysis (PD); 2) pilot study of 1month duration on patients with chronic kidney disease (CKD) who have not yet started RRT by dialysis; 3) main study of 6-months duration on PD patients or pre-dialysis CKD patients (depending on the outcome of studies 1 and 2). However, due to difficulties in recruiting PD patients the two pilot studies were merged into a single pilot study of 1-month duration for which both PD and predialysis CKD patients were recruited. In addition, the main study of 6-months duration was conducted on pre-dialysis CKD patients.

Patient recruitment was conducted by a nephrologist. Exercise testing and prescription were conducted by myself and another PhD student (whose research focused on muscle physiology). Blood sampling was carried out by myself or a nephrologist. All the analytical procedures reported in this thesis (including the methods development) were conducted by myself alone.

All patients were recruited from Nephrology Outpatient clinics at Leicester General Hospital. Exclusion criteria were age <18 years, pregnancy, and orthopaedic or cardiovascular disability that severely limited exercise capacity. Potential participants were fully informed both verbally and in writing about the purpose of the study and the experimental procedures to be performed, and were given at least 24 h to reach a decision. Patients willing to participate were then asked to provide written informed consent and underwent a medical examination to ensure their ability to participate in the study.
### 1-month uncontrolled study (Chapter 5)

Both PD patients and pre-dialysis CKD patients were recruited for the 1-month study. Thirty-three patients, including 7 PD patients and 26 pre-dialysis CKD patients, provided written informed consent to participate. A total of 25 patients, including 4 PD patients, completed the study. However, for the purpose of sample homogeneity, patients on PD were excluded from the analysis presented in this thesis. Therefore a total of 21 pre-dialysis patients were included in this study. All patients were assigned to an exercise programme of 1-month duration as detailed below. In addition, 10 of the patients included in this study were randomised to receive sufficient additional oral bicarbonate to raise plasma bicarbonate to approximately 29 mmol/l, while the others continued with their usual bicarbonate therapy (target plasma bicarbonate 24 mmol/l). Investigating the effect of the additional bicarbonate therapy is however beyond the scope of this thesis and therefore data from all the patients that participated in this study were pooled together. Nevertheless, a potential confounding effect from this therapy is addressed and data from patients on standard vs. additional bicarbonate therapy were also examined separately.

All patients performed an exercise test as described below at baseline and after 1-month of regular exercise. Resting venous blood samples were obtained on both occasions. Patients were asked to refrain from exercise during the preceding 24 h.

### 6-months controlled study (Chapter 6)

Forty pre-dialysis CKD patients were recruited for the 6-months study. The first 20 patients recruited were assigned to an exercise programme of 6-months duration as detailed below (15 of these patients were kept from the 1-month study, i.e. engaged in an additional period of 5-months of exercise training), while the other 20 patients continued with their habitual physical activity. In addition, 10 patients on each group were randomised to receive additional oral bicarbonate (target plasma bicarbonate 29 mmol/l) or to continue with their usual bicarbonate therapy (target plasma bicarbonate 24 mmol/l). Therefore, patients were assigned to four groups as follows: 1) control group (no exercise

and no additional bicarbonate therapy); 2) additional bicarbonate therapy only (no exercise); 3) exercise only (no additional bicarbonate therapy); and 4) exercise and additional bicarbonate therapy. However, as the scope of this thesis is to investigate the effect of exercise, the groups were collapsed irrespective of the additional bicarbonate therapy to form one exercise group and one non-exercise control group. Nevertheless, a potential confounding effect from the additional bicarbonate therapy was addressed by examining the data from patients in the exercise group as standard vs. additional bicarbonate therapy.

All patients performed an exercise test as described below at baseline and after 6-months. Resting venous blood samples were obtained on both occasions. Patients were asked to refrain from exercise during the preceding 24 h.

## Acute study (Chapter 4)

In addition to the above studies, the effect of acute exercise was also investigated. For this purpose two further venous blood samples were collected immediately after and 1 h after the baseline exercise test from all the patients that participated in the 1-month study but analysis were only conducted on patients that completed the study. However, patients on PD (n=4) and patients on immunosuppressive therapy (n=5) were excluded. In addition, 1 further patient was excluded because blood samples were not obtained following exercise. Therefore, a total of 15 pre-dialysis patients were included in this study.

### Pilot study (Chapter 3)

Before conducting the studies in patients with CKD, a preliminary investigation was conducted in healthy volunteers. However, as some procedures were still in development at that time, there are relevant disparities in relation to the procedures described here. Therefore, a full description of the methods used in that study is included in the respective individual chapter for clarity.

# 2.2. Exercise testing

At baseline, the exercise test consisted of 30 min walking on a motorised treadmill at a 1% gradient and at a speed that elicited a rating of perceived exertion (RPE) in the range of 12-14 ("somewhat hard") according to Borg (1982). RPE was recorded every 2 min and the treadmill speed was adjusted to maintain the RPE in the target range. The treadmill speed was also recorded every 2 min. At the end of each study (1-month or 6-months), the exercise test was repeated using exactly the same treadmill speed profile (i.e. same absolute exercise intensity) and the RPE response was recorded every 2 min.

## 2.3. Exercise programme

All patients that participated in the 1-month study and patients in the exercise group from the 6-months study were prescribed a home-based exercise programme, which consisted of at least 30 min walking, 5 times per week at a RPE in the range of 12-14 ("somewhat hard") for the total duration of the respective study (1-month or 6-months). As most CKD patients receive  $\beta$ -blocker therapy an overall heart rate (HR) range could not be used to prescribe the exercise intensity. Nevertheless, an individual HR range was established during the baseline exercise test by recording the HR response at the required RPE target range and this was also provided to each patient in conjunction with an HR monitor. For monitoring purposes, patients were asked to keep exercise session. In addition, patients were requested to attend the hospital gym once per month for a supervised exercise session to ensure compliance and to make any necessary adjustments to the exercise programme.

# 2.4. Blood sampling, handling and analysis

All venous blood samples, approximately 20 ml each, were collected by venepuncture from an antecubital vein using a 21 g butterfly needle cannula and a dry syringe, and immediately dispensed into two separate tubes as follows: approximately 7.5 ml into one S-Monovette tube (Sarstedt, Leicester, UK) containing K<sub>3</sub>EDTA (1.6 mg/ml) and approximately 12.5 ml into one universal tube containing heparin (16 units/ml).

## Haematology

For the following analysis K<sub>3</sub>EDTA-treated whole blood was used. Haemoglobin concentration was determined in duplicate using the cyanmethaemoglobin method. Haematocrit was determined by measuring packed cell volumes in triplicate on a microhaematocrit centrifuge. Plasma volume of resting blood samples was estimated from the haematocrit values. Plasma volume changes in post-exercise blood samples were estimated from the haemoglobin and haematocrit values according to Dill & Costill (1974) and all cell counts and plasma measurements were corrected for these changes relative to the resting blood sample in the acute study only (Chapter 4). Total and differential leukocyte counts were determined using an automated haematology analyser (Coulter Ac·T 5diff OV, Beckman Coulter, High Wycombe, UK).

### Plasma lactate

Duplicate 100  $\mu$ l aliquots of K<sub>3</sub>EDTA-treated whole blood were immediately deproteinised in 1ml ice-cold 0.3 M perchloric acid. These tubes were then centrifuged and the resulting supernatant was stored at -80°C for later fluorometric determination of plasma lactate concentration using the method described by Maughan (1982).

## Plasma markers of systemic inflammation

The remaining K<sub>3</sub>EDTA-treated whole blood was centrifuged at 1,500 g for 10 min in a refrigerated centrifuge at 4°C. The plasma obtained was aliquoted into

eppendorf tubes at 0.5 ml/tube and stored at -80°C for later determination of plasma concentrations of interleukin-6 (IL-6), interleukin-10 (IL-10), soluble tumour necrosis factor receptor I (sTNF-RI), soluble tumour necrosis factor receptor II (sTNF-RI) and C-reactive protein (CRP) by enzyme-linked immunosorbent assay (ELISA) as detailed in Section 2.5.

### Neutrophil degranulation

1 ml of heparinised whole blood was immediately added to an eppendorf tube containing 50 µl of 10 mg/ml bacterial extract solution (Stimulant, 84015, Sigma-Aldrich, Gillingham, Dorset, UK). The tube was sealed and blood and stimulant were mixed by gentle inversion and then incubated for 1 h at 37°C, being gently mixed again after 30 min. After incubation, the mixture was centrifuged for 2 min at 12,400 g and the resulting supernatant was stored at -80°C prior to analysis. A further 1 ml of heparinised whole blood was centrifuged at 1,500 g for 10 min in a refrigerated centrifuge at 4°C and the plasma obtained was stored at -80°C prior to analysis. Polymorphonuclear (PMN) cell elastase concentration was determined in both bacterially-stimulated and unstimulated (plasma elastase) samples using a commercially available ELISA kit (RD191021100, BioVendor GmbH, Heidelberg, Germany), according to the manufacturer's instructions. Bacterially-stimulated and unstimulated samples were pre-diluted 1:1000 and 1:50 in the dilution buffer provided, respectively. All samples were assayed in duplicate and all samples from the same patient were assayed in the same plate. The inter- and intra-assay coefficient of variation (CV) for all elastase ELISAs were 2.3% and 2.9%, respectively. Total bacterially-stimulated elastase release was calculated by subtracting plasma elastase concentration from the bacterially-stimulated elastase concentration. This was then divided by the neutrophil count to obtain the bacterially-stimulated elastase release per neutrophil.

### T-lymphocyte and monocyte activation

200 µl aliquots of heparinised whole blood were cultured in 12 x 75 mm polystyrene round bottom tubes with caps (BD Biosciences, Oxford, UK) with no

additive (unstimulated condition) or with 1 µg/ml staphylococcal enterotoxin B (SEB, S4881, Sigma-Aldrich, Gillingham, Dorset, UK). Triplicate tubes were set up for each condition and incubated at 37°C in a humid 5% CO<sub>2</sub> atmosphere for 20 h. After incubation, the whole blood aliquots were labelled in the respective culture tubes with cocktails of fluorochrome conjugated mouse monoclonal antibodies against human cell surface markers (all from BD Biosciences, Oxford, UK) as follows (one per condition): 1) lymphocyte surface markers: FITC conjugated anti-CD4 (555346), PE conjugated anti-CD69 (555531) and PE-Cy5 conjugated anti-CD8 (555368); 2) monocyte surface markers: FITC conjugated anti-CD14 (555397), PE conjugated anti-CD86 (555658) and PerCP conjugated anti-HLA-DR (347402); the remaining tube was left unstained. Labelling was carried out on ice for 20 min, and this was followed by erythrocyte lysis and leukocyte fixation, achieved by incubating samples for 10 min in the dark with FACS Lysing solution (BD Biosciences, Oxford, UK). Leukocytes were subsequently washed twice in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 2 mM EDTA and resuspended again in the same buffer for immediate flow cytometer acquisition. Samples were acquired on a flow cytometer (BD FACSCalibur, Oxford, UK) equipped with CellQuest software package (BD Biosciences, Oxford, UK). For samples labelled with lymphocyte markers, side scatter (SSC) vs. forward scatter (FSC) plots were used to gate on the lymphocyte population by morphology, and 30,000 lymphocytes were acquired per sample. For samples labelled with monocyte markers, 100,000 total cells were acquired per sample. Negative unstained control samples were also acquired. Software generated data files were stored for later analysis as detailed in Section 2.6. Flow cytometer settings were set up at the beginning of the study and kept constant for each patient samples.

### Peripheral blood mononuclear cell cytokine production

Peripheral blood mononuclear cells (PBMCs) were separated from approximately 5 ml of heparinised whole blood by density gradient centrifugation at 400 g for 30 min on Histopaque 1077 (Sigma-Aldrich, Gillingham, Dorset, UK). Cells were subsequently washed twice in sterile

isotonic salt solution (HBSS, Invitrogen, Paisley, UK, supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin and 10 mM HEPES) and resuspended in culture medium (RPMI 1640 with 2 mM L-Glutamine, Invitrogen, Paisley, UK, supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin, 100 µg/ml streptomycin and 50 mM HEPES). PBMCs were counted on a haemocytometer, and cell concentration adjusted to 10<sup>6</sup> PBMCs/ml. Aliquots of cells (200 µl) were cultured in flat bottom 96-well microplates in culture medium alone (unstimulated condition) or with 1 µg/ml SEB (S4881, Sigma-Aldrich, Gillingham, Dorset, UK). Quadruplicate wells were set up for each condition, and incubated at 37°C in a humid 5% CO<sub>2</sub> atmosphere for 8 days. After incubation, the microplates were centrifuged at 300 g for 10 min, and the resulting supernatants harvested and stored at -80°C for later determination of IL-6 and IL-10 concentrations by ELISA (detailed in Section 2.5). SEB-stimulated PBMCs IL-6 and IL-10 production were calculated by subtracting the respective unstimulated concentrations from the SEB-stimulated concentrations.

### Whole blood cytokine production

IL-6 concentration was also determined in the stimulated samples obtained as described above for the neutrophil degranulation assay by ELISA (using the method developed for culture supernatants detailed in Section 2.5). Bacterially-stimulated whole blood IL-6 production was calculated by subtracting plasma IL-6 concentration from the bacterially-stimulated concentration.

### 2.5. Enzyme-linked immunosorbent assays

### Interleukin-6 and interleukin-10 assays

ELISAs for detection of IL-6 and IL-10 levels in plasma samples were developed and optimised using the capture and detection antibodies included in BD OptEIA ELISA sets (IL-6: 555220 and IL-10: 555157, BD Biosciences, Oxford, UK), National Institute for Biological Standards and Control (NIBSC) standards (IL-6: 89/548 and IL-10: 93/722, NIBSC, Potters Bar, Hertfordshire, UK) and an ELISA Amplification System (19589-109, Invitrogen, Paisley, UK). 96-well ELISA plates (Nunc-Immuno 439454 MaxiSorp) were coated with the anti-human IL-6 or IL-10 capture antibody diluted at the lot-specific recommended dilution in 0.05 M sodium carbonate buffer at 100 µl/well, sealed and incubated overnight at 4°C. The next day, plates were washed 3 times (at 300 µl/well) with tris-buffered saline (TBS) with 0.05% Tween 20 (TBS-T) and blocked for 1 h at room temperature with 5% BSA (Probumin, Millipore, Illinois, USA) in TBS at 200 µl/well. Plates were subsequently washed 3 times with TBS-T and duplicates of samples (neat), standards (serially diluted in TBS with 10% FCS from 200 pg/ml to 0.78 pg/ml) and blank (TBS with 10% FCS) were added at 100 µl/well. Plates were again sealed and incubated overnight at 4°C. The following day, plates were washed 6 times with TBS-T and the biotinylated anti-human IL-6 or IL-10 detection antibody diluted at the lot-specific recommended dilution in TBS-T with 1% BSA was added at 100 µl/well. Plates were incubated for 2 h at room temperature and subsequently washed 7 times with 30 seconds soaks with TBS-T. Streptavidin (SAv)-alkaline phosphatase conjugate (554065, BD Biosciences, Oxford, UK) diluted 1:2000 in TBS with 1% BSA was then added at 100 µl/well and plates were incubated for a further 1 h at room temperature. After a final wash (7 times with 30 seconds soaks with TBS-T), the substrate and amplifier solutions (prepared according to the manufacturer's instructions) were added (at 50 µl/well each) in two subsequent steps (incubated for approximately 25 min at 25°C each). The reaction was stopped with 0.3 M sulphuric acid (at 50 µl/well) and the plates were immediately read at 490 nm. Samples' concentrations were determined by relation to a standard curve generated by plotting the standards' absorbances

against the log of the standards' concentrations using a four-parameter logistic equation (Graphpad Prism version 5, Graphpad Software, La Jolla, CA, USA). All samples from the same patient were assayed on the same plate. Duplicates with a CV above 10% were repeated. The inter- and intra-assay CV were 4.4% and 3.4% for IL-6 and 5.1% and 3.8% for IL-10, respectively.

ELISAs for detection of IL-6 and IL-10 levels in culture supernatants were developed and optimised using BD OptEIA ELISA sets (IL-6: 555220 and IL-10: 555157, BD Biosciences, Oxford, UK), which include capture and detection antibodies, SAv-horseradish peroxidase (HRP) conjugate and standards. 96well ELISA plates (Nunc-Immuno 439454 MaxiSorp) were coated with the antihuman IL-6 or IL-10 capture antibody diluted at the lot-specific recommended dilution in 0.05 M sodium carbonate buffer at 65 µl/well, sealed and incubated overnight at 4°C. The next day, plates were washed 3 times (at 300 µl/well) with PBS with 0.05% Tween 20 (PBS-T.05) and blocked for 1 h at room temperature with 10% FCS in PBS at 100 µl/well. Plates were subsequently washed 3 times with PBS-T.05 and duplicates of samples (neat and diluted 1:5 in PBS for unstimulated and SEB-stimulated samples, respectively), standards (serially diluted in PBS with 10% FCS from 600 pg/ml to 0.6 pg/ml and 1 ng/ml to 1 pg/ml for IL-6 and IL-10 assays, respectively) and blank (PBS with 10% FCS) were added at 50 µl/well. Plates were again sealed and incubated overnight at 4°C. The following day, plates were washed 5 times with PBS-T.05 and the working detector (biotinylated anti-human IL-6 or IL-10 detection antibody + SAv-HRP, both diluted at the lot-specific recommended dilutions in PBS with 10% FCS) was added at 50 µl/well. Plates were incubated for 1 h 30 min at room temperature and subsequently washed 7 times with 30 seconds soaks with PBS-T.05. Tetramethylbenzidine (TMB) substrate (1-Step Ultra TMB-ELISA, Pierce, Illinois, USA) was then added at 50 µl/well and plates were incubated for 30 min in the dark at room temperature. The reaction was stopped with 2 M sulphuric acid (50 µl/well) and the plates were immediately read at 450 nm. Samples' concentrations were determined by relation to a standard curve generated by plotting the log of the standards' absorbances against the log of the standards' concentrations using a four-parameter logistic equation (Graphpad Prism version 5, Graphpad Software, La Jolla, CA, USA). All samples from the same patient were assayed on the same plate. Duplicates with a CV above 10% were repeated, as were samples that fell beyond the dynamic range of each assay standard curve (adjusting the dilution factor accordingly). The inter- and intra-assay CV were 3.8% and 2.5% for IL-6 and 5.0% and 3.4% for IL-10, respectively.

#### Soluble tumour necrosis factor receptors I and II assays

ELISAs for detection of sTNF-RI and sTNF-RII in plasma samples were developed and optimised using the capture and detection antibodies and standards included in R&D DuoSet ELISA Development kits (sTNF-RI: DY225 and sTNF-RII: DY726, R&D Systems, Abingdon, UK). 96-well ELISA plates (Nunc-Immuno 439454 MaxiSorp) were coated with the anti-human sTNF-RI or sTNF-RII capture antibody (previously reconstituted according to the manufacturer's instructions) diluted 1:200 in 0.05 M sodium carbonate buffer at 65 µl/well, sealed and incubated overnight at 4°C. The next day, plates were washed 4 times (at 200 µl/well) with PBS with 0.1% Tween 20 (PBS-T.1) and blocked for 1 h at room temperature with 1% BSA (Probumin, Millipore, Illinois, USA) in PBS at 100 µl/well. Plates were subsequently washed 4 times with PBS-T.1 and duplicates of samples (diluted 1:10 in PBS), standards (serially diluted in PBS with 1% BSA from 10 ng/ml to 10 pg/ml and 18 ng/ml to 18 pg/ml for sTNF-RI and sTNF-RII assays, respectively) and blank (PBS with 1% BSA) were added at 50 µl/well. Plates were again sealed and incubated overnight at 4°C. The following day, plates were washed 4 times with PBS-T.1 and the biotinylated anti-human sTNF-RI or sTNF-RII detection antibody (previously reconstituted according to the manufacturer's instructions) diluted 1:200 in PBS was added at 50 µl/well. Plates were incubated for 2 h at room temperature and subsequently washed 4 times with PBS-T.1. HRP Avidin D (A-2004, Vector Laboratories, Peterborough, UK) diluted 1:2000 in PBS was then added at 50 µl/well and plates were incubated for a further 1 h at room temperature. After a final wash (4 times with PBS-T.1), an OPD substrate solution (S2045, Dako, Glostrup, Denmark), prepared according to the manufacturer's instructions, was added at 50 µl/well. Approximately 5 min after or when suitable colour has developed, the reaction was stopped with 1 M sulphuric acid (at 75 µl/well) and

the plates were immediately read at 490 nm. Samples' concentrations were determined by relation to a standard curve generated by plotting the standards' absorbances against the log of the standards' concentrations using a four-parameter logistic equation (Graphpad Prism version 5, Graphpad Software, La Jolla, CA, USA). All samples from the same patient were assayed on the same plate. Duplicates with a CV above 10% were repeated, as were samples that fell beyond the dynamic range of each assay standard curve (adjusting the dilution factor accordingly). The inter- and intra-assay CV were 3.6% and 2.6% for sTNF-RI and 4.5% and 2.9% for sTNF-RII, respectively.

### C-reactive protein assay

An ELISA for detection of CRP levels in plasma samples was developed and optimised using a method adapted from Pawluczyk et al. (2011). 96-well ELISA plates (Nunc-Immuno 439454 MaxiSorp) were coated with anti-human CRP rabbit polyclonal antibody (235752, Calbiochem, Merck Chemicals, Nottingham, UK) diluted to a final concentration of 5 µg/ml in 0.05 M sodium carbonate buffer at 65 µl/well, sealed and incubated overnight at 4°C. The next day, plates were washed 4 times (at 200 µl/well) with PBS-T.1 and blocked for 1 h at room temperature with 1% BSA in PBS at 100 µl/well. Plates were subsequently washed 4 times with PBS-T.1 and duplicates of samples (diluted 1:100 in PBS), CRP standards (85/506, NIBSC, Potters Bar, Hertfordshire, UK, serially diluted in PBS with 1% BSA from 1 µg/ml to 1 ng/ml) and blank (PBS with 1% BSA) were added at 50 µl/well. Plates were again sealed and incubated overnight at 4°C. The following day, plates were washed 4 times with PBS-T.1 and antihuman CRP mouse monoclonal antibody (ab8279, Abcam, Cambridge, UK) diluted 1:750 in PBS was added at 50 µl/well. Plates were incubated for 2 h at room temperature and subsequently washed 4 times with PBS-T.1. HRP conjugated anti-mouse immunoglobulins rabbit polyclonal antibody (P0260, Dako, Glostrup, Denmark) diluted 1:1000 in PBS was then added at 50 µl/well and plates were incubated for a further 1 h at room temperature. After a final wash (4 times with PBS-T.1), an OPD substrate solution (S2045, Dako, Glostrup, Denmark), prepared according to the manufacturer's instructions, was added at 50 µl/well. Approximately 5 min after or when suitable colour has

developed, the reaction was stopped with 1 M sulphuric acid (at 75 µl/well) and the plates were immediately read at 490 nm. Samples' concentrations were determined by relation to a standard curve generated by plotting the standards' absorbances against the log of the standards' concentrations using a fourparameter logistic equation (Graphpad Prism version 5, Graphpad Software, La Jolla, CA, USA). All samples from the same patient were assayed on the same plate. Duplicates with a CV above 10% were repeated, as were samples that fell beyond the dynamic range of each assay standard curve (adjusting the dilution factor accordingly). The inter- and intra-assay CV were 3.2% and 2.9%, respectively.

## 2.6. Flow cytometry data analysis

Flow cytometry data analysis was performed using FlowJo version 9 for Macintosh (Tree Star, Ashland, OR, USA). All data files from the same patient were analysed on the same day.

## T-lymphocyte analysis

SSC vs. FSC plots of all cells acquired were used to gate on the lymphocyte population by morphology (Figure 2.1 a). Subsequent SSC vs. FL1 or FL3 plots of the lymphocyte population were used to gate, respectively, on the CD4 positive population (CD4<sup>+</sup>, helper T-cells; Figure 2.1 b) or on the CD8 brightly positive population (CD8<sup>++</sup>, cytotoxic T-cells; Figure 2.1 c). This gating strategy for cytotoxic T-cells was used because it is known that all lymphocytes that express CD8 at high fluorescence intensities also express CD3 and that this population forms the majority of the total cytotoxic T-cell blood pool, while on the other hand, lymphocytes that express CD8 at low fluorescence intensities also include natural killer cells (Simpson et al., 2007; Campbell et al., 2008). Absolute numbers of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes were obtained by multiplying the percentages of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes by the corresponding total lymphocyte count (obtained as described in section 2.4). This analysis was carried for both unstimulated and SEB-stimulated samples, but as there were no significant differences between both, only SEB-stimulated data are presented. Further FL2 vs. FL1 or FL3 plots of CD4<sup>+</sup> or CD8<sup>++</sup> lymphocytes, respectively, were used to determine the percentage the CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes expressing CD69 and the geometric mean of fluorescence intensity (GMFI) of CD69 in these cells (Figure 2.1 d, e, f and g). The unstimulated samples were used to define the threshold of positive staining and only SEB-stimulated data are reported.



Figure 2.1. T-lymphocyte flow cytometry data analysis.

## Monocyte analysis

FL2 vs. FL1 plots of all cells acquired were used to gate on the CD14<sup>+</sup>CD86<sup>+</sup> cells (Figure 3.2 a). Subsequent FL3 vs. FL1 plots of the CD14<sup>+</sup>CD86<sup>+</sup> cells were used to gate on the CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> cells (Figure 3.2 b). Backgating analysis was then performed to ensure the gated cells fell on the monocyte region by morphology on SSC vs. FSC plots (Figure 3.2 c). Further FL2 or FL3 histogram plots of CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes were used to determine, respectively, the GMFI of CD86 and HLA-DR in these cells (Figure 3.2 d and f). Negative unstained controls were used to define the threshold of positive staining. This analysis was carried for both unstimulated and SEB-stimulated samples and SEB-stimulated data are reported as a ratio to the unstimulated condition.



Figure 2.2. Monocyte flow cytometry data analysis.

# 2.7. Statistical analysis

Statistical analysis was performed using PASW statistics version 18 (SPSS, Chicago, IL, USA). All statistical procedures are detailed in each individual chapter.

# 2.8. Glossary of key immune and inflammatory variables

**Leukocytes** (white blood cells): heterogeneous cells found in the circulation and various tissues with diverse functions related to the immune response.

**Neutrophil:** granulocyte characterised by its multi-lobed nucleus and granular cytoplasm; also called a polymorphonuclear phagocyte; acts non-specifically and so forms part of the innate immune system; involved in defence against bacteria.

**Lymphocyte:** mononuclear immune cell involved in the adaptive immune response; subpopulations of lymphocytes include T cells and B cells; NK cells are a further example of lymphocytes, but these are involved in the innate immune response.

**Monocyte:** circulating mononuclear phagocytic leukocyte that can ingest and destroy foreign material but also capable of antigen-presentation (initiation of the acquired immune response); differentiates into a macrophage upon migration into tissue.

**T cells:** heterogeneous population of lymphocytes comprising helper T cells and cytotoxic/supressor T cells.

**Helper T cell**: subset of T cell capable of recognising antigen and producing several cytokines that activate other immune cells; expresses the cluster designation marker **CD4** on its surface.

**Cytotoxic T cell:** subset of T cell capable of killing certain tumour and virally infected cells; expresses the cluster designation marker **CD8** on its surface.

**CD69:** lymphoid activation cell surface marker whose rapid expression makes it amenable for the early detection of T cell activation and for subset activation analysis.

**CD14**: cell surface marker that is preferentially expressed on monocytes/ macrophages.

**CD86** (B7-2): cell surface molecule that provides a co-stimulatory signal necessary for T cell activation; ligand for CD28 and CTLA-4 on the T cell surface.

**HLA-DR**: major histocompatibility complex (MHC) class II cell surface molecule; MHC molecules are involved in antigen presentation to T cells. Class I MHC proteins are present on virtually all nucleated cells, whereas class II MHC proteins are expressed on antigen-presenting cells.

**Degranulation:** the process by which neutrophils release their cytotoxic granules (e.g. elastase) into the phagolysosomal vacuole, formed in phagocytosis.

**C-reactive protein (CRP)**: acute phase protein released in response to inflammation and infection to help with tissue repair.

**Acute phase proteins**: heterogeneous class of serum glycoproteins that are released by liver cells in response to pro-inflammatory cytokines and increase in concentration during inflammation and infection.

Interleukin (IL)-6: pro-inflammatory cytokine.

Interleukin (IL)-10: anti-inflammatory cytokine.

**Soluble tumour necrosis factor receptors (sTNF-Rs):** soluble form of the natural occurring inhibitors of tumour necrosis factor (TNF), a pro-inflammatory cytokine.

**Cytokine:** soluble factor produced by many cells; involved in communication between immune cells, and between immune cells and other tissues/organs; cytokines include interleukins (IL), tumour necrosis factors (TNF), colony-stimulating factors (CSF) and interferons (IFN).

**Peripheral blood mononuclear cells (PBMCs):** mainly lymphocytes and monocytes.

# Chapter 3

Effect of an acute bout of moderate intensity aerobic exercise and *ex vivo* extracellular acidosis on T lymphocyte and monocyte activation in healthy volunteers

## 3.1. Abstract

The initial aim of this study was to pilot, in healthy volunteers, the exercise test protocol used in the later studies in patients with chronic kidney disease (CKD). In addition, given that metabolic acidosis and immune dysfunction are features of CKD but few studies have focused on the effect of extracellular pH on immune function, this study aimed to investigate the effect of *ex vivo* extracellular acidosis on T lymphocyte and monocyte activation before and after exercise.

Ten (5 males and 5 females) recreationally active healthy subjects (age  $25 \pm 2$  yrs; body mass  $66.8 \pm 9.2$  kg) ran or walked for 30 min on a motorised treadmill at a 1% gradient and at a speed that elicited a rating of perceived exertion in the range of 12-14 ("somewhat hard"). Venous blood samples were collected before and after exercise. Peripheral blood mononuclear cells were cultured in acidotic (pH 7.1) vs. neutral medium (pH 7.4) and T lymphocyte subset (CD4<sup>+</sup> and CD8<sup>+</sup>) activation (CD69 expression) and monocyte (CD14<sup>+</sup>HLA-DR<sup>+</sup>) activation (CD86 expression) were determined by flow cytometry following 20 h *in vitro* stimulation by staphylococcal enterotoxin B (SEB) and tetanus toxoid plus influenza vaccines (TT+FLU). Data were examined using two-factor repeated measures ANOVAs (exercise x medium). Fluorescence intensity for a surface antigen was calculated as geometric mean (GMFI) of all positive-staining cells and expressed as a ratio to unstimulated cells.

CD86 expression by CD14<sup>+</sup>HLA-DR<sup>+</sup> monocytes was greater on cells cultured in acidotic vs. neutral medium with both stimuli (main effect of medium; SEB:  $1.5 \pm 0.5$  vs.  $1.3 \pm 0.3$ , P < 0.05; TT+FLU:  $1.3 \pm 0.5$  vs.  $1.1 \pm 0.5$ , P < 0.01) but there was no significant effect of exercise. There were no significant effects of exercise or medium on CD69 expression by CD4<sup>+</sup> or CD8<sup>+</sup>T lymphocytes for both stimuli.

These findings suggest that *ex vivo* extracellular acidosis up-regulates CD86 expression by  $CD14^{+}HLA-DR^{+}$  monocytes yet has negligible effects on CD69 expression by either  $CD4^{+}$  or  $CD8^{+}$  lymphocytes. In addition, the moderate exercise protocol used does not appear to affect T lymphocyte and monocyte activation.

# 3.2. Introduction

The initial aim of this study was to pilot, in healthy volunteers, the exercise test protocol used in the later studies in patients with chronic kidney disease (CKD) and to establish the "normal" immune response to this protocol. Specifically, we determined the feasibility of achieving the desired rating of perceived exertion (RPE) range by manipulating the treadmill speed during the exercise test and investigated the effect of this moderate intensity aerobic exercise bout on total and differential blood leukocyte counts and T lymphocyte and monocyte activation. It was hypothesised that moderate intensity aerobic exercise would induce modest alterations in immune cells counts and would exert little influence on T lymphocyte and monocyte activation.

In addition, given that metabolic acidosis (Kopple *et al.*, 2005; Kraut & Kurtz, 2005) and immune dysfunction (Cohen *et al.*, 1997; Pesanti, 2001) are features of CKD and that few studies have focused on the effect of extracellular pH on immune cells and their function (Lardner, 2001; Kellum *et al.*, 2004), the present study was also designed to investigate the effect of *ex vivo* extracellular acidosis on T lymphocyte and monocyte activation before and after exercise.

## 3.3. Methods

### Subjects

Ten (5 males and 5 females) recreationally active healthy subjects (mean  $\pm$  SD: age 25  $\pm$  2 years; height 171  $\pm$  8 cm; body mass 66.8  $\pm$  9.2 kg) volunteered to participate in the study, which was approved by the Loughborough University Ethics Committee. All subjects were informed about the rationale for the study and the nature of the experimental procedures to be performed before providing written informed consent. Subjects were also required to complete a comprehensive health-screening questionnaire before taking part in the study. They did not report any symptoms of infection in the previous 4 weeks and none were currently on medication. Three of the subjects had received the others have been previously immunised against any strain of the virus. All of the subjects had received the tetanus vaccination as part of their immunisation programme.

### Preliminary testing

Prior to the experimental trial, each subject performed a 30 min familiarisation trial on a motorised treadmill (Run Race, TechnoGym UK Ltd, Bracknell, UK) at a 1% constant gradient to determine the walking/running speed that elicited a RPE in the range of 12-14 ("somewhat hard"). Subjects began walking/running at 5 km/h. RPE was recorded every 3 min using the Borg scale (Borg, 1982) and the treadmill speed was adjusted accordingly until a RPE in the target range was established. Heart rate (HR) was also recorded every 3 min using short-range radio telemetry (TechnoGym UK Ltd, Bracknell, UK).

#### **Experimental trial procedures**

For the main trial, subjects reported to the laboratory between 8:00 and 9:00 AM following an overnight fast. They were instructed to avoid strenuous exercise during the preceding 24 h. On arrival, subjects' body mass was recorded and a resting venous blood sample was obtained whilst seated. Subjects then began the exercise test protocol, which consisted of 30 min of

walking/running on the same treadmill used above at a 1% constant gradient and at the speed determined during the familiarisation trial. RPE and HR were recorded every 3 min using the methods described above and the treadmill speed was adjusted if necessary. Samples of expired air (1 min) were collected into Douglas bags after 12 min of exercise and every 6 min thereafter. An  $O_2/CO_2$  analyser (Servomex 1400B, Crowborough, UK) was used along with a dry gas meter (Harvard Apparatus, Edenbridge, UK) for the determination of  $O_2$ uptake ( $\dot{V}O_2$ ), CO<sub>2</sub> production ( $\dot{V}CO_2$ ) and the respiratory exchange ratio (RER). During the trial subjects were given *ad libitum* access to water and the volume ingested was noted. Immediately after exercise, a further venous blood sample was obtained with the subject seated before body mass was recorded. Laboratory conditions were 24.1 ± 0.7°C ambient temperature and 32 ± 6% relative humidity.

### **Blood sampling**

Venous blood samples (20 ml each) were obtained from an antecubital vein using a 21 g butterfly needle cannula and syringe, and were dispensed into three separate S-Monovette tubes (Sarstedt, Leicester, UK), one containing K<sub>3</sub>EDTA (1.6 mg EDTA/ml blood) and two containing lithium heparin (16 IU heparin/ml blood).

### Total and differential leukocyte counts and plasma volume

Blood collected into the K<sub>3</sub>EDTA S-Monovette tube (7.5 ml capacity, 5 ml blood) was used for haematological analysis, including haemoglobin, haematocrit and total and differential leukocyte counts using an automated haematology analyser (A<sup>c</sup> · T 5diff analyser, Beckman Coulter, UK). Plasma volume changes were estimated from the haemoglobin and haematocrit values according to Dill & Costill (1974) and cell counts were corrected for plasma volume changes relative to the resting blood sample.

## Cell cultures

Peripheral blood mononuclear cells (PBMCs) were separated from 15 ml blood collected into the two lithium heparin S-Monovette tubes (7.5 ml capacity each) by density gradient centrifugation on Histopaque 1077 (Sigma-Aldrich, Gillingham, Dorset, UK), washed twice in sterile isotonic salt solution (HBSS, Invitrogen, Paisley, UK, supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin and 10 mM HEPES) and resuspended at approximately 2 x 10<sup>6</sup> PBMCs/ml in culture medium (MEM Gibco 21090, Invitrogen, Paisley, UK, supplemented with 2 mM L-Glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin and 2% dialysed fetal bovine serum) at different pH: neutral (additionally containing 8.3 mM NaHCO<sub>3</sub> and 24.8 mM NaCL; pH 7.4) and acidotic (additionally containing 6.5 mM HCl and 33.2 mM NaCL; pH 7.1) medium. Aliquots of cells (1 ml) were cultured in flat-bottomed 24-well plates in each medium alone (Unstim), with 1 µg/ml staphylococcal enterotoxin B (SEB, Sigma-Aldrich, Gillingham, Dorset, UK), or with a mixture of adsorbed tetanus vaccine BP (Wellcome, London, UK) and influenza vaccine (Influvac sub-unit 2006/7, Solvay Healthcare, Southampton, UK) both at 1:500 final dilution (TT+FLU). Cells were incubated at 37°C in a humid 5% CO<sub>2</sub> atmosphere.

## Staining and flow cytometry

After 20 h in culture, PBMCs from each culture condition were harvested. The cells were pelleted, the supernatant removed and the cells were resuspended in ice-cold phosphate-buffered saline containing 0.1% bovine serum albumin and 2mM EDTA (PBS-BSA-EDTA). Aliquots of cells were labelled with cocktails of the following monoclonal antibodies against human cell surface markers (all obtained from BD Biosciences, Oxford, UK): lymphocyte surface markers FITC anti-CD4, PE-Cy5 anti-CD8, and PE anti-CD69; monocyte surface markers FITC anti-CD14, PerCP anti-HLA-DR and PE anti-CD86. Labelling was carried out on ice for 20 min and the cells subsequently washed once in PBS-BSA-EDTA, after which they were fixed with FACS Lyse (BD Biosciences, Oxford, UK) and resuspended again in PBS/BSA/EDTA. Samples were analysed on a flow cytometer (BD FACSCalibur, Oxford, UK) equipped with the CellQuest software package (BD Biosciences, Oxford, UK). For samples labelled with

lymphocyte markers, side scatter versus forward scatter plots were used to gate on the lymphocyte population by morphology, and 30,000 lymphocyte events were acquired per analysis. The percentage of lymphocytes expressing CD4 and CD8 was derived from histogram analysis of FL1 and FL3, respectively. CD4<sup>+</sup> and CD8<sup>+</sup> cells were gated into separate regions, and FL2 histogram plots of the cells in each of these regions were used to determine the percentage of the CD4<sup>+</sup> and CD8<sup>+</sup> cells expressing CD69 and the fluorescence intensity of CD69 in these cells. For samples labelled with monocyte markers, 100,000 cell events in total were acquired per analysis. FL1 and FL3 histograms were used to gate on CD14<sup>+</sup> and HLA-DR<sup>+</sup> cells, respectively, and a FL2 histogram plot of CD14<sup>+</sup>HLA-DR<sup>+</sup> cells was used to determine the fluorescence intensity of CD86. Negative unstained controls were used to define the threshold of positive staining for all surface markers. Fluorescence intensity for a surface antigen was calculated as geometric mean (GMFI) of all positive-staining cells and expressed as a ratio to unstimulated cells.

### Statistical analysis

All data are presented as mean values and standard deviations ( $\pm$  SD). Data were checked for normality and if a data set was not normally distributed, statistical analysis was performed on the logarithmic transformation of the data. Exercise test data, including RPE, HR,  $\dot{V}O_2$  and RER, were examined using a one-factor (time) repeated measures analysis of variance (ANOVA). Haematological data, including plasma volume and total and differential leukocyte counts were analysed using Student's paired t-tests (pre- vs. post-exercise). Flow cytometry data were examined using a two-factor [exercise (pre- and post-exercise) x medium (neutral and acidotic)] repeated measures ANOVA. For the ANOVAs, assumptions of homogeneity and sphericity in the data were checked and appropriate adjustments in the degrees of freedom were made according to Atkinson (2001). Any significant *F* ratios subsequently shown were assessed by using Student's paired t-tests with Bonferroni correction for multiple comparisons applied to the unadjusted *P* value. However, for the two-factor ANOVA if there was no significant interaction but significant

main effects were found, the main effects only are reported. Statistical significance was accepted at P < 0.05.

## 3.4. Results

### Treadmill speed

The mean treadmill speed obtained from the familiarisation trial and therefore the speed at the onset of the main trial exercise test was  $8.3 \pm 1.7$  km/h, ranging from 6.0 to 11.0 km/h. Only minor adjustments in the speed were required throughout the exercise test to ensure target RPE. Specifically, four of the subjects required slight speed increments during the initial stages of the exercise test, while speed was reduced for two subjects during the last stages of the exercise test. Treadmill speed at 15 and 30 min of exercise was  $8.4 \pm 1.7$  and  $8.3 \pm 1.9$  km/h, respectively.

### Rating of perceived exertion

RPE increased with exercise duration (main effect of time:  $F_{2,4} = 11.0$ , P < 0.001; Figure 3.1), yet significant differences were only found between RPE at 3 min and at 15, 24, and 27 min (P < 0.05 for all). RPE also tended to be lower at 3 min than at 18 (P = 0.078), 21 (P = 0.058), and 30 min (P = 0.079). At 3 and 6 min, respectively five and three of the subjects were exercising below the required target RPE range of 12-14. With the consequent treadmill speed adjustments, all subjects were exercising within this range from 9 min until the end of the exercise test.



Figure 3.1. Subjective rating of perceived exertion (RPE) in response to the exercise test. Data are mean  $\pm$  SD (n=10); main effect of time: P < 0.001, \*P < 0.05 vs. 15, 24 and 27 min.

### Heart rate, oxygen uptake and respiratory exchange ratio

HR increased with exercise duration (main effect of time:  $F_{3,5} = 29.4$ , P < 0.001; Figure 3.2). HR at 3, 15, and 30 min was  $142 \pm 10$ ,  $155 \pm 12$ , and  $161 \pm 12$  beats/min, respectively. A main effect of time ( $F_{1,6} = 5.2$ , P < 0.05; Table 3.1) was also found for  $\dot{VO}_2$  with values increasing as a function of exercise duration, yet no significant differences between any time points were found (Table 3.1). Additionally, RER was similar throughout the exercise (Table 3.1).



Figure 3.2. Heart rate (HR) in response to the exercise test. Data are mean  $\pm$  SD (n=10); main effect of time: P < 0.001.

Table 3.1. Oxygen uptake mean and respiratory exchange ratio in response to the exercise test. Data are mean  $\pm$  SD (n=10).

Time (min)							
18	24	30					
Oxygen uptake (I/min)* <sup>t</sup>							
1.96 ± 0.73	1.99 ± 0.72	2.05 ± 0.74					
Respiratory exchange ratio							
0.93 ± 0.11	0.92 ± 0.07	0.92 ± 0.06					
	Time ( 18 Oxygen upta 1.96 ± 0.73 Respiratory ex 0.93 ± 0.11	Time (min)   18 24   Oxygen uptake (l/min)*t   1.96 ± 0.73 1.99 ± 0.72   Respiratory exchange ratio   0.93 ± 0.11 0.92 ± 0.07					

main effect of time: \*<sup>t</sup> P < 0.05

### Body mass and plasma volume

Following exercise, body mass when corrected for fluid intake fell by  $0.4 \pm 0.2$  kg, varying between 0.2 and 0.7 kg. Likewise, plasma volume fell by  $3.9 \pm 4.6\%$  after exercise (*P* < 0.05).

### Total and differential blood leukocyte counts

Total and differential blood leukocyte counts are shown in Table 3.2. After exercise, total leukocyte concentration was elevated by 26% above resting levels (P < 0.005). Similarly, numbers of circulating neutrophils, lymphocytes and monocytes at post-exercise were 22, 35, and 28% respectively, higher than at pre-exercise (P < 0.05, P < 0.005 and P < 0.01, respectively).

	Pre-exercise	Post-exercise		
Leukocytes (x 10 <sup>9</sup> /l)	5.0 ± 1.1	6.3 ± 1.6***		
Neutrophils (x 10 <sup>9</sup> /l)	2.4 ± 0.8	2.9 ± 1.3*		
Lymphocytes (x 10 <sup>9</sup> /l)	1.8 ± 0.4	2.5 ± 0.5***		
Monocytes (x 10 <sup>9</sup> /l)	0.6 ± 0.2	0.7 ± 0.2**		

Table 3.2. Total and	differential	leukocyte	counts	in response	to the	exercise	test.	Data
are mean ± SD (n=10)		-		-				

\*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.005 vs. pre-exercise

### T lymphocyte subsets

There was no significant exercise x medium interaction and no significant main effect of medium for the % of CD4<sup>+</sup> cells in any condition. However, there was a significant main effect of exercise for all conditions (Unstim:  $F_{1,0} = 8.4$ , P < 0.05; SEB:  $F_{1,0} = 16.5$ , P < 0.01; TT+FLU:  $F_{1,0} = 17.8$ , P < 0.005; Table 3.3), with the % of CD4<sup>+</sup> cells decreasing following exercise (marginal means pre vs. post-exercise Unstim:  $35 \pm 8$  vs.  $31 \pm 8\%$ ; SEB:  $35 \pm 6$  vs.  $30 \pm 7$ ; TT+FLU:  $34 \pm 6$  vs.  $29 \pm 7\%$ ). There was neither significant exercise x medium interaction nor significant effects of medium or exercise for the % of CD8<sup>+</sup> cells in any condition (Table 3.3).

	Pre-exercise		Post-exercise		
	Neutral	Acidotic	Neutral	Acidotic	
% CD4⁺					
Unstim* <sup>e</sup>	35 ± 8	35 ± 7	31 ± 9	31 ± 8	
SEB** <sup>e</sup>	35 ± 6	34 ± 6	30 ± 8	31 ± 8	
TT+FLU*** <sup>e</sup>	34 ± 6	34 ± 7	28 ± 8	29 ± 6	
% CD8⁺					
Unstim	28 ± 10	29 ± 9	30 ± 13	30 ± 11	
SEB	29 ± 10	28 ± 10	31 ± 13	29 ± 11	
TT+FLU	32 ± 10	32 ± 11	34 ± 13	32 ± 12	

Table 3.3. Percentages of CD4<sup>+</sup> and CD8<sup>+</sup> cells following 20h culture in acidotic vs. neutral medium with different stimuli and in response to the exercise test. Data are mean  $\pm$  SD (n=10).

main effect of exercise:  $*^{e} P < 0.05$ ,  $**^{e} P < 0.01$  and  $***^{e} P < 0.005$ 

# Activation of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes

There was no significant exercise x medium interaction for the % of CD4<sup>+</sup> cells expressing CD69 in any condition, yet there was a significant main effect of exercise for Unstim ( $F_{1,0} = 5.7$ , P < 0.05; marginal means pre- vs. post-exercise:  $5 \pm 4$  vs.  $4 \pm 4$ %; Table 3.4). There was also a significant main effect of medium for Unstim ( $F_{1,0} = 7.5$ , P < 0.05; marginal means neutral vs. acidotic:  $5 \pm 4$  vs.  $4 \pm 5$ %; Table 3.4) and TT+FLU ( $F_{1,0} = 9.8$ , P < 0.05; marginal means neutral vs. acidotic:  $16 \pm 8$  vs.  $13 \pm 7$ %; Table 3.4). No significant interaction between exercise and medium or significant main effects of medium or exercise for the % of CD8<sup>+</sup> cells expressing CD69 were found for any condition (Table 3.4). There was no significant exercise x medium interaction or significant effects of medium or exercise for CD69 expression (GMFI) on both CD4<sup>+</sup> and CD8<sup>+</sup> cells in any condition (Figures 3.3 and 3.4).

	Pre-exercise		Post-exercise		
_	Neutral	Acidotic	Neutral	Acidotic	
% CD4 <sup>+</sup> CD69 <sup>+</sup>					
Unstim* <sup>e</sup> * <sup>m</sup>	5 ± 5	5 ± 6	4 ± 4	4 ± 5	
SEB	50 ± 22	45 ± 15	47 ± 19	44 ± 19	
TT+FLU* <sup>m</sup>	15 ± 8	14 ± 7	16 ± 8	12 ± 7	
% CD8⁺CD69⁺					
Unstim	5 ± 5	5 ± 6	4 ± 4	4 ± 5	
SEB	50 ± 22	45 ± 15	47 ± 20	44 ± 19	
TT+FLU	15 ± 8	14 ± 7	16 ± 8	12 ± 7	

Table 3.4. Percentages of  $CD4^+$  and  $CD8^+$  cells expressing CD69 following 20h culture in acidotic vs. neutral medium with different stimuli and in response to the exercise test. Data are mean  $\pm$  SD (n=10).

main effect of exercise: \*<sup>e</sup> *P* < 0.05 main effect of medium: \*<sup>m</sup> *P* < 0.05



Figure 3.3. CD69 expression (geometric mean fluorescence intensity expressed as a ratio to unstimulated cells) on CD4<sup>+</sup> cells following 20h culture in acidotic vs. neutral medium with a) SEB and b) TT+FLU and in response to the exercise test. Data are mean  $\pm$  SD (n=10).



Figure 3.4. CD69 expression (geometric mean fluorescence intensity expressed as a ratio to unstimulated cells) on CD8+ cells following 20h culture in acidotic vs. neutral medium with a) SEB and b) TT+FLU and in response to the exercise test. Data are mean  $\pm$  SD (n=10).
## Activation of CD14<sup>+</sup>HLA-DR<sup>+</sup> monocytes

There was no significant exercise x medium interaction and no significant effect of exercise for CD86 expression on CD14<sup>+</sup>HLA-DR<sup>+</sup> cells in any condition. However, there was a main effect of medium for both SEB ( $F_{1,0} = 6.8$ , P < 0.05; Figure 3.5a) and TT+FLU ( $F_{1,0} = 16.0$ , P < 0.01; Figure 3.5b), with CD86 expression on CD14<sup>+</sup>HLA-DR<sup>+</sup> cells being up-regulated by acidotic medium (marginal means neutral vs. acidotic SEB:  $1.3 \pm 0.3$  vs.  $1.5 \pm 0.5$ ; TT+FLU:  $1.1 \pm 0.5$  vs.  $1.3 \pm 0.5$ ).



Figure 3.5. CD86 expression (geometric mean fluorescence intensity expressed as a ratio to unstimulated cells) on CD14<sup>+</sup>HLA-DR<sup>+</sup> cells following 20h culture in acidotic vs. neutral medium with a) SEB (main effect of medium: P < 0.05) and b) TT+FLU (main effect of medium: P < 0.01) and in response to the exercise test. Data are mean  $\pm$  SD (n=10).

## 3.5. Discussion

The initial aim of this study was to pilot the exercise test protocol used in the later studies in patients with CKD. Additionally, in an attempt to mimic the CKD patients' acidotic milieu, T lymphocyte and monocyte activation by different stimuli were assessed by flow cytometry in PBMCs isolated from pre- and post-exercise blood samples and cultured in acidotic vs. neutral media. The main findings of the present study were that *ex vivo* extracellular acidosis up-regulated CD86 expression by CD14<sup>+</sup>HLA-DR<sup>+</sup> monocytes, yet had negligible effects on CD69 expression by either CD4<sup>+</sup> or CD8<sup>+</sup> T lymphocytes. In addition, the moderate exercise protocol used in the present study did not appear to have an effect on these activation markers.

The exercise protocol appears to have successfully induced the required response with only some of the subjects requiring minor adjustments of the treadmill speed to elicit the target RPE. Moreover, the adjustments made appear to be more related with the exercise time, with slight increases necessary at the early stages of exercise for some subjects and small decreases needed at the late stages for others. However, it is important to highlight that in the present study the treadmill speed was previously determined in a familiarisation trial and although this would also be ideal with the patients it was not possible due to logistical and financial restrictions. Therefore, it was decided that in the patients' studies we should record the RPE at smaller time intervals, i.e. every 2 min, to ensure that the required RPE is attained as quickly as possible.

The exercise protocol used in the present study only caused a modest elevation in the total number of circulating leukocytes, which in turn reflected similar relative increases in the numbers of circulating neutrophils, lymphocytes and monocytes. Furthermore, none of the cell counts exceeded the normal ranges. This is accordance with Nieman *et al.* (2005) where only mild and transient increases in total and differential leukocyte counts have been reported following a similar exercise protocol of 30 min of walking at 60-65% of  $\dot{VO}_{2max}$ . This response is not unexpected, since the magnitude of the exercise induced elevation in total and differential leukocyte counts is proportional to the intensity and duration of the exercise performed (Nieman *et al.*, 1994; Robson *et al.*, 1999). One limitation of the present study was that blood samples were not taken during the recovery from exercise and therefore we were not able to determine how long the cell counts remained elevated above pre-exercise levels. It is likely that total and differential leukocyte counts would have returned to resting levels within 1 hour following exercise of the intensity and duration used, as observed in Nieman *et al.* (2005). Nevertheless, we concluded that this needed to be assessed in the patients' studies and it was decided that one further blood sample should be obtained at 1 h post-exercise.

The main finding of this study was the higher CD86 expression by CD14<sup>+</sup>HLA-DR<sup>+</sup> monocytes cultured for 20 h in acidotic (pH 7.1) vs. neutral (pH 7.4) medium, with this result being consistent for both SEB and TT+FLU stimulated cells. Similar effects were reported by Vermeulen *et al.* (2004), who observed increased expression of CD86 by dendritic cells exposed to pH 6.5 compared with pH 7.3. This effect appears to be related to the length of exposure to the acidotic condition, because these effects were observed after 4 h and 24 h of incubation, but not after 30 min exposure. Interestingly, in CKD defective expression of CD86 on monocytes of dialysis but not pre-dialysis patients was reported (Girndt *et al.*, 2001*b*). We might be tempted to suggest that the results of the present study indicate that this defective CD86 expression in dialysis patients is not caused by acidosis. However, the present findings cannot be extrapolated to CKD. It is important to highlight that metabolic acidosis in CKD is a chronic condition and in the present study cells were only exposed to *ex vivo* acute acidosis.

CD86 (B7-2) is a molecule that provides a co-stimulatory signal necessary for T cell activation. Activation of T cells requires at least two signals: the primary signal occurs through the engagement of the T cell receptor (TCR) by the major histocompatibility complex (MHC)-antigen peptide complex on the antigen-presenting cell (APC) such as monocyte or dendritic cell; the secondary signal occurs through the engagement of CD28 on the T cell by B7-1 (CD80) or B7-2 (CD86) co-stimulatory molecules on the APC (Bretscher, 1999). Both signals are required for production of an effective immune response and in the absence

of CD28 co-stimulation, T cell receptor signalling alone results in anergy (Sharpe & Freeman, 2002). In the present study CD86 expression was assessed on CD14<sup>+</sup>HLA-DR<sup>+</sup> monocytes, i.e. CD14<sup>+</sup> monocytes expressing MHC class II molecule, and therefore monocytes involved in the activation of CD4<sup>+</sup> T cells. We might therefore have expected that the up-regulation of the co-stimulatory CD86 signal in acidotic medium would have resulted in an enhanced CD4<sup>+</sup> T cell activation. However, the results of the present study were not able to show this and no effect of extracellular acidosis on CD69 expression (a marker of T cell activation) by CD4<sup>+</sup> T cells was observed. In fact, the percentage of CD4<sup>+</sup> cells expressing CD69 was actually lower in acidotic compared with neutral medium for unstimulated and TT+FLU-stimulated cells but not for SEB. Although there is no clear explanation for this, it seems reasonable to note that the statistical main effects observed take into account the pre and post-exercise samples together, which might induce in erroneous conclusions about the effect of medium itself since the percentages of CD4<sup>+</sup> cells decrease considerably following exercise. To address this we have investigated the effect of medium independently on the pre-exercise samples and have found no differences in the percentages of CD4<sup>+</sup>CD69<sup>+</sup> cells between acidotic and neutral medium for any condition (data not shown). This suggests that the effects observed here are related to alterations in cell number. Furthermore, it should be emphasised that T cell activation involves a far more complex signalling cascade than the simplistic description presented above. It is interesting to note that cytotoxic T lymphocyte antigen 4 (CTLA-4) competes with CD28 for B7 ligands, such as CD86, and in fact it has much higher affinity than CD28 for these molecules. B7 engagement of CTLA-4 down-regulates T cell activation (Sansom, 2000). Therefore, although in the present study CD86 was up-regulated by acidotic medium, its higher affinity with CTLA-4 might have contributed to a down-regulation of T cell activation.

The moderate exercise protocol used in the present study had negligible effect on CD69 expression by CD4<sup>+</sup> and CD8<sup>+</sup> cells. Although there was a significant main effect of exercise for the percentage of CD4<sup>+</sup> cells expressing CD69 in unstimulated cells, this is probably just an effect of the decrease in CD4<sup>+</sup> cells observed following exercise. Previous studies that have determined CD69 expression on CD4<sup>+</sup> and CD8<sup>+</sup> cells following 75 min of cycling at approximately 75% of  $\dot{VO}_{2max}$  (Rønsen *et al.*, 2001) or 60 min of treadmill running at 95% of ventilatory threshold (Green *et al.*, 2003), have also found no effect of exercise. However, decreases in the CD69 expression on both CD4<sup>+</sup> and CD8<sup>+</sup> cells immediately after an incremental treadmill test to exhaustion have also been reported (Vider *et al.*, 2001). The present study support the findings of Rønsen *et al.* (2001) and Green *et al.* (2003). The inconsistent findings reported by Vider *et al.* (2001) are probably due to the nature of the exercise challenge that aimed to achieve exhaustion.

CD86 expression by CD14<sup>+</sup>HLA-DR<sup>+</sup> monocytes was also unaffected by the moderate exercise protocol used in the present study. This is in contrast to the study of Lancaster *et al.* (2005), which have reported a decrease of CD86 expression by CD14<sup>+</sup> monocytes following 1.5 h of cycling at 55% of maximal work rate at 34°C. The reasons of this inconsistency may be due to the obvious differences in exercise duration and intensity or in the ambient conditions.

In summary, the findings of the present study suggest that *ex vivo* extracellular acidosis up-regulates CD86 expression by CD14<sup>+</sup>HLA-DR<sup>+</sup> monocytes, yet has negligible effects on CD69 expression by either CD4<sup>+</sup> or CD8<sup>+</sup> T lymphocytes. In addition, the moderate exercise protocol used in the present study appears to have no effect on T lymphocyte and monocyte activation. However, it is important to note that the effects of *ex vivo* acidosis in healthy participants do not necessarily reflect the situation of the *in vivo* acidotic milieu in CKD.

# Chapter 4

Effect of an acute bout of moderate intensity aerobic exercise on immunity and inflammation in chronic kidney disease patients

## 4.1. Abstract

Chronic kidney disease (CKD) is associated with a complex state of immune dysfunction characterised by immune depression and immune activation (inducing inflammation). In healthy people, acute moderate exercise usually exerts little influence on immune and inflammatory responses, but it is not known what effect it may have in CKD patients given their compromised immunity and inflammatory status. Therefore, this study investigated the effects of an acute bout of moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients.

Fifteen (12 males and 3 females) pre-dialysis stage 3-5 CKD patients (age 59  $\pm$  10 yrs; BMI 27.1  $\pm$  5.1 kg/m<sup>2</sup>; eGFR 18.3  $\pm$  7.3 ml/min/1.73 m<sup>2</sup>) walked for 30 min on a motorised treadmill at a 1% gradient and at a speed that elicited a rating of perceived exertion in the range of 12-14 ("somewhat hard"). Venous blood samples were collected before, immediately after and 1 h after exercise. CD4<sup>+</sup> and CD8<sup>++</sup> lymphocyte activation (CD69 expression) and CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocyte activation (HLA-DR and CD86 expression) were determined by flow cytometry following 20 h *in vitro* stimulation by SEB. Elastase release from unstimulated (plasma elastase) and bacterially-stimulated neutrophils, and plasma inflammatory markers (IL-6, IL-10, sTNF-Rs and CRP) were determined by ELISA. Data were examined using one-factor repeated measures ANOVAs and Student's paired t-tests (Holm-Bonferroni correction).

Exercise had no effect on CD4<sup>+</sup> or CD8<sup>++</sup> lymphocyte activation (CD69) and CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocyte activation (HLA-DR and CD86). Plasma elastase concentration was elevated immediately after exercise, yet values returned to resting levels 1 h after exercise. Bacterially-stimulated elastase release per neutrophil did not change immediately after exercise but increased by 1 h after exercise. In addition, exercise induced elevations in the plasma concentrations of IL-6 (peaked immediately after), IL-10 (peaked 1 h after) and sTNF-RII (only elevated 1 h after) but had no effect on sTNF-RI and CRP levels.

These findings indicate that acute moderate aerobic exercise in pre-dialysis CKD patients seems to be safe from an immune and inflammatory perspective and it appears to improve immunosurveillance as well as to exert anti-inflammatory effects.

## 4.2. Introduction

Chronic kidney disease (CKD) is associated with profound alterations in the innate and adaptive immunity as described in Chapter 1. This leads to a complex state of immune dysfunction, in which signs of immune depression and immune activation paradoxically coexist (Descamps-Latscha *et al.*, 2002; Eleftheriadis *et al.*, 2007; Kato *et al.*, 2008). While functional immune cell deficiencies predispose patients with CKD to infectious complications, persistent immune cell activation contributes to a state of chronic inflammation that is associated with increased cardiovascular disease risk among these patients. Remarkably, infection and cardiovascular disease are leading causes of morbidity and mortality in CKD (Naqvi & Collins, 2006; Dalrymple & Go, 2008; Saran & DuBose, 2008; Stenvinkel, 2010).

In healthy people, acute moderate exercise usually exerts little influence on immune and inflammatory responses, but acute strenuous exercise (intensive and/or prolonged) exerts a temporary depression on many aspects of the immune function and induces an inflammatory response (Gleeson, 2007; Walsh *et al.*, 2011). However, little is known about the effects of acute exercise on immune and inflammatory measures in immunocompromised individuals and/or persons with a chronic inflammatory condition.

To our knowledge, no previous research has addressed the effects of acute exercise on immune and inflammatory parameters in patients with CKD. This is somewhat surprising because if we are to advocate exercise into the routine clinical care of patients with CKD, it seems paramount to determine if exercise is safe to their underlying compromised immunity and inflammatory status. In addition, examining the effects of acute exercise may elucidate some of the mechanisms behind the benefits that regular exercise participation may confer.

Therefore, the aim of this study was to investigate the effects of an acute bout of moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients.

# 4.3. Methods

# Patients

Patients that completed the 1-month study (n=25) were eligible for this study, i.e. blood samples were obtained following the baseline exercise test. However, patients on PD (n=4) and patients on immunosuppressive therapy (n=5) were excluded. In addition, 1 further patient was excluded because blood samples were not obtained following exercise. Therefore, a total of 15 pre-dialysis patients were included in this analysis and their characteristics are shown in Table 4.1.

Table 4.1	. Patients'	characteristics	(n=15)	).
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Gender (n males / n females)	12 / 3
Age* (years)	59 ± 10
Weight* (kg)	80 ± 15
Height* (cm)	172 ± 10
BMI* (kg/m²)	27.1 ± 5.1
<b>eGFR*</b> (ml/min/1.73 m <sup>2</sup> )	18.3 ± 7.3
CKD stage <sup># a)</sup> Stage 3 Stage 4 Stage 5	1 (7%) 8 (53%) 6 (40%)
Primary renal disease <sup># b)</sup> Renal Vascular Disease Cystic/poly Glomerulonephritis Diabetes Pyelonephritis Uncertain	2 (13%) 2 (13%) 3 (20%) 2 (13%) 2 (13%) 4 (27%)
Number of comorbidities <sup>#</sup> 1 2 3 4	5 (33%) 6 (40%) 1 (7%) 3 (20%)

Data are \*mean ± SD or <sup>#</sup>n (%)

BMI: body mass index Da eGFR: estimated glomerular filtration rate CKD: chronic kidney disease <sup>a)</sup> based on eGFR <sup>b)</sup> grouped according to UK Renal Registry (2010)

#### Experimental and analytical procedures

All patients performed an exercise test as described in Section 2.2. Venous blood samples were obtained at rest (pre-exercise), immediately after the exercise test (post-exercise) and 1 h into the recovery period (1 h post-exercise). All analytical procedures are detailed in Chapter 2.

#### Statistical analysis

Data are presented as mean ± standard deviation (SD) unless otherwise stated. Data were inspected for normality using the Shapiro-Wilk test. If a data set was not normally distributed, statistical analysis was performed on the logarithmic transformation of the data, but data were back transformed for presentation. Outliers were defined as values deviating more than 3 SDs from the mean and were excluded from analysis. Data were examined using a one-factor (time: pre-exercise, post-exercise, 1 h post-exercise) analysis of variance (ANOVA) with repeated measures design. For any significant unadjusted F values subsequently shown, sphericity of the data were determined using the Mauchly's test and appropriate adjustments in the degrees of freedom were made according to Atkinson (2001), i.e. Greenhouse-Geisser correction applied when Greenhouse-Geisser epsilson < 0.75 or Huynh-Feldt correction used for less severe asphericity. A priori planned Student's paired t-tests with Holm-Bonferroni correction for multiple comparisons applied to the unadjusted P value were then performed between specific time points (post-exercise vs. preexercise and 1 h post-exercise vs. pre-exercise) and effect sizes for these comparisons were calculated according to Cohen (1988) with correction for sample size (Hedges, 1981), by dividing the difference between means by the pooled SD. Statistical significance was accepted at P < 0.05.

## 4.4. Results

#### Exercise test outcomes

The average RPE response to exercise was  $13 \pm 1$ , and this was achieved at an average walking speed of  $4.4 \pm 1.5$  km/h (range 2.1 to 8.1 km/h). The RPE response to exercise at 2 min intervals is illustrated in Figure 4.1. Walking speed at 2, 16 and 30 min was  $2.9 \pm 0.9$ ,  $4.6 \pm 1.6$  and  $4.7 \pm 1.8$  km/h, respectively. There was an effect of exercise on plasma lactate concentration (Figure 4.2;  $F_{1,1} = 17.2$ , P = 0.001). Plasma lactate concentration was elevated above pre-exercise levels at post-exercise (P = 0.006, ES = 1.36) but it had returned to pre-exercise levels by 1 h post-exercise.



Figure 4.1. Rating of perceived exertion (RPE) response to exercise. Data are mean  $\pm$  SD (n=15).



Figure 4.2. Plasma lactate response to exercise. Data are mean and individual values (n=13).

#### Total and differential blood leukocyte counts

Total and differential blood leukocyte counts in response to exercise are shown in Table 4.2. Exercise induced an increase in total leukocyte concentration ( $F_{1,8}$ = 10.3, P = 0.001), which was more pronounced at post-exercise (P = 0.0001, ES = 0.44) but still observable at 1 h post-exercise (P = 0.010, ES = 0.27). These effects were mainly attributable to the observed effects of exercise on the numbers of circulating neutrophils ( $F_{1,4}$  = 10.7, P = 0.002) and lymphocytes ( $F_{2,0}$  = 13.0, P < 0.0005), as there was no effect of exercise on monocyte count. Neutrophil concentration was elevated above pre-exercise levels at postexercise (P = 0.0004, ES = 0.35) and to a greater extent at 1 h post-exercise (P= 0.002, ES = 0.47). Lymphocyte concentration was also elevated at postexercise (P = 0.005, ES = 0.53) but it had returned to pre-exercise levels by 1 h post-exercise.

	Pre-ex	Post-ex	1 h post-ex
Leukocytes (x 10 <sup>9</sup> /l)	6.6 ± 1.9	7.5 ± 1.9*	7.2 ± 2.2*
Neutrophils (x 10 <sup>9</sup> /l)	4.1 ± 1.4	4.6 ± 1.4*	4.9 ± 1.7*
Lymphocytes (x 10 <sup>9</sup> /l)	1.6 ± 0.6	1.9 ± 0.6*	1.5 ± 0.6
Monocytes (x 10 <sup>9</sup> /l)	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.2

Table 4.2. Total and differential blood leukocyte counts in response to exercise.

Data are mean ± SD (n=15)

\**P* < 0.05 vs. pre-ex

#### T lymphocyte subsets

Exercise induced alterations in the T lymphocyte subsets relative distribution, i.e.  $CD4^+/CD8^{++}$  ratio (Table 4.3;  $F_{2,0} = 18.1$ , P < 0.0005), which in comparison with pre-exercise was lower at post-exercise (P = 0.005, ES = 0.26) and higher at 1 h post-ex (P = 0.035, ES = 0.21). This effect was mostly due to the observed effect of exercise on  $CD8^{++}$  lymphocyte count (Table 4.3;  $F_{2,0} = 19.6$ , P < 0.0005), which was elevated at post-exercise (P = 0.001, ES = 0.24) but fell slightly below pre-exercise count at 1 h post-exercise (P = 0.040, ES = 0.16). There was no effect of exercise on  $CD4^+$  lymphocyte count (Table 4.3).

# Activation of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes

Exercise had no effect on the percentages of CD4<sup>+</sup> or CD8<sup>++</sup> lymphocytes expressing CD69 following 20 h *in vitro* SEB stimulation (Table 4.3). There were also no effects of exercise on the SEB-stimulated CD69 expression (GMFI) by either CD4<sup>+</sup>CD69<sup>+</sup> or CD8<sup>++</sup>CD69<sup>+</sup> lymphocytes (Table 4.3).

Table 4.3.  $CD4^+$  and  $CD8^{++}$  lymphocyte counts and  $CD4^+/CD8^{++}$  ratio, percentages of  $CD4^+$  and  $CD8^{++}$  lymphocytes expressing CD69 and CD69 expression by  $CD4^+CD69^+$  and  $CD8^{++}CD69^+$  lymphocytes after 20 h *in vitro* stimulation with staphylococcal enterotoxin B (SEB) in response to exercise.

	Pre-ex	Post-ex	1 h post-ex
T lymphocyte subsets			
CD4 <sup>+</sup> (x 10 <sup>9</sup> /l)	$0.5 \pm 0.3$	$0.6 \pm 0.3$	$0.5 \pm 0.3$
CD8 <sup>++</sup> <b>#</b> (x 10 <sup>9</sup> /l)	0.4 ± 0.3	0.5 ± 0.4*	0.3 ± 0.2*
CD4 <sup>+</sup> /CD8 <sup>++</sup> #	1.9 ± 1.2	1.7 ± 1.2*	2.2 ± 1.4*
CD69 <sup>+</sup> cells (%)			
CD4 <sup>+</sup>	23 ± 10	23 ± 11	23 ± 11
CD8 <sup>++</sup>	24 ± 11	26 ± 14	24 ± 11
<b>CD69</b> (GMFI)			
CD4 <sup>+</sup> CD69 <sup>+</sup>	141 ± 49	132 ± 42	143 ± 53
CD8 <sup>++</sup> CD69 <sup>+</sup> #	106 ± 51	101 ± 55	109 ± 60

GMFI: geometric mean of fluorescence intensity \*Statistical analysis performed on log-transformed data

 ity
 Data are mean ± SD (n=15)

 med data
 \*P < 0.05 vs. pre-ex</td>

# Activation of CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes

CD86 and HLA-DR expression (GMFI) by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes following 20 h *in vitro* SEB stimulation (expressed as a ratio to unstimulated condition) were also not affected by exercise (Table 4.4).

 Table 4.4. CD86 and HLA-DR expression by CD14+CD86+HLA-DR+ monocytes after 20 h

 *in vitro* stimulation with staphylococcal enterotoxin B (SEB) in response to exercise.

	Pre-ex	Post-ex	1 h post-ex
<b>CD86</b> (GMFI*)	1.9 ± 0.9	1.8 ± 0.8	1.9 ± 0.8
HLA-DR (GMFI*)	2.2 ± 1.0	2.2 ± 0.9	$2.2 \pm 0.9$

GMFI: geometric mean of fluorescence intensity \*Ratio to unstimulated condition

Data are mean ± SD (n=15)

#### Neutrophil degranulation (elastase release)

There was an effect of exercise on plasma elastase concentration, i.e. unstimulated elastase release (Table 4.5;  $F_{1,2} = 7.9$ , P = 0.015). Plasma elastase concentration was elevated above pre-exercise levels at post-exercise (P = 0.030, ES = 0.73), yet it had returned to pre-exercise levels by 1 h post-exercise. Total elastase release following 1 h *in vitro* stimulation with bacterial extract was also elevated following exercise (Table 4.5;  $F_{1,8} = 8.9$ , P = 0.003) and while a trend was already observed at post-exercise (P = 0.054, ES = 0.22) this was only evident at 1 h post-exercise (P = 0.007, ES = 0.54). When these data were corrected for changes in numbers of circulating neutrophils (i.e. expressed as bacterially-stimulated elastase release per cell), values were also elevated in response to exercise (Table 4.5;  $F_{1,3} = 6.6$ , P = 0.003), however this was only observed at 1 h post-exercise (P = 0.035, ES = 0.41).

Table 4.5. Plasma elastase	(unstimulated elastase	release) and tota	al and per neutrophil
elastase release following	1 h in vitro stimulation	with bacterial ex	ctract in response to
exercise.			

	Pre-ex	Post-ex	1 h post-ex
Plasma elastase (µg/l)			
	54 ± 19	74 ± 34*	52 ± 16
Total bacterially-stimulated elastase release (µg/I)			
	3398 ± 1489	3765 ± 1867	4359 ± 2033*
Bacterially-stimulated elastase release per neutrophil (fg/cell)			
	510 ± 180	516 ± 206	598 ± 245*
		Data ar	e mean ± SD (n=15)

ta are mean ± SD (n=15) \**P* < 0.05 vs. pre-ex

#### Plasma markers of systemic inflammation

Exercise induced an increase in plasma IL-6 levels (Figure 4.3;  $F_{2,0} = 13.3$ , P < 0.0005), which was more evident at post-exercise (P = 0.0004, ES = 0.33) but still noticeable at 1 h post-exercise (P = 0.004, ES = 0.25). Plasma IL-10 concentration was also elevated in response to exercise (Figure 4.4;  $F_{1,8} = 12.1$ , P < 0.0005), and although an increase was already apparent at post-exercise (P = 0.006, ES = 0.58) it was further increased by 1 h post-exercise (P = 0.001, ES = 1.12). Exercise also induced an elevation in plasma sTNF-RII levels (Figure 4.6;  $F_{2,0} = 4.3$ , P = 0.024), which was only evident at 1 h post-exercise (P = 0.027, ES = 0.30). There were no effects of exercise on plasma sTNF-RI (Figure 4.5) and CRP (Figure 4.7) concentrations.



**Figure 4.3. Plasma interleukin-6 (IL-6) response to exercise.** Data are mean and individual values (n=15); statistical analysis performed on log-transformed data.



Figure 4.4. Plasma interleukin-10 (IL-10) response to exercise. Data are mean and individual values (n=13).



Figure 4.5. Plasma soluble tumour necrosis factor receptor I (sTNF-RI) response to exercise. Data are mean and individual values (n=15).



Figure 4.6. Plasma soluble tumour necrosis factor receptor II (sTNF-RII) response to exercise. Data are mean and individual values (n=15).



**Figure 4.7. Plasma C-reactive protein (CRP) response to exercise.** Data are mean and individual values (n=15); statistical analysis performed on log-transformed data.

## 4.5. Discussion

This study investigated the effects of an acute bout of moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients. We found that exercise enhanced neutrophil responsiveness to a bacterial challenge, yet did not affect T lymphocyte and monocyte activation. In addition, exercise induced a systemic anti-inflammatory environment. Overall, exercise induced an immune and inflammatory response that is comparable to that observed in healthy individuals.

Exercise induced a modest increase in the peripheral blood leukocyte count, which peaked immediately after exercise reflecting the concomitant elevations in circulating neutrophil and lymphocyte numbers. However, 1 h after exercise cessation leukocyte concentration was still elevated, mostly due to a further increase in circulating neutrophils, as lymphocytes had already returned to preexercise values. On the other hand, circulating monocyte count does not appear to have been affected. Exercise-induced leukocytosis is a well established phenomenon (McCarthy & Dale, 1988; Walsh et al., 2011). It has been suggested that the leukocytosis observed immediately after exercise is likely to be produced by demargination of neutrophils and lymphocytes from the blood vessels due to increased shear stress and catecholamines, whereas the delayed leukocytosis is mainly attributable to a release of neutrophils from the bone marrow induced by elevated cortisol levels (McCarthy & Dale, 1988; McCarthy et al., 1991; Walsh et al., 2011). The magnitude of these changes is, however, related to the intensity and the duration of the exercise performed (Nieman et al., 1994; Robson et al., 1999; Walsh et al., 2011).

In contrast to the marked alterations induced by strenuous exercise (intensive or prolonged), Nieman *et al.* (2005) reported mild and transient increases in total and differential leukocyte counts following 30 min walking at 60-65% of  $\dot{V}O_{2max}$  in healthy women, whereas Markovitch *et al.* (2008) reported no effect of 30 min walking at 50% of  $\dot{V}O_{2max}$  in middle-aged healthy men. Our results are in agreement with Nieman *et al.* (2005) and the lack of effect observed by Markovitch *et al.* (2008) is likely to be intensity-related. Although we have not

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measured VO, during exercise, the average RPE response to exercise reported by Nieman et al. (2005) was identical to our study. Furthermore, the exercise protocol used in the present study was previously piloted in healthy volunteers and elicited similar alterations in total and differential leukocyte counts (Chapter 3). The neutrophilia observed 1 h after exercise might also be partially related to the circadian variation in circulating neutrophils, which are known to increase throughout the morning hours (Haus & Smolensky, 1999). In agreement with this, Nieman et al. (2005) reported that neutrophil concentration 1 h after exercise, albeit elevated in relation to pre- and post-exercise, did not differ significantly from a control sitting condition at this time point. As we did not include a resting trial, we may only speculate that the effects of exercise and circadian variation are likely to superimpose to each other. Most importantly, and given that neutrophilia, monocytosis and lymphocytopenia are common features of CKD (Eleftheriadis et al., 2007; Agarwal & Light, 2011), our data suggest that an acute bout of moderate intensity aerobic exercise in pre-dialysis CKD patients does not elicit abnormal alterations in total and differential leukocyte counts.

Exercise had little effect on the distribution of T lymphocyte subsets, which mainly reflected the small biphasic changes on the numbers of circulating CD8<sup>++</sup> lymphocytes – elevated immediately after exercise and falling below preexercise levels by 1 h into the recovery period. This pattern of mobilisation has been widely reported for T lymphocytes and its subpopulations (Gleeson & Bishop, 2005; Walsh et al., 2011) and appears to be largely dependent on the exercise intensity (Nieman et al., 1994). As expected for moderate intensity exercise, the effects on T lymphocyte subset counts reported in the present study were small and only CD8<sup>++</sup> lymphocytes appear to have been affected. CD8<sup>+</sup> lymphocytes have higher density of  $\beta_2$ -adrenergic receptors than CD4<sup>+</sup> lymphocytes and the density of these receptors increases with both exercise and exposure to catecholamines (Landmann, 1992; Shephard, 2003). Hence, the recruitment of CD8<sup>+</sup> lymphocytes during exercise into the circulation is usually greater than that for CD4<sup>+</sup> lymphocytes (Nieman et al., 1994; Gleeson & Bishop, 2005), which in the light of the exercise intensity used in the present study perhaps explains the lack of effect on CD4<sup>+</sup> lymphocytes. Again, our

results indicate that alterations in the circulating numbers of T lymphocyte subsets in response to an acute bout of moderate intensity aerobic exercise in pre-dialysis CKD patients follow the normal pattern.

SEB-stimulated activation of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes, assessed by CD69 expression, was unaffected by exercise. This is in agreement with our preliminary findings in healthy volunteers using the same exercise protocol (Chapter 3). Two other studies in well-trained men have found that the in vivo and mitogen-stimulated expression of CD69 on CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes did not significantly alter in response to 75 min of cycling at 75% of VO<sub>2max</sub> (Rønsen et al., 2001) or 60 min of treadmill running at 95% of ventilatory threshold (Green et al., 2003). However, decreases in the CD69 expression on both CD4<sup>+</sup> and CD8<sup>+</sup> cells immediately after an incremental treadmill test to exhaustion have also been reported in well-trained men (Vider et al., 2001) yet the nature of the exercise challenge is likely to explain this inconsistency. SEBstimulated activation of CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes, assessed by the expression of CD86 and HLA-DR molecules, was also unaffected by exercise. This is again consistent with our pilot work in healthy subjects (Chapter 3). Disturbances in T lymphocytes and monocytes have been implicated in the immune dysfunction state that accompanies CKD, in which signs of immune depression and activation coexist (Eleftheriadis et al., 2007; Kato et al., 2008). Our study suggests that an acute bout of moderate intensity aerobic exercise in pre-dialysis CKD patients does not hinder T lymphocyte and monocyte immune competence nor it seems to aggravate the "pre-activated" state of these cells.

Plasma elastase concentration was elevated above pre-exercise levels immediately after exercise, indicating that exercise induced a spontaneous neutrophil activation (degranulation *in vivo*). This is not unexpected because elevated plasma elastase levels have been consistently reported following various exercise protocols (Robson *et al.*, 1999; Peake, 2002; Walsh *et al.*, 2011). Even moderate intensity exercise bouts, such as 60 min of cycling at approximately 70% of maximal heart rate (Smith *et al.*, 1996) or 30 min of cycling at 70% of  $\dot{VO}_{2max}$  (Blannin *et al.*, 1996) have been shown to elevate the plasma concentration of elastase in healthy volunteers. Nevertheless, within 1 h

after exercise plasma elastase had returned to resting levels. Considering that neutrophils from patients with CKD are known to be primed (Sela *et al.*, 2005), this observation is particularly important as it suggests that the exercise-induced neutrophil activation was only transient. The effects of repetitive exercise, however, require further investigation.

Total bacterially-stimulated elastase release increased in response to exercise, however this most likely reflects the parallel neutrophilia (i.e. more cells, more elastase is released). When adjusted to take numbers of circulating neutrophils into account, bacterially-stimulated elastase release per neutrophil was unaltered immediately after exercise, but was increased 1 h after exercise, indicating enhanced neutrophil responsiveness to a bacterial challenge in the post-exercise period. This is in contrast to the reported reductions in stimulated neutrophil degranulation following numerous exercise protocols (Robson *et al.*, 1999; Peake, 2002; Walsh *et al.*, 2011). In fact, stimulated neutrophil degranulation has even been shown to be diminished 2.5 h after 30 min of cycling at 70% of  $\dot{VO}_{2max}$  in healthy subjects (Blannin *et al.*, 1996) and therefore, exercise intensity is unlikely to explain this difference.

In healthy people, the decreased functional capacity of the neutrophils following acute exercise has been, at least in part, attributed to the cortisol-induced release of less mature neutrophils from the bone marrow (Pyne, 1994; Peake, 2002). However, as neutrophil function is known to be impaired in CKD (Chonchol, 2006), in the current study this influx of "fresh" neutrophils may actually have contributed to the mobilisation of more functional neutrophils into the circulation. Although speculative this is perhaps supported by the fact that the increase in bacterially-stimulated elastase release per neutrophil coincided with the delayed neutrophilia (i.e. at 1 h post-exercise). Nonetheless, our results indicate improved ability of the neutrophils to respond to a bacterial challenge following an acute bout of moderate intensity aerobic exercise in pre-dialysis CKD patients. Given that neutrophil dysfunction is believed to increase the susceptibility to infections in CKD (Cohen *et al.*, 1997; Chonchol, 2006), a potential protective effect of exercise is encouraging. Having said this, we do not know the duration of any protective effect.

Plasma IL-6 concentration was moderately elevated in response to exercise, with peak levels occurring immediately after exercise and, albeit still elevated, appeared to be returning towards resting levels by 1 h after exercise termination. Exercise also induced an increase in plasma IL-10 concentration, and although levels were already elevated immediately after exercise, peak values were reached 1 h after the end of exercise. At this time, there was also a small increase in plasma sTNF-RII levels, while the plasma concentrations of sTNF-RI and CRP appeared to have been unaffected by exercise. It has been consistently reported that plasma IL-6 levels increase during exercise, with a magnitude that is determined by the combination of mode, intensity and duration of the exercise performed (Fischer, 2006; Pedersen & Febbraio, 2008). Two studies in disease-free subjects have investigated the IL-6 response to the same exercise mode and duration used in the current study and have provided conflicting findings - in agreement with our results, Nieman et al. (2005) reported an increase following 30 min walking at 60-65% of  $\dot{V}O_{2max}$ , while in contrast Markovitch et al. (2008) reported no effect of 30 min walking at 50% of  $\dot{V}O_{2max}$ . Although differences in subject characteristics (such as gender, age or fitness) may also be a factor to consider, it is more likely that exercise intensity explains this discrepancy. In agreement, 40 min of cycling at approximately 80% of maximal heart rate (RPE - "somewhat hard") led to an increase in plasma IL-6 levels, while 40 min of cycling at approximately 60% of maximal heart rate (RPE - "fairly light") did not, in middle-aged sedentary overweight men (Mendham et al., 2011).

In CKD, circulating plasma IL-6 levels are chronically elevated and have been associated with atherosclerosis and other uraemic complications (Stenvinkel *et al.*, 2002*b*; 2005). In fact, IL-6 appears to be the strongest predictor of outcome in CKD (Barreto *et al.*, 2010). This raises the question about the safety of an exercise-induced increase in plasma IL-6 levels in patients with CKD. However, it is now clear that contracting skeletal muscle is the main source of the IL-6 in circulation in response to exercise (Steensberg *et al.*, 2000; Toft *et al.*, 2011) and muscle-derived IL-6, the first so-called "myokine" described (Pedersen *et al.*, 2003), appears to mediate metabolic and anti-inflammatory effects, both locally and systemically in an hormone-like fashion (Petersen & Pedersen,

2005; Pedersen & Febbraio, 2008). In contrast to the cytokine cascade in sepsis, where IL-6 appearance is preceded by the pro-inflammatory TNF- $\alpha$  and IL-1 $\beta$ , in response to exercise IL-6 is typically the first cytokine present in the circulation, which is followed by the cytokine inhibitors IL-1ra and sTNF-Rs as well as the anti-inflammatory IL-10 (Petersen & Pedersen, 2005; Pedersen & Febbraio, 2008). The kinetics of the cytokine response to exercise observed in the present study are consistent with this. The greatest increase in plasma IL-6 levels observed immediately after exercise was followed by a marked increase in plasma IL-10 levels 1 h after exercise, and although plasma IL-6 concentration was still elevated at this time, it seemed to be returning towards resting values. Furthermore, a small increase in plasma sTNF-RII levels was also detected at this time. Given the moderate intensity of the exercise performed only mild increases in sTNF-Rs were expected, because even after strenuous exercise the increases in sTNF-Rs have been shown to be considerably lower than the marked increases in IL-6 and IL-10 (Ostrowski et al., 1999). However, the abnormally high resting levels of sTNF-Rs observed in our patients might have underestimated the observed effect of exercise on sTNF-RII as well as masked a possible effect on sTNF-RI.

On the other hand, plasma CRP concentration was clearly unaffected by exercise. This is somewhat expected since CRP levels only appear to be modestly elevated immediately following strenuous physical exertion (Kasapis & Thompson, 2005; Plaisance & Grandjean, 2006). However, we should acknowledge that our study is limited by the fact that we have only collected blood samples until 1 h after exercise and alterations in CRP levels are often not seen until a few hours or even the day after exercise (Kasapis & Thompson, 2005; Plaisance & Grandjean, 2006). In agreement, an increase in plasma CRP levels was reported 24 h after 40 min of cycling at approximately 80% of maximal heart rate in middle-aged sedentary overweight men, while no alterations were detected immediately or 3 h after exercise (Mendham *et al.*, 2011).

A recent systematic review has highlighted a major gap in our knowledge regarding the effects of acute exercise on inflammatory markers in patients with a chronic inflammatory disease (Ploeger *et al.*, 2009). To our knowledge this is

the first study to address this question in CKD and our results indicate that an acute bout of moderate intensity exercise in pre-dialysis CKD patients induces an anti-inflammatory environment and does not appear to exacerbate their underlying inflammatory status. An obvious limitation of our study is that we have not measured classical pro-inflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$ . However, there is convincing evidence from the literature to suggest that their levels would remain unaltered: it has been demonstrated that IL-6 infusion in humans enhances plasma IL-1ra, IL-10 and sTNF-RI but not TNF- $\alpha$  and IL-1 $\beta$  (Tilg *et al.*, 1994; Steensberg *et al.*, 2003). In addition, exercise and IL-6 infusion have been shown to inhibit the endotoxin-induced increase in circulating levels of TNF- $\alpha$  in healthy humans (Starkie *et al.*, 2003).

In summary, exercise enhanced neutrophil responsiveness to a bacterial challenge, while it did not affect T lymphocyte and monocyte activation. In addition, exercise induced a systemic anti-inflammatory environment. These findings suggest that an acute bout of moderate intensity aerobic exercise in pre-dialysis CKD patients seems to be safe from an immune and inflammatory perspective and it appears to improve immunosurveillance as well as to exert anti-inflammatory effects. However, it is not known whether these effects translate into a longer lasting state of immune and inflammatory protection.

# Chapter 5

Effect of 1-month of regular moderate intensity aerobic exercise on immunity and inflammation in chronic kidney disease patients

## 5.1. Abstract

Chronic kidney disease (CKD) is associated with a complex state of immune dysfunction characterised by immune depression, which predisposes CKD patients to infections, and by immune activation resulting in inflammation, which is associated with cardiovascular disease among these patients. It has been suggested that regular moderate exercise may enhance immune function and exert anti-inflammatory effects. However, such effects have been poorly investigated in CKD. Therefore, this study investigated the effects of 1-month of regular moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients.

Twenty-one (16 males and 5 females) pre-dialysis stage 3-5 CKD patients (age 58 ± 11 yrs; BMI 27.7 ± 4.6 kg/m<sup>2</sup>; eGFR 21.5 ± 8.3 ml/min/1.73 m<sup>2</sup>) exercised for 30 min, 5 times/week, for 1-month. The exercise programme consisted of brisk walking at a rating of perceived exertion (RPE) in the range of 12-14 ("somewhat hard"). At baseline and 1-month, patients performed a standard 30 min treadmill exercise test. Resting venous blood samples were collected on both occasions. SEB-stimulated CD4<sup>+</sup> and CD8<sup>++</sup> lymphocyte (CD69 expression) and CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocyte activation (HLA-DR and CD86 expression) were determined by flow cytometry. Elastase release from unstimulated (plasma elastase) and bacterially-stimulated neutrophils, plasma inflammatory markers (IL-6, IL-10, sTNF-Rs and CRP), and cytokine levels in SEB-stimulated PBMCs and bacterially-stimulated whole blood cultures were measured by ELISA. Data were examined using Student's paired t-tests.

RPE response to the exercise test at 1 month was lower than at baseline. After 1month, CD86 expression by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes was down-regulated, while HLA-DR expression, albeit showing a similar trend, did not change significantly. CD4<sup>+</sup> and CD8<sup>++</sup> lymphocyte activation (CD69) and neutrophil degranulation responses also remained unaltered. Plasma IL-6 levels were reduced, while plasma IL-10 levels were increased, resulting in a decreased IL-6/IL-10 ratio. Plasma sTNF-Rs levels were also reduced, while plasma CRP levels remained unchanged. IL-6 levels in SEBstimulated PBMCs and bacterially-stimulated whole blood cultures were also reduced.

These findings indicate that regular moderate aerobic exercise in pre-dialysis CKD patients seems to be safe from an immune and inflammatory perspective and it appears to exert anti-inflammatory effects, most likely mediated by a down-regulation of monocyte activation. Longer duration and controlled trials are needed to confirm these potential effects.

## 5.2. Introduction

The profound alterations in innate and adaptive immunity associated with chronic kidney disease (CKD) lead to a complex state of immune dysfunction, in which signs of immune depression and immune activation paradoxically coexist (as described in Chapter 1). Immune cell suppression predisposes patients with CKD to infections (Cohen *et al.*, 1997; Descamps-Latscha *et al.*, 2002; Eleftheriadis *et al.*, 2007; Kato *et al.*, 2008), yet persistent immune cell activation contributes to a state of chronic inflammation, evidenced by elevations in circulating levels of inflammatory mediators such as tumour necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-6 and IL-8 (Pereira *et al.*, 2008; 2009). These are associated with increased cardiovascular disease risk among these patients, with infection and cardiovascular disease the leading causes of morbidity and mortality in CKD (Naqvi & Collins, 2006; Dalrymple & Go, 2008; Saran & DuBose, 2008; Stenvinkel, 2010).

It has been suggested that regular participation in moderate intensity exercise may enhance certain aspects of immune function and may exert antiinflammatory effects (Petersen & Pedersen, 2005; Senchina & Kohut, 2007; Haaland *et al.*, 2008; Beavers *et al.*, 2010; Gleeson *et al.*, 2011; Nieman, 2011). These are believed to contribute, at least in part, to the lower risk of infection and cardiovascular disease that is observed in physically active individuals in comparison with their sedentary counterparts (Gleeson *et al.*, 2011; Nieman, 2011). However, the impact of regular exercise on immune and inflammatory parameters in patients with CKD is still unclear. The majority of the limited literature in this area has been focused on the effects of exercise in haemodialysis patients (Bronas, 2009), with little attention given to pre-dialysis CKD patients.

Therefore, the aim of this study was to investigate the effects of 1-month of regular moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients.

# 5.3. Methods

# Patients

Thirty-three CKD patients (26 pre-dialysis patients and 7 patients on PD) were recruited for the 1-month study. A total of 25 patients, including 4 patients on PD, completed the study, while the other 8 patients dropped out. However, patients on PD were excluded from the analysis. Therefore, the present study includes a total of 21 pre-dialysis patients and their characteristics at baseline are shown in Table 5.1.

Table 5.1. Patients' characteristics at baseline (n=21).

Gender (n males / n females)	16 / 5
Age* (years)	58 ± 11
Weight* (kg)	82 ± 15
Height* (cm)	172 ± 9
BMI* (kg/m²)	27.7 ±4.6
<b>eGFR*</b> (ml/min/1.73 m <sup>2</sup> )	21.5 ± 8.3
CKD stage <sup># a)</sup> Stage 3 Stage 4 Stage 5	5 (24%) 10 (48%) 6 (29%)
Primary renal disease <sup># b)</sup> Renal Vascular Disease Cystic/poly Glomerulonephritis Diabetes Pyelonephritis Uncertain Other	4 (19%) 2 (10%) 4 (19%) 2 (10%) 2 (10%) 5 (24%) 2 (10%)
Number of comorbidities <sup>#</sup> 1 2 3 4	8 (38%) 7 (33%) 2 (10%) 4 (19%)
Immunosuppressive therapy <sup># c)</sup>	5 (24%)
BMI: body mass index	Data are *mean ± SD or <sup>#</sup> n (%)

BMI: body mass index Data are \*mean ± SD of eGFR: estimated glomerular filtration rate CKD: chronic kidney disease a) based on eGFR b) grouped according to UK Renal Registry (2010) c) excluded from the analysis of immune and inflammatory parameters

## Experimental and analytical procedures

All patients were prescribed a home-based exercise programme as described in Section 2.3. At baseline and after 1-month of regular exercise, all patients performed an exercise test as described in Section 2.2. Resting venous blood samples were obtained on both occasions. All analytical procedures are detailed in Chapter 2.

#### Statistical analysis

Data are presented as mean  $\pm$  standard deviation (SD) unless otherwise stated. Data were inspected for normality using the Shapiro-Wilk test. If a data set was not normally distributed, statistical analysis was performed on the logarithmic transformation of the data, but data were back transformed for presentation. Outliers were defined as values deviating more than 3 SDs from the mean and were excluded from analysis. Data were examined using Student's paired ttests (baseline vs. 1-month). Effect sizes were calculated according to Cohen (1988) with correction for sample size (Hedges, 1981), by dividing the difference between means by the pooled SD. Statistical significance was accepted at *P* < 0.05.

## 5.4. Results

#### Exercise test outcomes

The average walking speed during both exercise tests was  $4.3 \pm 1.3$  km/h (range 2.1 to 8.1 km/h). The RPE response to baseline and 1-month exercise tests is illustrated in Figure 5.1. At 1-month, the RPE response to exercise was lower than at baseline (Table 5.2; ES = 1.78 and ES = 1.74 for average and area under curve, respectively). Likewise, the plasma lactate response to the 1-month exercise test was lower than at baseline (Table 5.2; ES = 0.95).



Figure 5.1. Rating of perceived exertion (RPE) response to baseline and 1-month exercise tests. Data are mean  $\pm$  SD (n=21).

Table 5.2. Exercise test outcomes at baseline and 1-month.

	Baseline	1-month	Р
RPE (average)	13 ± 1	11 ± 1	0.00001
<b>RPE</b> (area under curve)	183 ± 21	138 ± 31	0.00002
$\Delta$ plasma lactate (mmol/l)	1.2 ± 1.1	$0.4 \pm 0.4$	0.008

RPE: rating of perceived exertion  $\Delta$  = post-ex – pre-ex

Data are mean ± SD (n=21)

#### **Body composition**

After 1-month of regular exercise there was a negligible decrease in body weight (Table 5.3; ES = 0.07), which led to a minor reduction in BMI (Table 5.3; ES = 0.08).

## Total and differential blood leukocyte counts

The exercise intervention had no effect on the total and differential numbers of circulating leukocytes (Table 5.3). Haematocrit, haemoglobin and plasma volume were also unaltered (Table 5.3).

	Baseline	1-month	Р
Body composition*			
Weight (kg)	82.6 ± 15.7	81.5 ± 15.1	0.011
<b>BMI</b> (kg/m <sup>2</sup> )	27.8 ± 4.7	27.4 ± 4.6	0.013
General haematology*			
Haematocrit (%)	36.5 ± 4.5	36.7 ± 4.4	0.807
Haemoglobin (g/dl)	13.4 ± 1.6	13.3 ± 1.8	0.710
Plasma volume (%)	63.5 ± 4.5	63.4 ± 4.4	0.810
Total and differential blood leu	ıkocyte counts <sup>#</sup>		
Leukocytes (x 10 <sup>9</sup> /l)	6.9 ± 2.2	6.7 ± 2.2	0.561
Neutrophils (x 10 <sup>9</sup> /I	4.3 ± 1.5	4.0 ± 1.5	0.192
Lymphocytes (x 10 <sup>9</sup> /l)	1.7 ± 0.7	1.8 ± 0.8	0.312
Monocytes (x 10 <sup>9</sup> /l)	0.6 ± 0.2	0.6 ± 0.2	0.612

Table 5.3. Body composition, general haematology and total and differential blood leukocyte counts at baseline and 1-month.

BMI: body mass index

Data are mean  $\pm$  SD (\*n=21 or <sup>#</sup>n=16)

#### T lymphocyte subsets

There were no effects of the exercise intervention on the numbers of  $CD4^+$  and  $CD8^{++}$  lymphocytes (Table 5.4). Consequently, the  $CD4^+/CD8^{++}$  ratio also remained unchanged (Table 5.4).

# Activation of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes

The percentages of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes expressing CD69 following 20 h *in vitro* SEB stimulation were unaffected by the exercise programme (Table 5.4). Similarly, SEB-stimulated CD69 expression (GMFI) by either CD4<sup>+</sup>CD69<sup>+</sup> or CD8<sup>++</sup>CD69<sup>+</sup> lymphocytes was also unaltered (Table 5.4).
Table 5.4. CD4 <sup>+</sup> and CD8 <sup>++</sup> lymphocyte counts and CD4 <sup>+</sup> /CD8 <sup>++</sup> ratio, percentages of CD4 <sup>+</sup>
and CD8 <sup>++</sup> lymphocytes expressing CD69 and CD69 expression by CD4 <sup>+</sup> CD69 <sup>+</sup> and
CD8 <sup>++</sup> CD69 <sup>+</sup> lymphocytes after 20 h <i>in vitro</i> stimulation with staphylococcal enterotoxin
B (SEB) at baseline and 1-month.

	Baseline	1-month	Ρ
T lymphocyte subsets			
CD4 <sup>+</sup> (x 10 <sup>9</sup> /l)	$0.6 \pm 0.3$	$0.6 \pm 0.3$	0.989
CD8 <sup>++</sup> (x 10 <sup>9</sup> /l)	$0.5 \pm 0.3$	$0.5 \pm 0.3$	0.876
CD4 <sup>+</sup> /CD8 <sup>++</sup>	1.7 ± 0.7	1.6 ± 0.5	0.519
CD69 <sup>+</sup> cells (%)			
CD4 <sup>+</sup>	25 ± 10	24 ± 14	0.727
CD8 <sup>++</sup>	27 ± 11	25 ± 12	0.412
<b>CD69</b> (GMFI)			
CD4 <sup>+</sup> CD69 <sup>+</sup>	135 ± 32	132 ± 34	0.845
CD8 <sup>++</sup> CD69 <sup>+</sup>	92 ± 30	95 ± 39	0.813

GMFI: geometric mean of fluorescence intensity

Data are mean ± SD (n=16)

Data are mean  $\pm$  SD (n=16)

# Activation of CD14+CD86+HLA-DR+ monocytes

CD86 expression (GMFI) by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes following 20 h *in vitro* SEB stimulation (expressed as a ratio to unstimulated condition) was down-regulated after the exercise intervention (Table 5.5; ES = 0.49), while HLA-DR expression by the same cells did not change significantly (Table 5.5).

Table 5.5. CD86 and HLA-DR expression by CD14+CD86+HLA-DR+ monocytes after 20	h
in vitro stimulation with staphylococcal enterotoxin B (SEB) at baseline and 1-month.	

	Baseline	1-month	<b>P</b> *
<b>CD86</b> (GMFI <sup>#</sup> )	1.9 ± 0.8	1.6 ± 0.9	0.029
HLA-DR (GMFI <sup>#</sup> )	$2.0 \pm 0.6$	1.7 ± 0.6	0.224

GMFI: geometric mean of fluorescence intensity

<sup>#</sup>Ratio to unstimulated condition

\*Statistical analysis performed on log-transformed data

# Neutrophil degranulation (elastase release)

The exercise intervention had no effect on plasma elastase concentration (unstimulated elastase release) nor on the total and per neutrophil elastase release following 1 h *in vitro* stimulation with bacterial extract (Table 5.6).

Table 5.6. Plasma elastase (unstimulated elastase release) and total and per neutrophil elastase release following 1 h *in vitro* stimulation with bacterial extract at baseline and 1-month.

	Baseline	1-month	Р
Plasma elastase (µg/l)			
	66 ± 22	67 ± 27	0.910
Total bacterially-stimulated	elastase release	(µg/I)	
	3274 ± 1617	3296 ± 1416	0.931
Bacterially-stimulated elastase release per neutrophil* (fg/cell)			
	498 ± 147	541 ± 284	0.833

\*Statistical analysis performed on log-transformed data Data are mean ± SD (n=16)

#### Plasma markers of systemic inflammation

Following 1-month of regular exercise, plasma IL-6 concentration was decreased below baseline levels (Figure 5.2; ES = 0.41), while plasma IL-10 concentration was increased (Figure 5.3; ES = 0.78). Consequently, there was a decline in the plasma IL-6/IL-10 ratio (Figure 5.4; ES = 0.61). Plasma sTNF-RI (Figure 5.5) and sTNF-RII (Figure 5.6) levels were also reduced following the exercise intervention (ES = 0.44 and ES = 0.62, respectively), while plasma CRP levels remained unchanged (Figure 5.7).



**Figure 5.2. Plasma interleukin-6 (IL-6) at baseline and 1-month**. Data are mean and individual values (n=16); statistical analysis performed on log-transformed data.



Figure 5.3. Plasma interleukin-10 (IL-10) at baseline and 1-month. Data are mean and individual values (n=14).



Figure 5.4. Plasma interleukin-6 to interleukin-10 (IL-6/IL-10) ratio at baseline and 1month. Data are mean and individual values (n=16); statistical analysis performed on logtransformed data.



Figure 5.5. Plasma soluble tumour necrosis factor receptor I (sTNF-RI) at baseline and 1month. Data are mean and individual values (n=16).



Figure 5.6. Plasma soluble tumour necrosis factor receptor II (sTNF-RII) at baseline and 1-month. Data are mean and individual values (n=16).



**Figure 5.7. Plasma C-reactive protein (CRP) at baseline and 1-month.** Data are mean and individual values (n=16); statistical analysis performed on log-transformed data.

# **PBMCs cytokine production**

IL-6 production by PBMCs following 8 days *in vitro* SEB stimulation was decreased following the exercise intervention (Table 5.7; ES = 0.95), while SEB-stimulated PBMCs IL-10 production remained unchanged (Table 5.7). Accordingly, the ratio of IL-6 to IL-10 production by SEB-stimulated PBMCs at 1-month was lower than at baseline (Table 5.7; ES = 1.36).

# Whole blood cytokine production

Whole blood IL-6 production following 1 h *in vitro* stimulation with bacterial extract was also lowered following 1-month of regular exercise (Table 5.7; ES=0.60).

Table 5.7. Peripheral blood mononuclear cells (PBMCs) cytokine production following 8 days *in vitro* stimulation with staphylococcal enterotoxin B (SEB) and whole blood cytokine production following 1 h *in vitro* stimulation with bacterial extract at baseline and 1-month.

	Baseline	1-month	<b>P</b> *
SEB-stimulated PBMCs c	ytokine production		
IL-6 (ng/I)	1.8 ± 1.7	1.1 ± 1.9	0.004
IL-10 (ng/l)	1.1 ± 0.7	1.1 ± 0.8	0.355
IL-6/IL-10 ratio	2.2 ± 2.0	$0.7 \pm 0.7$	0.007

#### Bacterially-stimulated whole blood cytokine production

IL-6 (pg/l) 19.9 ± 12.1 14.2 ± 7.8 0.04 <sup>2</sup>	11
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IL: interleukin

Data are mean ± SD (n=16)

\*Statistical analysis performed on log-transformed data

# Effect of additional bicarbonate therapy

Ten of the patients that participated in this study were randomised to receive sufficient additional oral bicarbonate to raise plasma bicarbonate to approximately 29 mmol/l, while the remaining patients continued with their usual bicarbonate therapy (target plasma bicarbonate 24 mmol/l).

To investigate the effects of the additional bicarbonate therapy data were examined using an ANOVA with mixed design: within-subjects (time: baseline vs. 1-month) x between-subjects (treatment: standard vs. bicarbonate).

As expected, there was an effect of the additional bicarbonate therapy on plasma bicarbonate concentration (F = 10.9, P = 0.005). At 1-month, plasma bicarbonate levels were elevated above baseline levels in the bicarbonate group (1-month: 26.8 ± 2.6 mmol/l vs. baseline: 22.6 ± 3.2 mmol/l, P = 0.006) but remained unaltered in the standard group (1-month: 22.3 ± 3.4 mmol/l vs. baseline: 22.6 ± 2.7 mmol/l, P = 0.813).

However, there were no effects of the additional bicarbonate therapy on any of the immune and inflammatory parameters studied.

# 5.5. Discussion

This study investigated the effects of 1-month of regular moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients. We found that the exercise intervention down-regulated monocyte activation, while it did not affect T lymphocyte activation or neutrophil degranulation responses. In addition, the exercise intervention exerted anti-inflammatory effects at the systemic level and in PBMC and whole blood cultures.

After 1-month of regular exercise, the RPE response to a standardised exercise test was lower than at baseline: patients' perception of the same absolute exercise load dropped, in average, from "somewhat hard" to "fairly light". In agreement, the plasma lactate response to the acute exercise test was also reduced after 1-month. Previous studies have shown that aerobic exercise training can improve exercise tolerance and capacity in pre-dialysis CKD patients (Clyne et al., 1991; Eidemak et al., 1997; Boyce et al., 1997; Leehey et al., 2009; Toyama et al., 2010; Mustata et al., 2010). Our data confirm and extend these findings by showing that even 1-month of regular exercise appears to be sufficient to bring about significant improvements in exercise tolerance in pre-dialysis CKD. It may be argued that our study is limited by the lack of determination of peak exercise capacity. However, such measurements may not necessarily reflect the patients' capacity to perform daily living activities, which are more likely to demand sustained effort at submaximal levels (Koufaki & Kouidi, 2010). Therefore, the observed improvements in submaximal exercise tolerance are arguably even more clinically relevant.

Circulating total and differential blood leukocyte counts were not affected by regular exercise. This is consistent with previous research in healthy elderly adults (Haaland *et al.*, 2008). However, a recent study has shown that leukocyte, neutrophil and monocyte counts were reduced following 6-weeks of moderate aerobic exercise training in overweight women with coronary risk factors (Michishita *et al.*, 2010). In addition, the reported decreases in monocyte and neutrophil counts were related to the decreases in fasting triglyceride and BMI and the increases in insulin sensitivity and exercise capacity (Michishita *et al.*)

*al.*, 2010). Hence, although our study failed to detect this, it is possible that regular exercise participation may reduce neutrophil and monocyte counts in those with a chronic inflammatory condition. On the other hand, we have previously reported that an acute bout of moderate intensity aerobic exercise in pre-dialysis CKD patients elicits modest alterations in total and differential blood leukocyte counts (Chapter 4). Given that disturbances in total and differential blood leukocytes are common in CKD (Eleftheriadis *et al.*, 2007; Agarwal & Light, 2011), it is important to highlight that regular exercise appears to be safe in this regard.

Regular exercise had no effect on the in vivo and in vitro neutrophil degranulation responses, as indicated by the unaltered plasma elastase levels and bacterially-stimulated elastase release after 1-month of regular exercise. Little is known about the effects of moderate exercise training on neutrophil function (Walsh et al., 2011) but there is cross-sectional evidence suggesting that long-term physical activity may counteract the age-associated decline in neutrophil function. Yan et al. (2001) reported that physically active older men (who exercised at least twice a week for a minimum of 1 hour for more than 3 years) had higher neutrophil phagocytic activity than their sedentary counterparts and that this function was preserved at the level of a group of younger subjects. Hence, it is conceivable that exercise interventions of longer duration than the one used in the present study are required for beneficial effects of exercise on neutrophil function to become apparent. On the other hand, given that plasma elastase levels are elevated in CKD (Costa et al., 2008; Caimi et al., 2009; Polańska et al., 2010) and that primed neutrophils have been implicated in the chronic state of systemic inflammation and oxidative stress that accompanies CKD (Sela et al., 2005), it is important to note that the present findings indicate that regular exercise in pre-dialysis CKD patients does not appear to exacerbate their resting neutrophils' activation state, even though transient spontaneous neutrophil activation might occur during each acute exercise bout (Chapter 4). In addition, although the current study does not show that the enhanced neutrophil responsiveness to an *in vitro* bacterial challenge observed following an acute bout of exercise (Chapter 4) translates into a chronic adaptation, it should also be highlighted that regular

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exercise does not appear to be detrimental to the neutrophil function of predialysis CKD patients, which is particularly important considering that neutrophil function is impaired in CKD (Chonchol, 2006)

T lymphocyte subset counts and their relative distribution also remained unaltered following the exercise programme, which is again in agreement with several reports in healthy older adults (Haaland *et al.*, 2008; Simpson & Guy, 2010). A single study in a group of CKD patients on haemodialysis has also reported no alterations in the circulating CD4<sup>+</sup> and CD8<sup>+</sup> lymphocyte counts or the CD4<sup>+</sup>/CD8<sup>+</sup> ratio following 6-months of exercise training (Daniilidis *et al.*, 2004). In CKD, although lymphocytopenia is commonly described, cell numbers are usually in the lower normal range and the CD4<sup>+</sup>/CD8<sup>+</sup> ratio also remains within normal levels (Girndt *et al.*, 2001*a*; Eleftheriadis *et al.*, 2007). Our data are consistent with this.

Regular exercise had no effect on the SEB-stimulated activation of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes, assessed by the early activation marker CD69. There is limited information concerning the effects of exercise training on T lymphocyte activation. Only two studies to date have addressed this guestion using the CD69 marker. Phytohemagglutinin (PHA)-stimulated CD69 expression by CD4<sup>+</sup> and CD8<sup>+</sup> cells remained unaltered following 10-months of moderate aerobic exercise training in older adults (Lee et al., 2004). Likewise, 12-months of resistance training had no effect on the percentages of CD3<sup>+</sup>, CD4<sup>+</sup> or CD8<sup>+</sup> cells expressing CD69 in elderly women (Raso et al., 2007). Interestingly, in the first study PHA-induced T lymphocyte proliferation and CD25 (IL-2 receptor  $\alpha$ ) expression were increased following the exercise intervention (Kohut & Senchina, 2004; Lee et al., 2004), whereas in the latter the percentages of CD3<sup>+</sup>, CD4<sup>+</sup> or CD8<sup>+</sup> cells expressing CD25 or HLA-DR did not change, as did not numbers of CD4<sup>+</sup> or CD8<sup>+</sup> cells expressing the co-stimulatory molecule CD28 nor did PHA-stimulated T lymphocyte proliferation (Raso et al., 2007). In fact, studies that have used other markers of T lymphocyte activation have yielded conflicting results. Kapasi et al. (2003) found no effect of 8-months of a mixed endurance and resistance exercise training programme in frail elderly nursing home residents on the percentages of CD3<sup>+</sup>, CD4<sup>+</sup> or CD8<sup>+</sup> cells expressing CD28 or CD25 and the percentages of  $CD3^+$  and  $CD8^+$  cells

expressing HLA-DR. Of note, PHA-stimulated T lymphocyte proliferation was also unaffected. In contrast, increased numbers and percentages of CD4<sup>+</sup> cells expressing CD28 were reported after 6-months of moderate aerobic exercise training in a group of elderly males and females (Shimizu et al., 2008) and increased percentages of PHA-stimulated CD4<sup>+</sup> cells expressing CD25 were reported during 12-months of moderate aerobic exercise training in older men but not older women (Broadbent & Gass, 2008). It can be argued that when studying T lymphocyte activation, the assessment of activation markers following in vitro activation by appropriate stimuli is more relevant than the ex vivo spontaneous levels of these markers. However, obvious differences between the studies described above, including the length of the intervention, the exercise regimen (mode, intensity and duration) and subjects' characteristics. make comparisons difficult. Nevertheless. although inconclusive, the available evidence points towards some favourable effects of regular, particularly aerobic exercise, on several aspects of T lymphocyte function but it appears that long-term interventions (> 6-months) are required (Kohut & Senchina, 2004; Haaland et al., 2008; Simpson & Guy, 2010). The fact that most cross-sectional studies have consistently reported enhanced T lymphocyte function in physically active older adults (probably for longer periods than what is commonly used in exercise intervention studies) compared with their sedentary counterparts (Kohut & Senchina, 2004; Simpson & Guy, 2010) also supports the idea that long-term regular exercise participation is needed for the benefits to occur. Therefore, although it appears from the current findings that T lymphocyte activation is not affected by regular exercise in pre-dialysis CKD patients, longer duration trials are warranted to fully elucidate this.

On the other hand, it is important to highlight that the majority of the research described above was conducted in sedentary but otherwise healthy older adults, and little is known about the effects of regular exercise on immunocompromised individuals, such as CKD patients. T lymphocyte deficiencies have been widely reported in CKD (Girndt *et al.*, 2001*a*; Eleftheriadis *et al.*, 2007; Kato *et al.*, 2008). Of particular interest is the fact that despite reduced T lymphocyte proliferative activity, an increased number of CD3<sup>+</sup> cells expressing CD69 (assessed *ex vivo* and after PHA and anti-CD3

stimulation) has been described in pre-dialysis and haemodialysis patients (albeit to a greater extent in the latter group), as compared to healthy controls (Meier *et al.*, 2002). Given that higher percentages of CD3<sup>+</sup> or CD3<sup>+</sup>CD69<sup>+</sup> cells expressing anexin V or CD95 (apoptosis markers) were also described in patients vs. controls, it is believed that in patients with CKD a significant proportion of activated T cells ultimately do not proliferate but become apoptotic (Meier et al., 2002). The present study indicates that regular moderate aerobic exercise does not influence CD69 expression by T lymphocytes and therefore it does not appear to aggravate their activated state. Nevertheless, our study is limited by its short-duration and the lack of a control group. As mentioned above, chronic T lymphocyte function adaptations only appear to occur after longer periods of exercise engagement. In addition, CD69 expression by T lymphocytes has been reported to increase overtime in a group of CKD patients on haemodialysis (Meier et al., 2000), so similar findings could be anticipated in pre-dialysis patients. Therefore, longer duration and controlled trials are needed to establish the impact of regular exercise on T lymphocyte activation in predialysis CKD patients.

CD86 expression by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes following *in vitro* SEBstimulation was down-regulated after the exercise intervention, whereas HLA-DR expression by the same cells, although apparently showing a similar trend, did not change significantly. These results suggest that regular exercise may reduce monocyte activation in pre-dialysis CKD patients. To our knowledge no previous research has investigated the effects of chronic exercise on these monocyte cell surface molecules, while a single study has reported that CD86 and HLA-DR expression by CD14<sup>+</sup> monocytes were down-regulated following acute prolonged exercise (Lancaster et al., 2005). However, there is emerging evidence that exercise training and increased physical activity levels downregulate Toll-like receptor (TLR) 4 expression by CD14<sup>+</sup> monocytes (McFarlin et al., 2004; Stewart et al., 2005; McFarlin et al., 2006), which is a recognised potential mechanism for the anti-inflammatory role of exercise (Gleeson et al., 2006; Flynn & McFarlin, 2006; Gleeson et al., 2011). Our results are in agreement with these findings because activation of TLRs up-regulates major histocompatibility complex (MHC) class II (HLA-DR) and co-stimulatory

molecule expression (CD80/86) on the antigen processing cell (APC) such as the monocyte or dendritic cell (Takeda *et al.*, 2003) and therefore it is possible that the down-regulation of CD86 and possibly HLA-DR were mediated by a down-regulation of TLR 4. Although we have not used the conventional TLR 4 ligand, that is, lipopolysaccharide, as a stimuli in our *in vitro* activation assays, it should be noted that SEB has already been shown to up-regulate TLR 4 in human monocytes (Hopkins *et al.*, 2005).

Another explanation for the observed down-regulation of monocyte activation may lie in a reduced proportion of circulating CD16<sup>+</sup> monocytes. The recently updated classification of monocyte heterogeneity acknowledges the existence subsets: classical (CD14<sup>++</sup>CD16<sup>-</sup>), of three monocyte intermediate (CD14<sup>++</sup>CD16<sup>+</sup>) and non-classical (CD14<sup>+</sup>CD16<sup>++</sup>) (Ziegler-Heitbrock et al., 2010). However, in earlier studies the latter two subsets were often denoted as (CD14<sup>+</sup>)CD16<sup>+</sup> monocytes, which have traditionally been labelled proinflammatory monocytes and are elevated in various inflammatory conditions, including CKD (Ziegler-Heitbrock, 2007). Interestingly, CD16<sup>+</sup> monocytes express higher levels of CD86 and HLA-DR in comparison with classical monocytes (Gordon & Taylor, 2005; Ziegler-Heitbrock, 2007; Zawada et al., 2011) and therefore a reduction in the proportion of these cells in the circulation following the exercise intervention could also explain our findings. In fact, evidence exists that regular exercise appears to reduce the proportion of proinflammatory monocytes in the blood compartment (Timmerman et al., 2008), which is proposed as another mechanism whereby exercise exerts its antiinflammatory effects (Gleeson et al., 2011). Of note, pro-inflammatory, specifically CD14<sup>++</sup>CD16<sup>+</sup> monocytes, have been recently shown to be independently associated with cardiovascular outcome in both dialysis (Heine et al., 2008) and pre-dialysis (Rogacev et al., 2011) patients. Hence, research addressing the impact of regular exercise on the different monocyte subsets in CKD patients seems highly warranted.

On the other hand, we have to acknowledge the possible detrimental role of a down-regulation of monocyte activation in the orchestration of the adaptive immune response. T cell activation requires at least two signals. The interaction between MHC-antigen peptide complex on the APC with the T cell

receptor provides the first signal, while the second signal occurs through the interaction between co-stimulatory cell surface molecules (CD80/86) on the APC and CD28 on the T cell membrane. Importantly, in the absence of co-stimulatory signalling, T cells fail to respond effectively and are rendered anergic (Sharpe & Freeman, 2002).

In CKD, T lymphocyte function is clearly impaired and it is currently acknowledged that this can be attributed to the defective interaction between the APC and the T lymphocyte (Girndt et al., 2001a; Eleftheriadis et al., 2007; Kato et al., 2008). Several aspects of this interaction have been shown to be impaired, although it should be noted that most of this evidence comes from studies conducted on haemodialysis patients (Eleftheriadis et al., 2007). Of particular interest is a study by Girndt et al. (2001b), which has shown that ex vivo monocyte CD86 expression was reduced in haemodialysis patients (also confirmed after in vitro stimulation with either interferon-y or cyclic adenosine monophosphate), while HLA-DR expression was not affected. Furthermore, the defective CD86 expression was associated with the clinical immune defect (lower levels in non-responders than responders to hepatitis B vaccination), as well as with the *in vitro* lymphocyte proliferative response (Girndt *et al.*, 2001b). However, this defect was not observed in pre-dialysis patients, suggesting a possible role for the dialysis procedure itself and/or the severity of the renal function impairment (Girndt et al., 2001b). It is also important to highlight that while Girndt et al. (2001b) reported that monocyte HLA-DR expression was normal, this was only assessed ex vivo and a previous study has shown that although monocytes recently isolated from haemodialysis patients do not express less HLA-DR than those from healthy individuals, their HLA-DR expression is impaired after in vitro stimulation with hepatitis B surface antigen (Stachowski et al., 1994). Nevertheless, from the evidence above it is not clear whether or not CD86 and HLA-DR expression are compromised in pre-dialysis patients.

However, there is emerging evidence implicating CKD-associated TLR disturbances in the APC dysfunction (Kato *et al.*, 2008). As mentioned above, activation of TLRs up-regulates CD86 and HLA-DR expression (Takeda *et al.*, 2003), and therefore it is possible that the defective expression of these

molecules are mediated through TLR deficiencies. Indeed, evidence of a downregulation of TLR 4 expression exists for both pre-dialysis (Ando *et al.*, 2006; Koc *et al.*, 2011) and haemodialysis (Kuroki *et al.*, 2007; Koc *et al.*, 2011) patients, while two conflicting studies in haemodialysis patients have reported an up-regulation of TLR 4 expression (Gollapudi *et al.*, 2010; Kim *et al.*, 2011). As previously discussed it is plausible that the exercise-induced downregulation of monocyte activation observed in the present study was mediated through a down-regulation of TLR 4 expression. Hence, it is possible that the anti-inflammatory effects of exercise in CKD might be accompanied by some degree of impairment of the adaptive immune response.

In the present study we have used SEB as the stimuli for the *in vitro* activation assays. In contrast to conventional antigens, SEB does not require processing and presentation and acts through cross-linking the MHC on the APC with the V $\beta$  domain of the TCR, resulting in the stimulation of a large proportion of T cells that is CD4-independent and therefore both CD4<sup>+</sup> and CD8<sup>+</sup> cells are stimulated (Bueno et al., 2007). Importantly, proliferation of T lymphocytes after SEB stimulation also requires co-stimulation (Krummel et al., 1996; Mittrücker et al., 1996). Therefore, it could be argued that the exercise-induced downregulation of monocyte activation observed in the present study did not impair T lymphocyte function because SEB-stimulated activation of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes was maintained after the exercise intervention. However, we have to acknowledge that the use of CD69 expression as an indicator of maintained T lymphocyte function is limited in CKD. Indeed, as noted above, an increased number of CD69<sup>+</sup> T lymphocytes is actually associated with lower T lymphocyte proliferative capacity (Meier et al., 2002; 2005). In addition, stimulation of T cells by SEB in the absence of co-stimulation (CD28-deficient mice) does not appear to influence the early events of T cell activation, such as CD69 expression (Mittrücker et al., 1996). Therefore, we cannot refute the possibility that a downregulation of monocyte activation could be associated with reduced T lymphocyte responsiveness and future research should address the impact of regular exercise on other aspects of the T lymphocyte function of CKD patients.

On the other hand, it has been demonstrated that monocytes from CKD patients are "pre-activated" and overproduce pro-inflammatory cytokines (Girndt

et al., 2001a; Descamps-Latscha et al., 2002; Eleftheriadis et al., 2007), typically by assessing the concentration of pro-inflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-8, in supernatants from LPS-stimulated PBMC cultures (Girndt et al., 1995; Higuchi et al., 1997; Morita et al., 1997; Sardenberg et al., 2004) but also in supernatants from LPS-stimulated whole blood cultures (Gollapudi et al., 2010) or through intracellular staining following LPS stimulation by flow cytometry (Girndt et al., 1998). Haemodialysis treatment is an obvious candidate to explain such findings, but studies that have included pre-dialysis patients have also shown that pro-inflammatory cytokine production is increased, albeit to a smaller extent than in haemodialysis patients (Higuchi et al., 1997; Sardenberg et al., 2004; Mantuano et al., 2007), indicating that uraemia per se also plays a role. In the present study, we found that IL-6 production by PBMCs following SEB stimulation was reduced after the exercise intervention, while IL-10 production by the same cells was maintained, resulting in a marked reduction in the ratio of IL-6 to IL-10 production. In addition, the exercise-induced reduction in IL-6 production was confirmed in bacteriallystimulated whole blood cultures. These findings are in agreement with mounting evidence indicating that regular exercise can improve the PBMC cytokine profile after in vitro stimulation through reductions in pro-inflammatory and/or increases in anti-inflammatory cytokine production (Smith et al., 1999; Stewart et al., 2005; Sloan et al., 2007; Timmerman et al., 2008; Phillips et al., 2010; Ortega et al., 2010), and provide the first evidence of similar effects in pre-dialysis CKD patients. Importantly, the reduced production of pro-inflammatory cytokines by PBMCs has been proposed as another possible mechanism mediating the antiinflammatory effects of exercise (Nicklas & Brinkley, 2009). Hence, it seems that the apparent exercise-induced down-regulation of the immune response (discussed above) might be a small price to pay for the anti-inflammatory effects. Interestingly, the production of IL-6, TNF- $\alpha$  and IL-10 by LPS-stimulated PBMCs from haemodialysis patients have also been associated with the clinical immune defect – non-responders to hepatitis B vaccination produced higher levels of IL-6 and TNF- $\alpha$ , while responders produced normal levels of these cytokines but higher levels of IL-10 (Girndt et al., 1995). Undoubtedly, immune system dysfunction in CKD is a multifactorial process, where at the immune cell level signs of functional deficiency coexist with signs of activation. It makes

intuitive sense that persistent cellular activation will further impair the functional defects. This perhaps also explains the apparently conflicting findings from a recent study, which has reported that monocytes from infection prone predialysis CKD patients exhibited significantly reduced synthesis of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-8 in response to LPS challenge compared with those from control subjects (Ando *et al.*, 2006). Nevertheless, seeming paradoxical it can be argued that in pre-dialysis CKD patients a down-regulation of the immune/inflammatory response through exercise training may contribute to an enhanced immune function in the longer term.

The potential anti-inflammatory effects of regular exercise were confirmed at the systemic level. Plasma IL-6 concentration was reduced after 1-month of regular exercise, while plasma IL-10 concentration was increased, resulting in a decreased ratio of IL-6 to IL-10 in the circulation (a proposed indicator of the overall inflammatory status). Plasma sTNF-Rs levels were also reduced, while plasma CRP levels remained unaltered. These findings are very promising because the persistent inflammation that accompanies CKD is associated with cardiovascular disease (the leading cause of death in CKD) and other uraemic complications (Carrero & Stenvinkel, 2010). The cytokine network is clearly altered in CKD (Stenvinkel et al., 2005; Carrero et al., 2008). Plasma IL-6 levels are elevated and have consistently been shown to predict all cause and cardiovascular mortality in CKD (Stenvinkel et al., 2002b; 2005). Of interest, the predictive value of plasma IL-6 has been recently confirmed in pre-dialysis CKD patients (Barreto et al., 2010). IL-10 has been regarded as one of the most important anti-inflammatory immune-regulating cytokines (Moore et al., 2001). Hence, as a result of the underlying persistent inflammation, it is not surprising that plasma IL-10 levels are usually found to be elevated in CKD (Morita et al., 1997; Moser et al., 2003; Sardenberg et al., 2004). On the other hand, haemodialysis patients with lower levels of plasma IL-10 appear to be at a higher risk for atherosclerosis (Seyrek et al., 2005). In agreement, haemodialysis patients genetically predisposed to produce higher levels of IL-10 show better immune balance (Girndt et al., 2001c) and are at lower risk of cardiovascular events (Girndt et al., 2002). In this context, it can be argued that the balance between IL-6 and IL-10 might be a more valid parameter to draw

conclusions about the overall inflammatory status. The sTNF-Rs, which are the extracellular form of the natural occurring inhibitors of TNF but also likely "footprints" of its activity (Brockhaus, 1997), are also elevated in CKD (Pereira *et al.*, 1994; Descamps-Latscha *et al.*, 1995; Ankersmit *et al.*, 2001). Altogether the evidence presented above clearly illustrates the significance of our findings.

However, in contrast to the favourable effects on plasma cytokines we have failed to detect an effect of regular exercise on plasma CRP. There is no obvious explanation for this. Although it is also well established that CRP levels are elevated and predict all cause and cardiovascular mortality in CKD (Lacson & Levin, 2004; Wanner *et al.*, 2009), CRP is a rather non-specific marker and therefore it might be difficult to detect changes in small patient cohorts. In addition, CRP is currently thought to be a simple marker rather than a causal factor for cardiovascular disease (Zhang *et al.*, 2007; Zacho *et al.*, 2008) and therefore it can be argued that an effect of exercise may be detectable in the longer-term. Notwithstanding, it is noteworthy that IL-6 has been consistently reported to be a stronger predictor of all cause and cardiovascular outcome in CKD, than any other inflammatory marker, including CRP (Tripepi *et al.*, 2005; Honda *et al.*, 2006; Zoccali *et al.*, 2006; Pachaly *et al.*, 2008; Wetmore *et al.*, 2010).

Previous studies reporting effects of regular exercise on systemic inflammatory markers in CKD patients have yielded conflicting results (Załuska *et al.*, 2002; Castaneda *et al.*, 2004; Nindl *et al.*, 2004; Cheema *et al.*, 2007*a*; 2007*b*; Kopple *et al.*, 2007; Toussaint *et al.*, 2008; Leehey *et al.*, 2009; Wilund *et al.*, 2010; Afshar *et al.*, 2010; 2011; Cheema *et al.*, 2011). It is noteworthy that in most of these studies systemic inflammatory markers have been secondary study outcomes and therefore it is not surprising that the widely used inflammatory marker CRP is reported in all of them. Exercise training reduced CRP levels in 6 studies (Załuska *et al.*, 2002; Castaneda *et al.*, 2004; Nindl *et al.*, 2004; Cheema *et al.*, 2007*a*; 2007*b*; Afshar *et al.*, 2010; 2011; Cheema *et al.*, 2010; 2011; Cheema *et al.*, 2004; Nindl *et al.*, 2004; Nindl *et al.*, 2004; Cheema *et al.*, 2007*a*; 2007*b*; Afshar *et al.*, 2010; 2011; Cheema *et al.*, 2004; Nindl *et al.*, 2004; Cheema *et al.*, 2007*a*; 2007*b*; Afshar *et al.*, 2010; 2011; Cheema *et al.*, 2004; Cheema *et al.*, 2007; Toussaint *et al.*, 2008; Leehey *et al.*, 2009; Wilund *et al.*, 2010). It is important to highlight that only 2 of these studies have focused on pre-dialysis patients (Castaneda *et al.*, 2004; Leehey *et al.*, 2009). Castaneda *et al.* (2004) reported that 12-weeks

of resistance exercise training reduced CRP and IL-6 levels, while Leehey et al. (2009) reported that 24-weeks of aerobic exercise training had no effect on CRP levels. On the other hand, data from cytokine measurements is only available in 3 other studies in haemodialysis patients (Kopple et al., 2007; Wilund et al., 2010; Cheema et al., 2011). Besides the lack of effect on CRP levels, Kopple et al. (2007) reported no effect of approximately 21-weeks of aerobic (intradialytic) or resistance (before dialysis) training or both on IL-6 and TNF-α levels, whilst Wilund et al. (2010) also reported no effect of 4-months of intradialytic aerobic exercise on IL-6 levels. It is worth mentioning that in the study of Kopple et al. (2007) a trend for a reduction in CRP and IL-6 levels was apparent following aerobic exercise training but not after resistance exercise training or a combination of both regimens. Intriguingly, Cheema et al. (2011) have recently reported that 12-weeks of intradialytic progressive resistance training had no effect on TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-10 and IL-12 levels, while a reduction in CRP levels had been previously reported (Cheema et al., 2007a). Altogether the available literature indicates that the effect of regular exercise on the inflammatory status of CKD patients is still unresolved. The present study points towards a strong anti-inflammatory role of moderate aerobic exercise in pre-dialysis CKD patients. While our study is limited by its short duration and uncontrolled design, it is strengthened by the simultaneous measurement of several immune and inflammatory parameters and therefore provides novel insights into the anti-inflammatory role of exercise in CKD.

Some of the mechanisms whereby exercise might have exerted its antiinflammatory effects have already been noted above. In addition, it has been proposed that the anti-inflammatory effects of regular exercise may be mediated by the induction of an anti-inflammatory environment with each bout of exercise (Petersen & Pedersen, 2005; Gleeson *et al.*, 2011). We have previously demonstrated that exercise of the same mode, intensity and duration as prescribed in the present study is capable of such effect in pre-dialysis CKD patients (Chapter 4). Another mechanism possibly mediating the antiinflammatory effects of exercise is the reduction of visceral fat mass, with a subsequent decreased release of adipokines, following exercise training (Pedersen, 2009; Gleeson *et al.*, 2011). In the present study, there were minor, yet statistically significant, reductions in body weight and BMI. Of note, exercise can reduce visceral fat even in the absence of any weight loss (Ross & Bradshaw, 2009). In a separate arm of this study full body dual energy x-ray absorptiometry scans revealed that there was an increase in lean body mass (baseline:  $49.4\pm 2.2$  kg vs. 1-month:  $50.1 \pm 2.3$  kg) and a reduction in fat mass (baseline:  $28.1 \pm 2.4$  kg vs. 1-month:  $27.5 \pm 2.4$  kg) following the exercise intervention (Clapp, 2010). These findings are suggestive of a possible involvement of the adipocytes in the anti-inflammatory effects of exercise observed in the present study.

In summary, these findings suggest that regular moderate intensity aerobic exercise in pre-dialysis CKD patients seems to be safe from an immune and inflammatory perspective. Further, a 1-month home-based exercise programme appears to exert anti-inflammatory effects, most likely mediated by a down-regulation of monocyte activation. Longer duration and controlled trials are needed to confirm these potential effects.

# Chapter 6

Effect of 6-months of regular moderate intensity aerobic exercise on immunity and inflammation in chronic kidney disease patients

# 6.1. Abstract

We have previously reported that a 1-month exercise intervention in pre-dialysis CKD patients exerted anti-inflammatory effects, likely mediated by the observed down-regulation of monocyte activation, yet did not affect T lymphocyte activation and neutrophil degranulation responses. However, the study was limited by its short-duration and lack of control. Therefore, the present study investigated the effects of 6-months of regular moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients using a controlled design.

Eighteen (11 M, 7 F) pre-dialysis stage 3-5 CKD patients (age 62 ± 8 yrs; eGFR 25.3 ± 7.9 ml/min/1.73 m<sup>2</sup>) exercised for 30 min, 5 times/week, for 6-months. The exercise programme consisted of brisk walking at a rating of perceived exertion (RPE) in the range of 12-14. A further 14 (8M, 6F) pre-dialysis stage 3-5 CKD patients (age 56 ± 15 yrs; eGFR 29.3 ± 5.6 ml/min/1.73 m<sup>2</sup>) acted as non-exercise controls. Resting venous blood samples were collected at baseline and after 6-months. SEB-stimulated CD4<sup>+</sup> and CD8<sup>++</sup> lymphocyte (CD69 expression) and CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocyte activation (HLA-DR and CD86 expression) were determined by flow cytometry. Elastase release from unstimulated (plasma elastase) and bacterially-stimulated neutrophils, and plasma inflammatory markers (IL-6, IL-10, sTNF-Rs and CRP) were measured by ELISA. Data were examined using ANOVAs with mixed design and Student's t-tests (Holm-Bonferroni correction).

Average RPE response to a standardised 30 min treadmill walking exercise test after the training period was lower than at baseline in exercisers only. After 6-months, CD86 and HLA-DR expression by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes was down-regulated in exercisers, but up-regulated in controls. Expression of CD69 by both CD4<sup>+</sup>CD69<sup>+</sup> and CD8<sup>++</sup>CD69<sup>+</sup> lymphocytes was down-regulated in exercisers, but did not change in controls. Plasma IL-6 showed a tendency to reduction and IL-10 to increase in exercisers but was unaltered in controls. Plasma IL-6/IL-10 ratio, reflecting overall inflammatory status, was reduced in exercisers, but unchanged in controls. Plasma sTNF-RI were reduced and sTNF-RII tended to be reduced in exercisers but unaltered in controls. Plasma CRP levels and neutrophil degranulation responses were not affected by the exercise intervention.

These findings confirm that regular walking exercise is safe from an immune and inflammatory perspective and has the potential to be an effective anti-inflammatory therapy in pre-dialysis CKD patients.

# 6.2. Introduction

Previous work presented in this thesis suggests that a 1-month home-based exercise programme in pre-dialysis CKD patients is associated with an antiinflammatory effect, as evidenced by lower inflammatory cytokine levels in the circulation and released from stimulated cell cultures, and most likely related to the observed down-regulation of monocyte activation (Chapter 5). This is important given the chronically inflamed state of patients with CKD and the strong association between chronic systemic inflammation and cardiovascular disease; the major cause of mortality in these patients (Stenvinkel, 2010). On the other hand, 1-month of regular exercise had no effect had on T lymphocyte activation and neutrophil degranulation responses (Chapter 5).

While these findings are encouraging the study was of short duration and had an uncontrolled design. It is interesting to note that the available evidence from exercise intervention studies assessing immune, particularly T lymphocyte function in healthy individuals is suggestive that longer interventions are required for alterations to occur (Kohut & Senchina, 2004). Furthermore, the fact that the evidence from cross-sectional studies between physically active and sedentary individuals is much more compelling regarding immune and inflammatory measures compared with data from exercise interventions, further support the idea that long-term regular exercise participation might be needed for stronger effects to occur (Kohut & Senchina, 2004; Beavers *et al.*, 2010).

Therefore, the aim of this study was to investigate the effects of 6-months of regular moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients using a controlled design.

# 6.3. Methods

#### Patients

Forty CKD pre-dialysis patients were recruited. The first 20 patients recruited were assigned to an exercise programme of 6-months duration, while the other 20 patients continued with their habitual physical activity (control group). Fifteen of the patients in the exercise group were carried into this study from the 1-month study (Chapter 5) and engaged in an additional period of 5-months of exercise training. For this reason randomisation was not possible.

#### Experimental and analytical procedures

Patients in the exercise group were prescribed a home-based exercise programme as described in Section 2.3. All patients performed an exercise test as described in Section 2.2 at baseline and after 6-months. Resting venous blood samples were obtained on both occasions. All analytical procedures are detailed in Chapter 2.

#### Statistical analysis

Data are presented as mean ± standard deviation (SD) unless otherwise stated. Data were inspected for normality using the Shapiro-Wilk test. If a data set was not normally distributed, statistical analysis was performed on the logarithmic transformation of the data, but data were back transformed for presentation. Outliers were defined as values deviating more than 3 SDs from the mean and were excluded from analysis. Baseline data were initially screened using Student's independent t-tests (exercise vs. control). All data was then examined using an analysis of variance (ANOVA) with mixed design: within-subjects (time: baseline vs. 6-months) x between-subjects (group: exercise vs. control). Assumption of homogeneity was checked using Levene's test. Where significant time x group interactions were observed, data were further examined using *a priori* planned Student's t-tests with Holm-Bonferroni correction as follows: paired (baseline vs. 6-months) for each group and independent (exercise vs. control) for 6-months data. Effect sizes for these comparisons were calculated according to Cohen (1988) with correction for sample size (Hedges, 1981), by dividing the difference between means by the pooled SD. Where significant differences between groups were observed at baseline and for other related variables, data were further examined by computing the absolute (6-months – baseline) or relative (percentage of difference from baseline) changes over 6-months for each group and using Student's independent t-tests (exercise vs. control). Statistical significance was accepted at P < 0.05.

# 6.4. Results

### Patients' characteristics at baseline

Eighteen patients in the exercise group and 14 non-exercise controls completed the study. Reasons for dropping out were progression to dialysis (n=2), change in personal circumstances (n=2), and lack of motivation (n=4). Baseline characteristics of patients that completed the study are shown in Table 6.1. There was a similar distribution by gender among the groups (exercise: 61% males and 39% females vs. control: 57% males and 43% females) and the groups did not differ in terms of age (P = 0.201) and anthropometric characteristics (weight: P = 0.271, height: P = 0.619, BMI: P = 0.327). Kidney function was also comparable between groups (eGFR: P = 0.533). The majority of the patients on each group were in stages 3 and 4 of CKD.

Exercise group	Control group
(n=18)	(n=14)
11 / 7	8 / 6
62 ± 8	56 ± 15
80.2 ± 15.2	87.7 ± 20.9
171 ± 11	173 ± 12
27.5 ± 4.4	29.3 ± 5.5
25.3 ± 7.9	29.3 ± 5.5
7 (39%) 8 (44%) 3 (17%)	6 (43%) 7 (50%) 1 (7%)
3 (17%) 2 (11%) 3 (17%) 1 (6%) 2 (11%) 0 4 (22%) 3 (17%)	0 1 (7%) 4 (29%) 1 (7%) 0 1 (7%) 6 (43%) 1 (7%)
0 8 (44%) 5 (28%) 2 (11%) 3 (17%) 5 (28%)	2 (14%) 6 (43%) 2 (14%) 3 (21%) 1 (7%) 3 (21%)
	Exercise group (n=18) 11 / 7 62 $\pm$ 8 80.2 $\pm$ 15.2 171 $\pm$ 11 27.5 $\pm$ 4.4 25.3 $\pm$ 7.9 7 (39%) 8 (44%) 3 (17%) 2 (11%) 3 (17%) 1 (6%) 2 (11%) 3 (17%) 1 (6%) 2 (11%) 3 (17%) 1 (6%) 2 (11%) 3 (17%) 5 (28%) 2 (11%) 3 (17%) 5 (28%)

Table 6.1. Patients' characteristics at baseline for each group.

Data are \*mean ± SD or <sup>#</sup>n (%)

BMI: body mass indexData areeGFR: estimated glomerular filtration rateCKD: chronic kidney diseasea) based on eGFRb) grouped according to UK Renal Registry (2010)c) excluded from the analysis of immune and inflammatory parameters

#### **Exercise test outcomes**

The average walking speed during both exercise tests did not differ between groups (exercise:  $4.3 \pm 1.3$  km/h vs. control:  $4.0 \pm 1.2$  km/h, P = 0.479). The RPE response to both exercise tests for each group is illustrated in Figure 6.1. RPE response did not differ between groups at baseline (Table 6.2; P = 0.693and P = 0.759 for average and area under curve, respectively) but it was affected by the exercise intervention (Table 6.2; time x group interaction for average: F = 5.5, P = 0.026 and area under curve: F = 5.0, P = 0.034). At 6months, the RPE response in the exercise group was lower than at baseline (Table 6.2; average: P = 0.0003, ES = 1.75 and area under curve: P = 0.00004, ES = 1.72), and also lower than the control group at that time (Table 6.2; average: P = 0.029, ES = 0.84 and area under curve: P = 0.032, ES = 0.83). In contrast, RPE response did not change in the control group (Table 6.2; average: P = 0.141, ES = 0.56 and area under curve: P = 0.124, ES = 0.61). Plasma lactate response to exercise did not differ between groups at baseline (Table 6.2; P = 0.586) and it was unaffected by the exercise intervention (Table 6.2).



Figure 6.1. Rating of perceived exertion (RPE) response to baseline and 6-months exercise tests for a) exercise group and b) control group. Data are mean  $\pm$  SD (n=18 ex; n=14 ctrl).

	Exercis	xercise group Control group		ol group
	Baseline	6-months	Baseline	6-months
<b>RPE</b> (average)	12.4 ± 0.6	10.4 ± 1.5* <sup>#</sup>	12.3 ± 0.7	11.6 ± 1.5
<b>RPE</b> (area under curve)	180 ± 18	124 ± 43* <sup>#</sup>	178 ± 18	159 ± 41
<b>∆ plasma lactate</b> (mmol/l)	1.0 ± 1.1	0.6 ± 0.9	0.8 ± 0.7	0.8 ± 0.7
DDE: rating of paragive	devertion	Data	ara maan + SD (	n=19 ov: n=14 otrl)

 Table 6.2. Exercise test outcomes at baseline and 6-months for each group.

RPE: rating of perceived exertion  $\Delta$  = post-ex – pre-ex

Data are mean ± SD (n=18 ex; n=14 ctrl) \**P* < 0.05 vs. baseline \**P* < 0.05 vs. control

## **Body composition**

The exercise intervention did not affect weight or BMI (Table 6.3). However, weight and consequently BMI were lower at 6-months in comparison with baseline (Table 6.3; main effects of time for weight: F = 8.3, P = 0.007 and BMI: F = 9.9, P = 0.004).

## Total and differential blood leukocyte counts

Total and differential blood leukocyte counts at baseline and 6-months for each group are given in Table 6.3. At baseline, there were no differences between groups in numbers of circulating leukocytes (P = 0.562), neutrophils (P = 0.556), lymphocytes (P = 0.973) or monocytes (P = 0.659) and neither of these parameters were affected by the exercise intervention. There were also no time x group interactions nor main effects of time for haematocrit, haemoglobin or plasma volume (Table 6.3).

	Exercise group		Control group	
	Baseline	6-months	Baseline	6-months
Body composition*				
Weight (kg)	80.2 ± 15.2	78.5 ± 15.1	87.7 ± 20.9	85.9 ± 19.5
<b>BMI</b> (kg/m²)	27.5 ± 4.4	27.0 ± 4.5	29.3 ± 5.5	28.6 ± 4.9
General haematolog	<i>ay*</i>			
Haematocrit (%)	36.9 ± 4.5	37.4 ± 3.7	36.3 ± 3.2	35.6 ± 3.8
<b>Haemoglobin</b> (g/dl)	13.6 ± 1.7	13.4 ± 1.4	13.5 ± 1.8	12.6 ± 2.5
Plasma volume (%)	63.1 ± 4.5	62.6 ± 3.7	63.7 ± 3.2	64.4 ± 3.8
Total and differentia	l blood leukocy	/te counts <sup>#</sup>		
Leukocytes (x 10 <sup>9</sup> /l)	6.7 ± 2.4	6.6 ± 2.1	6.2 ± 1.5	5.9 ± 1.6
Neutrophils (x 10 <sup>9</sup> /l	4.3 ± 1.8	4.2 ± 1.6	3.9 ± 1.3	3.6 ± 1.2
Lymphocytes (x 10 <sup>9</sup> /l)	1.5 ± 0.6	1.6 ± 0.6	1.5 ± 0.3	1.6 ± 0.5
Monocytes (x 10 <sup>9</sup> /l)	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2

Table 6.3. Body composition, general haematology and total and differential blood leukocyte counts at baseline and 6-months for each group.

BMI: body mass index

Data are mean ± SD

(\*n=18 or <sup>#</sup>n=13 ex; \*n=14 or <sup>#</sup>n=11 ctrl)

#### T lymphocyte subsets

T lymphocyte subset counts are shown in Table 6.4. At baseline, there were no differences among groups in the numbers of CD4<sup>+</sup> (P = 0.652) and CD8<sup>++</sup> (P = 0.785) lymphocytes. Therefore, the CD4<sup>+</sup>/CD8<sup>++</sup> ratio was also comparable between groups (Table 6.4; P = 0.491). There were no time x group interactions for any of these measures (Table 6.4).

## Activation of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes

The percentages of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes expressing CD69 following 20 h *in vitro* SEB stimulation did not differ between groups at baseline (Table 6.4; P = 0.645 and P = 0.790, respectively) and were also unaffected by the exercise intervention (Table 6.4). However, there were main effects of time for both CD4<sup>+</sup> (F = 21.5, P < 0.0005) and CD8<sup>++</sup> (F = 7.4, P = 0.013) percentage of positive cells for CD69, which were lower at 6-months in comparison with baseline (Table 6.4). SEB-stimulated CD69 expression (GMFI) by either CD4<sup>+</sup>CD69<sup>+</sup> or CD8<sup>++</sup>CD69<sup>+</sup> lymphocytes at baseline tended to be higher in the exercise group in comparison with the control group (Table 6.4; P = 0.056 and P = 0.053, respectively). There was a time x group interaction (F = 6.1, P =0.024) and a main effect of time (F = 6.9, P = 0.017) for CD69 expression by CD4<sup>+</sup>CD69<sup>+</sup> lymphocytes (Table 6.4), which was down-regulated following the exercise intervention in the exercise group (Table 6.4; P = 0.017, ES = 0.59) while it did not change in the control group (Table 6.4; P = 0.912). Likewise, a time x group interaction (F = 13.0, P = 0.002) and a main effect of time (F = 4.6, P = 0.046) were also found for CD69 expression by CD8<sup>++</sup>CD69<sup>+</sup> lymphocytes (Table 6.4), which was also down-regulated after 6-months in the exercise group (Table 6.4; P = 0.010, ES = 0.65) but remained unaltered in the control group (Table 6.4; P = 0.266). Comparisons of the relative changes over 6months (i.e. percentage of difference from baseline) between groups in SEBstimulated CD69 expression (GMFI) by either CD4<sup>+</sup>CD69<sup>+</sup> (Figure 6.2) or  $CD8^{++}CD69^{+}$  (Figure 6.3) lymphocytes confirmed these effects (ES = 1.09 and ES = 1.70, respectively).

Table 6.4. CD4<sup>+</sup> and CD8<sup>++</sup> lymphocyte counts and CD4<sup>+</sup>/CD8<sup>++</sup> ratio, percentages of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes expressing CD69 and CD69 expression by CD4<sup>+</sup>CD69<sup>+</sup> and CD8<sup>++</sup>CD69<sup>+</sup> lymphocytes after 20 h *in vitro* stimulation with staphylococcal enterotoxin B (SEB) at baseline and 6-months for each group.

	Exercise group		Contro	ol group
	Baseline	6-months	Baseline	6-months
T lymphocyte sub	sets			
CD4 <sup>+</sup> (x 10 <sup>9</sup> /l)	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.3
CD8 <sup>++ #</sup> (x 10 <sup>9</sup> /l)	$0.4 \pm 0.3$	0.4 ± 0.3	0.3 ± 0.1	0.3 ± 0.1
CD4 <sup>+</sup> /CD8 <sup>++</sup> #	2.2 ± 1.6	2.4 ± 2.0	2.3 ± 1.0	2.4 ± 1.0
CD69 <sup>+</sup> cells (%)				
$CD4^+$	24 ± 10	20 ± 8	26 ± 8	22 ± 8
CD8 <sup>++</sup>	30 ± 15	24 ± 10	31 ± 10	28 ± 8
<b>CD69</b> (GMFI)				
$CD4^{+}CD69^{+}$	175 ± 55	143 ± 53*	131 ± 41	130 ± 42
CD8 <sup>++</sup> CD69 <sup>+</sup>	110 ± 44	85 ± 32*	77 ± 23	82 ± 23

GMFI: geometric mean of fluorescence intensity \*Statistical analysis performed on log-transformed data

Data are mean ± SD (n=10 ex; n=10 ctrl)

\*P < 0.05 vs. baseline



**Figure 6.2.** Relative changes over 6-months in SEB-stimulated CD69 expression (GMFI) by CD4<sup>+</sup>CD69<sup>+</sup> lymphocytes for each group. Data are mean and individual values (n=10 ex; n=10 ctrl).



**Figure 6.3. Relative changes over 6-months in SEB-stimulated CD69 expression (GMFI) by CD8<sup>++</sup>CD69<sup>+</sup> lymphocytes for each group.** Data are mean and individual values (n=10 ex; n=10 ctrl).

# Activation of CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes

CD86 expression (GMFI) by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes following 20 h in vitro SEB stimulation (expressed as a ratio to unstimulated condition) at baseline was higher in the exercise group in comparison with the control group (Table 6.5; P = 0.015), while HLA-DR expression by the same cells did not differ significantly (Table 6.5; P = 0.108). There was a time x group interaction for CD86 expression by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes (Table 6.5; F = 24.8, P < 0.0005), which was down-regulated following the exercise intervention in the exercise group (Table 6.5; P = 0.004, ES = 1.27) but in contrast it was upregulated in the control group (Table 6.5; P = 0.018, ES = 0.76). Similarly, a time x group interaction was also found for HLA-DR expression by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes (Table 6.5; F = 0.6, P = 0.006), which was also down-regulated after 6-months in the exercise group (Table 6.5; P = 0.037, ES = 0.72) and up-regulated in the control group (Table 6.5; P = 0.043, ES = 0.51). Comparisons of the relative changes over 6-months (i.e. percentage of difference from baseline) between groups in SEB-stimulated CD86 (Figure 6.4) and HLA-DR (Figure 6.5) expression (GMFI expressed as a ratio to unstimulated condition) by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes confirmed these effects (ES = 2.12 and ES = 1.17, respectively).

Table 6.5. CD86 and HLA-DR expression by CD14+CD86+HLA-DR+ monocytes after 20 h
in vitro stimulation with staphylococcal enterotoxin B (SEB) at baseline and 6-months for
each group.

	Exercise group		Control group	
	Baseline	6-months	Baseline	6-months
<b>CD86</b> (GMFI <sup>a)</sup> )	2.1 ± 0.8 <sup>#</sup>	1.3 ± 0.3*	1.3 ± 0.5	1.7 ± 0.6*
HLA-DR (GMFI <sup>a)</sup> )	3.0 ± 1.7	2.0 ± 0.7*	1.9 ± 0.5	2.3 ± 0.9*

GMFI: geometric mean of fluorescence intensity

<sup>a)</sup>Ratio to unstimulated condition

Statistical analysis performed on log-transformed data

Data are mean ± SD (n=11 ex; n=10 ctrl) \**P* < 0.05 vs. baseline \**P* < 0.05 vs. control



Figure 6.4. Relative changes over 6-months in SEB-stimulated CD86 expression (GMFI expressed as a ratio to unstimulated condition) by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes for each group. Data are mean and individual values (n=11 ex; n=10 ctrl).



Figure 6.5. Relative changes over 6-months in SEB-stimulated HLA-DR expression (GMFI expressed as a ratio to unstimulated condition) by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes for each group. Data are mean and individual values (n=11 ex; n=10 ctrl).
#### Neutrophil degranulation (elastase release)

At baseline, there were no differences between groups for plasma elastase concentration, i.e. unstimulated elastase release (P = 0.833) or total and per neutrophil elastase release following 1 h *in vitro* stimulation with bacterial extract (P = 0.667 and P = 0.992, respectively) and the exercise intervention had no effect on any of these measures (Table 6.6).

Table 6.6. Plasma elastase (unstimulated elastase release) and total and per neutrophil elastase release following 1 h *in vitro* stimulation with bacterial extract at baseline and 6-months for each group.

	Exercise group		Control group		
	Baseline	6-months	Baseline	6-months	
Plasma elastase (	ug /l)				
	68 ± 22	63 ± 22	70 ± 18	62 ± 9	
Total bacterially-stimulated elastase release* (µg /l)					
	3703 ± 1892	3586 ± 2107	3150 ± 1636	2837 ± 1134	
Bacterially-stimulated elastase release per neutrophil* (fg /cell)					
	522 ± 121	539 ± 174	530 ± 144	535 ± 141	
*Statistical analysis per	Statistical analysis performed on log-transformed data			Data are mean ± SD (n=13 ex; n=11 ctrl)	

#### Plasma markers of systemic inflammation

At baseline, there were no differences between groups for plasma IL-6 (Figure 6.6; P = 0.632), IL-10 (Figure 6.7; P = 0.148), IL-6/IL-10 ratio (Figure 6.8; P = 0.318), sTNF-RII (Figure 6.10; P = 0.556) and CRP (Figure 6.11; P = 0.897) but plasma sTNF-RI concentration was higher in the exercise group in comparison with the control group (Figure 6.9; P = 0.009). There were time x group interactions for plasma IL-10 (Figure 6.7; F = 4.8, P = 0.043), IL-6/IL-10 ratio (Figure 6.8; F = 9.3, P = 0.006) and sTNF-RI (Figure 6.9; F = 7.5, P = 0.013). Plasma IL-10 levels tended to be increased after 6-months in the exercise group (Figure 6.7; P = 0.074, ES = 0.66) but did not change in the control group (Figure 6.7; P = 0.333). Plasma IL-6/IL-10 ratio was reduced following the

exercise intervention in the exercise group (Figure 6.8; P = 0.024; ES = 0.65) while it remained unchanged in the control group (Figure 6.8; P = 0.117). Plasma sTNF-RI concentration also decreased in the exercise group (Figure 6.9; P = 0.044, ES = 0.20) and remained unaltered in the control group (Figure 6.9; P = 0.134). In addition, there was a main effect of group for plasma sTNF-RI concentration (Figure 6.9; F = 6.3, P = 0.020) with higher overall values observed in the exercise group. Trends for time x group interactions were also observed for plasma levels of IL-6 (Figure 6.6; F = 4.2, P = 0.054) and sTNF-RII (Figure 6.10; F = 3.4, P = 0.082), which tended to be reduced after 6-months in the exercise group (Figure 6.6; P = 0.060, ES = 0.40 and Figure 6.10; P =0.064, ES = 0.25, respectively) but remained unaltered in the control group (Figure 6.6; P = 0.429 and Figure 6.10; P = 0.504, respectively). There was no time x group interaction for plasma CRP concentration (Figure 6.11). Comparisons of the absolute changes over 6-months (i.e. absolute difference from baseline) between groups confirmed the statistical outcomes of the time x group interactions but are useful in the interpretation of the observed effects and trends as the absolute changes observed for each marker were in opposite directions (Table 6.7). Absolute changes over 6-months were different between groups for plasma IL-10, IL-6/IL-10 and sTNF-RI (ES = 0.93, ES = 1.27 and ES = 1.15, respectively) and tend to differ for plasma IL-6 and sTNF-RII (ES = 0.88) and ES = 0.78, respectively), while absolute changes over 6-months in plasma CRP did not differ between groups (ES = 0.40).



Figure 6.6. Plasma interleukin-6 (IL-6) at baseline and 6-months for each group. Data are mean and individual values (n=12 ex; n=10 ctrl); statistical analysis performed on log-transformed data; time x group interaction (trend): P = 0.054.



Figure 6.7. Plasma interleukin-10 (IL-10) at baseline and 6-months for each group. Data are mean and individual values (n=10 ex; n=9 ctrl); statistical analysis performed on log-transformed data; time x group interaction: P = 0.043.



Figure 6.8. Plasma interleukin-6 to interleukin-10 (IL-6/IL-10) ratio at baseline and 6-months for each group. Data are mean and individual values (n=12 ex; n=11 ctrl); statistical analysis performed on log-transformed data; time x group interaction: P = 0.006; \*P < 0.05 vs. baseline.



Figure 6.9. Plasma soluble tumour necrosis factor receptor I (sTNF-RI) at baseline and 6months for each group. Data are mean and individual values (n=13 ex; n=10 ctrl); statistical analysis performed on log-transformed data; time x group interaction: P = 0.013; \*P < 0.05vs. baseline;  $^{#}P < 0.05$  vs. control.



Figure 6.10. Plasma soluble tumour necrosis factor receptor II (sTNF-RII) at baseline and 6-months for exercise and control groups. Data are mean and individual values (n=12 ex; n=10 ctrl); statistical analysis performed on log-transformed data; time x group interaction (trend): P = 0.082.



Figure 6.11. Plasma C-reactive protein (CRP) at baseline and 6-months for exercise and control groups. Data are mean and individual values (n=13 ex; n=9 ctrl); statistical analysis performed on log-transformed data.

	Exercise group	Control group	<b>P</b> *
<b>∆ IL-6</b> (pg/ml)	-2.2 ± 3.6	0.4 ± 2.1	0.054
<b>△ IL-10</b> (pg/ml)	1.5 ± 2.3	-0.5 ± 1.7	0.043
Δ IL-6/IL-10 ratio	-1.0 ± 1.2	0.2 ± 0.6	0.006
Δ sTNF-RI (ng/ml)	-0.5 ± 0.7	$0.3 \pm 0.7$	0.013
Δ sTNF-RII (ng/ml)	-1.7 ± 3.2	1.1 ± 3.9	0.082
<b>Δ CRP</b> (μg/ml)	-0.9 ± 2.2	0.6 ± 2.2	0.370

Table 6.7. Absolute changes over 6-months in plasma markers of systemic inflammation for each group.

IL: interleukin

sTNF-R: soluble tumour necrosis factor receptor

Data are mean ± SD (n=see corresponding figure)

CRP: C-reactive protein  $\Delta$  = 6-months – baseline

\*Statistical analysis performed on log-transformed data

# Effect of additional bicarbonate therapy

Ten of the 20 patients initially recruited for each group were randomised to receive additional oral bicarbonate (target plasma bicarbonate 29 mmol/l) or to continue with their usual bicarbonate therapy (target plasma bicarbonate 24 mmol/l).

Eight of the 18 patients in the exercise group who completed the study, and 6 of the 14 patients in the control group who completed the study received additional bicarbonate therapy.

To investigate the effects of the additional bicarbonate therapy on the exercise group data were examined using an ANOVA with mixed design: within-subjects (time: baseline vs. 6-months) x between-subjects (treatment: standard vs. bicarbonate).

As expected, there was an effect of the additional bicarbonate therapy on plasma bicarbonate concentration (F = 7.0, P = 0.017). At 6-months, plasma bicarbonate levels were elevated above baseline levels in the bicarbonate group (6-months:  $27.4 \pm 1.3 \text{ mmol/l}$  vs. baseline:  $23.1 \pm 3.2 \text{ mmol/l}$ , P = 0.009) but remained unchanged in the standard group (6-months:  $25.0 \pm 2.5 \text{ mmol/l}$  vs. baseline:  $23.9 \pm 2.6 \text{ mmol/l}$ , P = 0.137).

However, there were no effects of the additional bicarbonate therapy on any of the immune and inflammatory parameters studied.

### 6.5. Discussion

This study investigated the effects of 6-months of regular moderate intensity aerobic exercise on measures of immunity and inflammation in pre-dialysis CKD patients. We found that the exercise intervention down-regulated T-lymphocyte and monocyte activation and that these effects were accompanied by improvements in the systemic inflammatory status. In contrast, the exercise intervention had no effect on the neutrophil degranulation responses.

The exercise intervention improved exercise tolerance in the exercise group only, as indicated by the markedly lower RPE response to the exercise test at 6-months than at baseline, an effect that was not observable in the control group. This confirms the findings from our previous 1-month uncontrolled study (Chapter 5) and adds to previous evidence that aerobic exercise training can improve exercise tolerance and capacity in pre-dialysis CKD patients (Clyne et al., 1991; Eidemak et al., 1997; Boyce et al., 1997; Leehey et al., 2009; Toyama et al., 2010; Mustata et al., 2010). CKD is associated with an accelerated decline in physical function, reaching levels that significantly impact on clinical outcomes, such as morbidity, quality of life and, ultimately, survival (Kosmadakis et al., 2010). Here, we provide evidence that a home-based involving walking intervention exercise can bring about significant improvements in the pre-dialysis CKD patients' ability to perform sustained effort at submaximal levels; an intensity likely to reflect their capacity to perform daily living activities.

In contrast with the findings from our previous 1-month uncontrolled study (Chapter 5), the plasma lactate response to the acute exercise test was unaffected by the exercise intervention. Despite not reaching statistical significance it should be noted, however, that the acute lactate response was nearly halved in the exercise group at 6-months while it remained absolutely unaltered in the control group. As expected with moderate intensity exercise, the exercise-induced rise in plasma lactate levels was only small, which perhaps makes it difficult to detect statistical significant changes. In addition, it is noteworthy that a separate part of this study has suggested that the expected training effect on the lactate response to acute exercise appears to be

augmented by the administration of additional bicarbonate (Clapp, 2010). This appears to be because a significant reduction in the lactate response was observed at 6-months in the group of patients from the exercise group that received additional bicarbonate therapy. Although we have continuously monitored heart rate (HR) during exercise testing, it was concluded that the HR response to the acute exercise test was an unreliable measure of exercise tolerance. The reason for this is that there were changes in the patients' prescription of anti-hypertensive medication dosages throughout the study, which, in addition, differed between the exercise and control groups (Kosmadakis *et al.*, 2011). Hence, HR data is not presented here.

Total and differential blood leukocyte counts were not affected by the exercise intervention, confirming the findings from our previous 1-month uncontrolled study (Chapter 5). Disturbances in total and differential blood leukocytes are often reported in CKD (Eleftheriadis *et al.*, 2007; Agarwal & Light, 2011). We have previously reported that acute exercise of the same mode, intensity and duration as prescribed here elicits alterations in total and differential blood leukocyte counts after exercise (Chapter 4). In this context, the present findings primarily indicate that the exercise regimen used is safe, as there is no indication that the acute changes that might occur with each bout of exercise affect the resting cell counts from pre-dialysis CKD patients when such exercise is performed on a regular basis.

The exercise intervention had no effect on the *in vivo* and *in vitro* neutrophil degranulation responses, which is again in agreement with our previous findings (Chapter 5). In CKD, neutrophils are known to be in a primed state, which is associated with the state of systemic inflammation and oxidative stress that accompanies the disease (Sela *et al.*, 2005). We have previously reported that acute exercise of the same mode, intensity and duration as prescribed here induces spontaneous neutrophil activation after exercise in pre-dialysis CKD patients (Chapter 4). The present data confirm that when such exercise is performed regularly, it does not aggravate the activation state of neutrophils from pre-dialysis CKD patients. Furthermore, neutrophil function is impaired in CKD, which is believed to contribute to the high incidence of infections (Chonchol, 2006). We have previously shown that improved neutrophil

responsiveness to an *in vitro* bacterial challenge is observed following an acute bout of exercise in pre-dialysis CKD patients (Chapter 4). The present findings confirm that such an effect does not appear to translate into improved resting neutrophil function when patients engage in regular exercise, but as importantly, confirm that regular exercise does not appear to hinder neutrophil immune competence in pre-dialysis CKD patients. On the other hand, given that data from the control patients are not suggestive of altered *in vivo* and *in vitro* neutrophil degranulation responses over the 6-months study period, it remains to be determined if engagement in regular exercise may confer protection against a progressive exacerbation of neutrophil activation and a progressive decline in neutrophil function over the longer term. The cross-sectional evidence that long-term physical activity may counteract the age-associated decline in neutrophil function (Yan *et al.*, 2001) is certainly supportive of potential benefits.

In agreement with our previous findings (Chapter 5) and a previous study in haemodialysis patients (Daniilidis *et al.*, 2004), T lymphocyte subset counts and their relative distribution, i.e. CD4<sup>+</sup>/CD8<sup>++</sup> ratio, were also unaffected by the exercise intervention. The percentages of CD4<sup>+</sup> and CD8<sup>++</sup> lymphocytes expressing CD69 following *in vitro* SEB stimulation were not affected by the exercise intervention, however CD69 expression (GMFI) by these cells was down-regulated in the exercise group, while it remained unaltered in the controls. These results suggest that regular exercise reduces T lymphocyte activation in pre-dialysis CKD patients. This is a very promising effect considering that it has been consistently demonstrated that T lymphocytes are chronically activated in CKD (Descamps-Latscha *et al.*, 1995; Meier *et al.*, 2002; Moser *et al.*, 2003; Litjens *et al.*, 2006).

Given that T lymphocyte "pre-activation" in CKD patients is associated with premature apoptosis and impaired proliferation of these cells (Ankersmit *et al.*, 2001; Meier *et al.*, 2002; Moser *et al.*, 2003; Meier *et al.*, 2005; 2007), it may be speculated that the down-regulation of T lymphocyte activation through regular exercise is actually enhanced positive effect for the function of these cells. Of interest, TNF-RI is a well known cell death-inducing receptor (Ashkenazi & Dixit, 1998; Naudé *et al.*, 2011) and previous studies have used the concentrations of

plasma sTNF-RI as an indicator of the susceptibility of T cells to undergo increased activation-induced cell death in CKD patients (Ankersmit *et al.*, 2001; Moser *et al.*, 2003). In the present study, plasma sTNF-RI levels were reduced in the exercise group and remained unaltered in the control group. These findings match the T lymphocyte activation data and suggest, albeit indirectly, that a down-regulation of T lymphocyte activation following the exercise intervention was accompanied by reduced apoptosis of these cells. Clearly, the impact of regular exercise on T lymphocyte apoptosis and function in CKD patients warrants further investigation.

The exercise intervention also affected monocyte activation. CD86 and HLA-DR expression (GMFI) by CD14<sup>+</sup>CD86<sup>+</sup>HLA-DR<sup>+</sup> monocytes following *in vitro* SEB-stimulation were down-regulated in the exercise group, but up-regulated in the control group. These results confirm our previous work (Chapter 5) indicating that regular exercise reduces monocyte activation in pre-dialysis CKD patients. It is likely that this down-regulation of monocyte activation have contributed, at least in part, to the observed effects on T lymphocyte activation, as this occurs through interactions between the monocyte and the T cell. On the other hand, we can only attribute the up-regulation of monocyte activation that was observed in the control group to a progressive state of monocyte activation that accompanies CKD progression (Descamps-Latscha *et al.*, 1995).

Possible mechanisms mediating the down-regulation of monocyte activation following exercise training have been discussed in Chapter 5. Briefly, given that exercise training down-regulates monocyte Toll-like receptor (TLR) 4 expression (Gleeson *et al.*, 2006; Flynn & McFarlin, 2006) and that activation of TLRs up-regulates monocyte expression of HLA-DR and CD86 (Takeda *et al.*, 2003), it is conceivable that the down-regulation of CD86 and HLA-DR observed in the present study was mediated by a down-regulation of TLR 4. An alternative explanation proposed was that the down-regulation of monocyte activation following regular exercise may be related to a reduced proportion of circulating CD16<sup>+</sup> monocytes, which are known to express higher levels of CD86 and HLA-DR in comparison with classical monocytes (Takeda *et al.*, 2003). It is interesting to note that IL-10 production is low-to-absent in CD16<sup>+</sup> monocytes (Ziegler-Heitbrock, 2007). Although we did not perform direct

measurements of monocyte production of IL-10 in the present study, we did observe a trend for increased systemic levels of IL-10 in the exercise group, which may provide some indirect support for a possible shift into a subset of circulating monocytes that are able to produce IL-10 following the exercise intervention. With these initial findings in mind, the effects of regular exercise on the different monocyte subsets and their cytokine production profiles in CKD patients certainly deserve further research.

The effects of regular exercise on systemic markers of inflammation are in agreement with our previous findings (Chapter 5). In the exercise group, plasma IL-6 concentration tended to be reduced after 6-months, while plasma IL-10 concentration tended to be increased, resulting in a significantly decreased ratio of IL-6 to IL-10 in the circulation. Plasma sTNF-RI and sTNF-RII levels were and tended to be reduced, respectively. In contrast, CRP levels remained unaltered. None of these markers were, however, altered in the control group over the 6-months study period. Although it may be argued that these effects were not as statistically sound as desired because of the limited sample size, they certainly provide evidence of the potential anti-inflammatory role of regular exercise engagement in pre-dialysis CKD patients. This is further strengthened by the accompanying anti-inflammatory effects that were observed at the cell level. The significance and the underlying mechanisms of these anti-inflammatory effects of regular exercise in pre-dialysis CKD patients have already been thoroughly discussed (Chapter 5).

An obvious limitation of our study is its non-randomised design and therefore the intervention group might have included the patients who where wiling to exercise whereas the control group might have included those who where unwilling. However, this research project had a pragmatic design and when we had some difficulties in recruiting patients to the 1-month pilot study we opted to invite them to carry on to the 6-month trial and therefore making randomisation unviable. Notwithstanding, we were also concerned to minimize the risk of "contamination", i.e. that patients in the study who met during their regular clinical care as well as for study sessions may influence each others' exercise patterns. In this small study, such contamination could have been a significant confounder. Given that we have used a sequential design (i.e. only exercisers, and later non-exercisers, were involved in the study at any one time) such potential confounder was avoided.

In summary, these findings confirm that regular moderate intensity aerobic exercise in pre-dialysis CKD patients is safe from an immune and inflammatory perspective. The 6-months home-based exercise programme exerted anti-inflammatory effects at both systemic and cell level. These findings suggest that regular walking exercise has the potential to be an effective anti-inflammatory therapy in pre-dialysis CKD patients.

Chapter 7

General Discussion

The studies reported in this thesis provide compelling evidence that walking exercise is safe from an immune and inflammatory perspective and has the potential to be an effective anti-inflammatory therapy in pre-dialysis CKD patients.

In Chapter 4, it was demonstrated that a single bout of walking exercise induced an overall immune and inflammatory response that was comparable to that observed in healthy individuals, with no indication of harmful effects to patients' underlying state of immune dysfunction. Acute exercise induced a normal pattern of mobilisation of immune cells. Concerning immune cell function, acute exercise had no effect on T lymphocyte and monocyte activation, while it actually improved neutrophil responsiveness to a bacterial challenge in the recovery period. In addition, acute exercise induced a systemic anti-inflammatory environment, evidenced by the marked elevation in circulating levels of plasma IL-10 following exercise, which was most likely mediated by the observed increase in plasma IL-6 levels.

In Chapters 5 and 6, it was demonstrated that regular walking exercise exerted anti-inflammatory effects, with no apparent detrimental effects to patients' immune and inflammatory status. Regular exercise led to improvements in the systemic inflammatory status (ratio of pro-inflammatory IL-6 to anti-inflammatory IL-10 cytokine levels) that were accompanied, and most likely mediated, by a down-regulation of T lymphocyte (only evident after 6-months) and monocyte activation. In addition, a reduction in IL-6 production in PBMC and whole blood cultures was also observed, though this was only assessed in the 1-month study. On the other hand, regular exercise had no effect on circulating immune cell numbers and neutrophil degranulation responses.

Considering the well-known immune deficient and inflammatory state that accompanies CKD (Kato *et al.*, 2008; Carrero & Stenvinkel, 2010), it is somewhat surprising that this was the first investigation on the impact of acute exercise on measures of immunity and inflammation in CKD patients. Nevertheless, it is reassuring that no detrimental effects of acute exercise on these measures were observed. In fact, acute exercise appeared to improve neutrophil immune competence in the recovery period. Although it was later

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found that when patients engaged in regular exercise such effect did not translate into improved resting neutrophil function, it is still possible that there is a window of protection following each exercise bout. Because we have only collected blood samples up to 1 h after exercise it would be interesting that future studies determine the duration of this effect beyond this time point. In addition, it would be relevant to investigate if this improvement in neutrophil responsiveness still occurs when patients become accustomed to exercise, i.e. if acute exercise confers a temporary protective effect when patients are engaged in regular exercise.

Although acute exercise induced an increase in plasma IL-6 levels, this is likely to be originated from the contracting skeletal muscle during exercise and unlikely to be preceded by elevations in pro-inflammatory cytokines. In contrast to the cytokine cascade in sepsis, where IL-6 appearance is preceded by the pro-inflammatory TNF- $\alpha$  and IL-1 $\beta$ , in response to exercise IL-6 is typically the first cytokine present in the circulation (Petersen & Pedersen, 2005; Pedersen & Febbraio, 2008). In fact, the increase in plasma IL-6 levels is the most likely cause of the subsequent elevations in plasma IL-10 and sTNF-Rs levels, and therefore the key mediator of the exercise-induced anti-inflammatory environment. Given that this is one of the postulated mechanisms for the antiinflammatory effects of regular exercise (Petersen & Pedersen, 2005; Gleeson et al., 2011), which we have observed in the later studies of this thesis, it would be relevant that future studies attempt to determine the exercise intensity that elicit the greatest acute anti-inflammatory effects in pre-dialysis CKD patients, without obviously compromising their underlying inflammatory state. An ideal study would examine the effects of different exercise intensities on the time course of changes in circulating cytokines (dose-response study design). Of interest, a recent study in healthy volunteers has suggested that compared with 55% and 65% of  $\dot{\textit{V}}\rm{O}_{_{2max}}$  , 60 min of treadmill running at 75% of  $\dot{\textit{V}}\rm{O}_{_{2max}}$  results in a greater increase in circulating IL-6 and IL-1ra but not TNF-α levels (Scott et al., 2011). On the other hand, the cytokine response to acute exercise should also be assessed at different time points during a regular exercise programme to ensure that the anti-inflammatory effects are still occurring when patients become adapted to exercise.

It can be argued that the observed down-regulation of T lymphocyte and monocyte activation are signs of a detrimental effect of regular exercise on the orchestration of the adaptive immune response. T lymphocyte function is clearly impaired in CKD and it is currently acknowledged that this can be attributed to the defective interaction between the APC and the T lymphocyte (Girndt et al., 2001a; Eleftheriadis et al., 2007; Kato et al., 2008). In fact, defective monocyte CD86 expression has been reported in haemodialysis patients (Girndt et al., 2001b). Although this defect was not observed in pre-dialysis patients, evidence of a down-regulation of TLR 4 expression exists for both pre-dialysis (Ando et al., 2006; Koc et al., 2011) and haemodialysis (Kuroki et al., 2007; Koc et al., 2011) patients. Given that exercise training in healthy individuals downregulates monocyte TLR 4 expression (Gleeson et al., 2006; Flynn & McFarlin, 2006) and that activation of TLRs up-regulates monocyte expression of HLA-DR and CD86 (Takeda et al., 2003) it is possible that the observed downregulation of CD86 and HLA-DR was mediated by a down-regulation of TLR 4. Although the impact of regular exercise on monocyte TLR expression in CKD patients needs to be determined in future studies, it appears that the antiinflammatory effects of regular exercise in CKD might be accompanied by some degree of impairment of the adaptive immune response.

However, it is important to recall that the complex state of immune dysfunction that accompanies CKD is characterised by immune depression as well as by immune activation. Monocytes from CKD patients are "pre-activated" and overproduce pro-inflammatory cytokines (Girndt *et al.*, 1995; Higuchi *et al.*, 1997; Morita *et al.*, 1997; Girndt *et al.*, 1998; Sardenberg *et al.*, 2004). Likewise, T lymphocytes from CKD patients show increased expression of early activation markers and heightened apoptotic turnover (Stachowski *et al.*, 1991; Meier *et al.*, 2000; Ankersmit *et al.*, 2001; Meier *et al.*, 2002; Moser *et al.*, 2003; Meier *et al.*, 2005; Litjens *et al.*, 2006; Meier *et al.*, 2007; 2008). Of note, data from non-exercise control patients in our study indicates a progressive state of monocyte activation over the study period. Hence, in the light of our findings it seems that the apparent exercise-induced down-regulation of the immune response might be a small price to pay for the anti-inflammatory effects. On the other hand, seeming paradoxical, besides contributing to reduce inflammation, a down-

regulation of the "pre-activated" state of immune cells might actually contribute to improved immune response in the longer term. The present findings certainly indicate that the down-regulation of T lymphocyte and monocyte activation is associated with reduced inflammation, evidenced at the systemic level and in stimulated cell cultures, but if this is associated with improved immune response remains unknown.

Taken together, our findings do not support that regular exercise can enhance immune function in CKD patients. Apart from the complexities associated with interpreting T lymphocyte and monocyte activation data in this regard, it was observed that regular exercise had no effect on the numbers of circulating immune cells and neutrophil function. However, given that these measures also remained unaltered in the non-exercise control patients over the study period, it is possible that regular exercise might still confer benefits over the longer term. Clearly, future studies should investigate the effects of longer exercise interventions on other functional aspects of the immune cells, preferably measures that are less likely to reflect their activated state.

Given the strong links between inflammation and cardiovascular disease, the leading cause of death in CKD (Stenvinkel, 2010), the present findings are of major clinical relevance and further support the incorporation of exercise in the routine clinical care of patients with CKD. It is important to highlight that exercise rehabilitation in CKD is far behind other chronic disease conditions and although current guidelines state that patients with CKD should be encouraged to exercise (National Collaborating Centre for Chronic Conditions, 2008), there are no specific exercise guidelines for this population. On the other hand, although there is mounting evidence of the beneficial effects of exercise in CKD, it should be noted that the vast majority of exercise research in CKD has been conducted in ESRD, particularly haemodialysis patients (Johansen, 2005; Cheema & Singh, 2005; Painter, 2005; Johansen, 2007; 2008; Cheema, 2008; Segura-Ortí, 2010; Segura-Ortí & Johansen, 2010; Smart & Steele, 2011). While there is no doubt that exercise rehabilitation is important for the ESRD population, the potential benefits of incorporating exercise therapy at earlier stages of the disease are arguably even greater because there are more predialysis patients and they have a far greater likelihood of cardiovascular death

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than progression to ESRD (Drey *et al.*, 2003; Keith *et al.*, 2004; John *et al.*, 2004; Go *et al.*, 2004; Foley *et al.*, 2005). Our findings provide evidence that a home-based regular walking programme has the potential to reduce the high risk of cardiovascular disease in pre-dialysis CKD patients.

It should be noted that although we have asked the patients to fill in exercise diaries, there was no way to quantify exactly how much exercise the patients actually performed, which might have led to some inter-individual variability in the outcomes. Future studies should use a more accurate and objective method of assessing exercise training compliance, such as an accelerometer.

Obviously the anti-inflammatory effects of regular exercise reported in the present thesis need to be confirmed in larger cohorts of pre-dialysis CKD patients. In addition, given the close links between wasting, inflammation and atherosclerosis in CKD (Stenvinkel *et al.*, 1999; 2002*b*), future studies should also address the impact of resistance exercise. There is initial evidence that regular resistance exercise can also induce anti-inflammatory effects in pre-dialysis CKD patients (Castaneda *et al.*, 2004), but a more in-depth study of such effects is warranted. Ideally, investigating both exercise modes alone and in combination in representative groups of patients at different CKD stages would definitely help tailoring future exercise guidelines for this population.

In conclusion, this thesis provides convincing evidence that exercise has the potential to be an effective anti-inflammatory therapy in pre-dialysis CKD patients and may in this way reduce the high risk of cardiovascular disease in these very vulnerable patients. However, more research is needed to fully elucidate the impact of exercise on the complex state of immune dysfunction that accompanies CKD.

# References

- Afshar R, Shegarfy L, Shavandi N & Sanavi S (2010). Effects of aerobic exercise and resistance training on lipid profiles and inflammation status in patients on maintenance hemodialysis. *Indian J Nephrol* **20**, 185–189.
- Afshar R, Emany A, Saremi A, Shavandi N & Sanavi S (2011). Effects of intradialytic aerobic training on sleep quality in hemodialysis patients. *Iran J Kidney Dis* **5**, 119–123.
- Agarwal R & Light RP (2011). Patterns and prognostic value of total and differential leukocyte count in chronic kidney disease. *Clin J Am Soc Nephrol* **6**, 1393–1399.
- Almeras C & Argilés A (2009). The general picture of uremia. Semin Dial 22, 329–333.
- Amore A & Coppo R (2002). Immunological basis of inflammation in dialysis. *Nephrol Dial Transplant* **17** Suppl 8, 16–24.
- Ando M, Shibuya A, Tsuchiya K, Akiba T & Nitta K (2006). Reduced expression of Toll-like receptor 4 contributes to impaired cytokine response of monocytes in uremic patients. *Kidney Int* **70**, 358–362.
- Ankersmit HJ, Deicher R, Moser B, Teufel I, Roth G, Gerlitz S, Itescu S, Wolner E, Boltz-Nitulescu G & Kovarik J (2001). Impaired T cell proliferation, increased soluble deathinducing receptors and activation-induced T cell death in patients undergoing haemodialysis. *Clin Exp Immunol* **125**, 142–148.
- Ansell D, Roderick P, Steenkamp R & Tomson CRV (2010). UK Renal Registry 12th Annual Report (December 2009): chapter 7: survival and causes of death of UK adult patients on renal replacement therapy in 2008: national and centre-specific analyses. *Nephron Clin Pract* **115** Suppl 1, c117–c144.
- Ashkenazi A & Dixit VM (1998). Death receptors: signaling and modulation. *Science* **281**, 1305–1308.
- Atkinson G (2001). Analysis of repeated measurements in physical therapy research. *Phys Ther Sport* **2**, 194–208.
- Baik I, Curhan GC, Rimm EB, Bendich A, Willett WC & Fawzi WW (2000). A prospective study of age and lifestyle factors in relation to community-acquired pneumonia in US men and women. Arch Intern Med 160, 3082–3088.
- Barreto DV, Barreto FC, Liabeuf S, Temmar M, Lemke H-D, Tribouilloy C, Choukroun G, Vanholder R, Massy ZA & European Uremic Toxin Work Group (EUTox) (2010). Plasma interleukin-6 is independently associated with mortality in both hemodialysis and predialysis patients with chronic kidney disease. *Kidney Int* 77, 550–556.
- Batty GD & Lee I-M (2004). Physical activity and coronary heart disease. BMJ 328, 1089–1090.
- Beavers KM, Brinkley TE & Nicklas BJ (2010). Effect of exercise training on chronic inflammation. *Clin Chim Acta* **411**, 785–793.
- Berlin JA & Colditz GA (1990). A meta-analysis of physical activity in the prevention of coronary heart disease. *Am J Epidemiol* **132**, 612–628.
- Blair SN & Morris JN (2009). Healthy hearts--and the universal benefits of being physically active: physical activity and health. *Ann Epidemiol* **19**, 253–256.
- Blair SN, Cheng Y & Holder JS (2001). Is physical activity or physical fitness more important in defining health benefits? *Med Sci Sports Exerc* **33**, S379–S399.

- Blannin AK, Gleeson M, Brooks S & Cave R (1996). Acute effect of exercise on human neutrophil degranulation (Abstract). *J Physiol* **495**, 140P.
- Borg GA (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* **14**, 377–381.
- Boyce ML, Robergs RA, Avasthi PS, Roldan C, Foster A, Montner P, Stark D & Nelson C (1997). Exercise training by individuals with predialysis renal failure: cardiorespiratory endurance, hypertension, and renal function. *Am J Kidney Dis* **30**, 180–192.
- Bretscher PA (1999). A two-step, two-signal model for the primary activation of precursor helper T cells. *Proc Natl Acad Sci USA* **96**, 185–190.
- Broadbent S & Gass G (2008). Aerobic training increases the stimulated percentage of CD4+CD25+ in older men but not older women. *Eur J Appl Physiol* **103**, 79–87.
- Brockhaus M (1997). Soluble TNF receptor: what is the significance? *Intensive Care Med* 23, 808–809.
- Bronas UG (2009). Exercise training and reduction of cardiovascular disease risk factors in patients with chronic kidney disease. *Adv Chronic Kidney Dis* **16**, 449–458.
- Bruunsgaard H (2005). Physical activity and modulation of systemic low-level inflammation. J Leukoc Biol 78, 819–835.
- Bueno C, Criado G, McCormick JK & Madrenas J (2007). T cell signalling induced by bacterial superantigens. *Chem Immunol Allergy* **93**, 161–180.
- Burden R, Tomson C & Guideline Development Committee Joint Specialty Committee on Renal Disease of the Royal College of Physicians of London and the Renal Association (2005). Identification, management and referral of adults with chronic kidney disease: concise guidelines. *Clin med* 5, 635–642.
- Byrne C, Ford D, Gilg J, Ansell D & Feehally J (2010a). UK Renal Registry 12th Annual Report (December 2009): chapter 3: UK ESRD incident rates in 2008: national and centrespecific analyses. *Nephron Clin Pract* **115** Suppl 1, c9–c39.
- Byrne C, Steenkamp R, Castledine C, Ansell D & Feehally J (2010*b*). UK Renal Registry 12th Annual Report (December 2009): chapter 4: UK ESRD prevalent rates in 2008: national and centre-specific analyses. *Nephron Clin Pract* **115** Suppl 1, c41–c67.
- Caimi G, Carollo C, Montana M, Vaccaro F & Presti Lo R (2009). Elastase, myeloperoxidase, nitric oxide metabolites and oxidative status in subjects with clinical stable chronic renal failure on conservative treatment. *Clin Hemorheol Microcirc* **43**, 253–258.
- Campbell JP, Guy K, Cosgrove C, Florida-James GD & Simpson RJ (2008). Total lymphocyte CD8 expression is not a reliable marker of cytotoxic T-cell populations in human peripheral blood following an acute bout of high-intensity exercise. *Brain Behav Immun* 22, 375–380.
- Carrero JJ & Stenvinkel P (2009). Persistent inflammation as a catalyst for other risk factors in chronic kidney disease: a hypothesis proposal. *Clin J Am Soc Nephrol* **4**, S49–S55.
- Carrero JJ & Stenvinkel P (2010). Inflammation in end-stage renal disease--what have we learned in 10 years? Semin Dial 23, 498–509.
- Carrero JJ, Yilmaz MI, Lindholm B & Stenvinkel P (2008). Cytokine dysregulation in chronic kidney disease: how can we treat it? *Blood Purif* **26**, 291–299.
- Carrero JJ, Park S-H, Axelsson J, Lindholm B & Stenvinkel P (2009). Cytokines, atherogenesis, and hypercatabolism in chronic kidney disease: a dreadful triad. *Semin Dial* **22**, 381–386.

- Castaneda C, Gordon PL, Parker RC, Uhlin KL, Roubenoff R & Levey AS (2004). Resistance training to reduce the malnutrition-inflammation complex syndrome of chronic kidney disease. *Am J Kidney Dis* **43**, 607–616.
- Cheema B, Abas H, Smith B, O'Sullivan A, Chan M, Patwardhan A, Kelly J, Gillin A, Pang G, Lloyd B & Singh MF (2007a). Progressive exercise for anabolism in kidney disease (PEAK): a randomized, controlled trial of resistance training during hemodialysis. *J Am Soc Nephrol* **18**, 1594–1601.
- Cheema B, Abas H, Smith B, O'Sullivan A, Chan M, Patwardhan A, Kelly J, Gillin A, Pang G, Lloyd B & Singh MF (2007b). Randomized controlled trial of intradialytic resistance training to target muscle wasting in ESRD: the Progressive Exercise for Anabolism in Kidney Disease (PEAK) study. Am J Kidney Dis 50, 574–584.
- Cheema BSB (2008). Review article: Tackling the survival issue in end-stage renal disease: time to get physical on haemodialysis. *Nephrology (Carlton)* **13**, 560–569.
- Cheema BSB & Singh MAF (2005). Exercise training in patients receiving maintenance hemodialysis: a systematic review of clinical trials. *Am J Nephrol* **25**, 352–364.
- Cheema BSB, Abas H, Smith BCF, O'Sullivan AJ, Chan M, Patwardhan A, Kelly J, Gillin A, Pang G, Lloyd B, Berger K, Baune BT & Singh MAF (2011). Effect of resistance training during hemodialysis on circulating cytokines: a randomized controlled trial. *Eur J Appl Physiol* **111**, 1437–1445.
- Chonchol M (2006). Neutrophil dysfunction and infection risk in end-stage renal disease. *Semin Dial* **19**, 291–296.
- Clapp EL (2010). The Effect of Therapeutic Exercise and Metabolic Acidosis on Skeletal Muscle Wasting in Chronic Kidney Disease. Doctoral Thesis. Loughborough University.
- Clyne N, Ekholm J, Jogestrand T, Lins LE & Pehrsson SK (1991). Effects of exercise training in predialytic uremic patients. *Nephron* **59**, 84–89.
- Cohen G, Haag-Weber M & Hörl WH (1997). Immune dysfunction in uremia. *Kidney Int Suppl* **62**, S79–S82.
- Cohen JW (1988). *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Lawrence Erlbaum Associates, Hillsdale, NJ.
- Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van Lente F & Levey AS (2007). Prevalence of chronic kidney disease in the United States. *JAMA* **298**, 2038–2047.
- Costa E, Rocha S, Rocha-Pereira P, Nascimento H, Castro E, Miranda V, Faria MDS, Loureiro A, Quintanilha A, Belo L & Santos-Silva A (2008). Neutrophil activation and resistance to recombinant human erythropoietin therapy in hemodialysis patients. *Am J Nephrol* **28**, 935–940.
- Dalrymple LS & Go AS (2008). Epidemiology of acute infections among patients with chronic kidney disease. *Clin J Am Soc Nephrol* **3**, 1487–1493.
- Dalrymple LS, Mohammed SM, Mu Y, Johansen KL, Chertow GM, Grimes B, Kaysen GA & Nguyen DV (2011). Risk of cardiovascular events after infection-related hospitalizations in older patients on dialysis. *Clin J Am Soc Nephrol* **6**, 1708–1713.
- Dammin GJ, Couch NP & Murray JE (1957). Prolonged survival of skin homografts in uremic patients. *Ann N Y Acad Sci* **64**, 967–976.

- Daniilidis M, Kouidi E, Giagoudaki F, Fleva A, Nikolaides P, Vasilaki A, Deligiannis A & Tourkantonis A (2004). The immune response in hemodialysis patients following physical training. *Sport Sci Health* **1**, 11–16.
- Department of Health (2005). National Service Framework for Renal Services Part Two: Chronic kidney disease, acute renal failure and end of life care. Department of Health, London.
- Descamps-Latscha B, Herbelin A, Nguyen AT, Roux-Lombard P, Zingraff J, Moynot A, Verger C, Dahmane D, de Groote D & Jungers P (1995). Balance between IL-1 beta, TNF-alpha, and their specific inhibitors in chronic renal failure and maintenance dialysis. Relationships with activation markers of T cells, B cells, and monocytes. *J Immunol* **154**, 882–892.
- Descamps-Latscha B, Jungers P & Witko-Sarsat V (2002). Immune system dysregulation in uremia: role of oxidative stress. *Blood Purif* **20**, 481–484.
- Dill DB & Costill DL (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* **37**, 247–248.
- Drey N, Roderick P, Mullee M & Rogerson M (2003). A population-based study of the incidence and outcomes of diagnosed chronic kidney disease. *Am J Kidney Dis* **42**, 677–684.
- Eidemak I, Haaber AB, Feldt-Rasmussen B, Kanstrup IL & Strandgaard S (1997). Exercise training and the progression of chronic renal failure. *Nephron* **75**, 36–40.
- Eleftheriadis T, Antoniadi G, Liakopoulos V, Kartsios C & Stefanidis I (2007). Disturbances of acquired immunity in hemodialysis patients. *Semin Dial* **20**, 440–451.
- Erikssen G (2001). Physical fitness and changes in mortality: the survival of the fittest. *Sports Med* **31**, 571–576.
- Feehally J, Griffith KE, Lamb EJ, O'donoghue DJ & Tomson CRV (2008). Early detection of chronic kidney disease. *BMJ* **337**, a1618.
- Fischer CP (2006). Interleukin-6 in acute exercise and training: what is the biological relevance? *Exerc Immunol Rev* **12**, 6–33.
- Flynn MG & McFarlin BK (2006). Toll-like receptor 4: link to the anti-inflammatory effects of exercise? *Exerc Sport Sci Rev* **34**, 176–181.
- Foley RN (2006). Infections and cardiovascular disease in patients with chronic kidney disease. *Adv Chronic Kidney Dis* **13**, 205–208.
- Foley RN (2007). Infections in patients with chronic kidney disease. *Infect Dis Clin North Am* **21**, 659–672.
- Foley RN (2008). Infectious complications in chronic dialysis patients. *Perit Dial Int* **28** Suppl 3, S167–S171.
- Foley RN, Parfrey PS & Sarnak MJ (1998). Epidemiology of cardiovascular disease in chronic renal disease. *J Am Soc Nephrol* **9**, S16–S23.
- Foley RN, Murray AM, Li S, Herzog CA, McBean AM, Eggers PW & Collins AJ (2005). Chronic kidney disease and the risk for cardiovascular disease, renal replacement, and death in the United States Medicare population, 1998 to 1999. J Am Soc Nephrol 16, 489–495.
- Fondell E, Lagerros YT, Sundberg CJ, Lekander M, Bälter O, Rothman KJ & Bälter K (2011). Physical activity, stress, and self-reported upper respiratory tract infection. *Med Sci Sports Exerc* 43, 272–279.

- Girndt M, Köhler H, Schiedhelm-Weick E, Meyer zum Büschenfelde KH & Fleischer B (1993). T cell activation defect in hemodialysis patients: evidence for a role of the B7/CD28 pathway. *Kidney Int* **44**, 359–365.
- Girndt M, Köhler H, Schiedhelm-Weick E, Schlaak JF, Meyer zum Büschenfelde KH & Fleischer B (1995). Production of interleukin-6, tumor necrosis factor alpha and interleukin-10 in vitro correlates with the clinical immune defect in chronic hemodialysis patients. *Kidney Int* **47**, 559–565.
- Girndt M, Sester U, Kaul H & Köhler H (1998). Production of proinflammatory and regulatory monokines in hemodialysis patients shown at a single-cell level. *J Am Soc Nephrol* **9**, 1689–1696.
- Girndt M, Sester M, Sester U, Kaul H & Köhler H (2001*a*). Molecular aspects of T- and B-cell function in uremia. *Kidney Int Suppl* **78**, S206–S211.
- Girndt M, Sester M, Sester U, Kaul H & Köhler H (2001*b*). Defective expression of B7-2 (CD86) on monocytes of dialysis patients correlates to the uremia-associated immune defect. *Kidney Int* **59**, 1382–1389.
- Girndt M, Sester U, Sester M, Deman E, Ulrich C, Kaul H & Köhler H (2001*c*). The interleukin-10 promoter genotype determines clinical immune function in hemodialysis patients. *Kidney Int* **60**, 2385–2391.
- Girndt M, Kaul H, Sester U, Ulrich C, Sester M, Georg T & Köhler H (2002). Anti-inflammatory interleukin-10 genotype protects dialysis patients from cardiovascular events. *Kidney Int* 62, 949–955.
- Gleeson M (2007). Immune function in sport and exercise. J Appl Physiol 103, 693–699.
- Gleeson M & Bishop NC (2005). The T cell and NK cell immune response to exercise. *Ann Transplant* **10**, 43–48.
- Gleeson M, McFarlin B & Flynn M (2006). Exercise and Toll-like receptors. *Exerc Immunol Rev* **12**, 34–53.
- Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS & Nimmo MA (2011). The antiinflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol* **11**, 607–615.
- Go AS, Chertow GM, Fan D, McCulloch CE & Hsu C-Y (2004). Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* **351**, 1296–1305.
- Gollapudi P, Yoon J-W, Gollapudi S, Pahl MV & Vaziri ND (2010). Leukocyte toll-like receptor expression in end-stage kidney disease. *Am J Nephrol* **31**, 247–254.
- Gordon S & Taylor PR (2005). Monocyte and macrophage heterogeneity. *Nat Rev Immunol* **5**, 953–964.
- Green KJ, Rowbottom DG & Mackinnon LT (2003). Acute exercise and T-lymphocyte expression of the early activation marker CD69. *Med Sci Sports Exerc* **35**, 582–588.
- Haaland DA, Sabljic TF, Baribeau DA, Mukovozov IM & Hart LE (2008). Is regular exercise a friend or foe of the aging immune system? A systematic review. *Clin J Sport Med* **18**, 539–548.
- Handschin C & Spiegelman BM (2008). The role of exercise and PGC1alpha in inflammation and chronic disease. *Nature* **454**, 463–469.
- Hansson GK (2005). Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* **352**, 1685–1695.

- Hansson GK & Hermansson A (2011). The immune system in atherosclerosis. *Nat Immunol* **12**, 204–212.
- Haus E & Smolensky MH (1999). Biologic rhythms in the immune system. *Chronobiol Int* **16**, 581–622.
- Hauser AB, Stinghen AEM, Kato S, Bucharles S, Aita C, Yuzawa Y & Pecoits-Filho R (2008). Characteristics and causes of immune dysfunction related to uremia and dialysis. *Perit Dial Int* 28 Suppl 3, S183–S187.
- Hedges LV (1981). Distributional theory for Glass's estimator of effect size and related estimators. *J Educ Stat* **6**, 107–128.
- Heine GH, Ulrich C, Seibert E, Seiler S, Marell J, Reichart B, Krause M, Schlitt A, Köhler H & Girndt M (2008). CD14(++)CD16+ monocytes but not total monocyte numbers predict cardiovascular events in dialysis patients. *Kidney Int* **73**, 622–629.
- Herzog CA, Asinger RW, Berger AK, Charytan DM, Díez J, Hart RG, Eckardt K-U, Kasiske BL, McCullough PA, Passman RS, DeLoach SS, Pun PH & Ritz E (2011). Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* **80**, 572–586.
- Higuchi T, Yamamoto C, Kuno T, Mizuno M, Takahashi S & Kanmatsuse K (1997). Increased production of interleukin-1beta and interleukin-1 receptor antagonist by peripheral blood mononuclear cells in undialyzed chronic renal failure. *Nephron* **76**, 26–31.
- Honda H, Qureshi AR, Heimbürger O, Bárány P, Wang K, Pecoits-Filho R, Stenvinkel P & Lindholm B (2006). Serum albumin, C-reactive protein, interleukin 6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. *Am J Kidney Dis* **47**, 139–148.
- Hopkins PA, Fraser JD, Pridmore AC, Russell HH, Read RC & Sriskandan S (2005). Superantigen recognition by HLA class II on monocytes up-regulates toll-like receptor 4 and enhances proinflammatory responses to endotoxin. *Blood* **105**, 3655–3662.
- Ishani A, Collins AJ, Herzog CA & Foley RN (2005). Septicemia, access and cardiovascular disease in dialysis patients: the USRDS Wave 2 study. *Kidney Int* **68**, 311–318.
- James MT, Laupland KB, Tonelli M, Manns BJ, Culleton BF, Hemmelgarn BR & Alberta Kidney Disease Network (2008). Risk of bloodstream infection in patients with chronic kidney disease not treated with dialysis. Arch Intern Med 168, 2333–2339.
- James MT, Quan H, Tonelli M, Manns BJ, Faris P, Laupland KB, Hemmelgarn BR & Alberta Kidney Disease Network (2009). CKD and risk of hospitalization and death with pneumonia. *Am J Kidney Dis* **54**, 24–32.
- James MT, Hemmelgarn BR & Tonelli M (2010). Early recognition and prevention of chronic kidney disease. *Lancet* **375**, 1296–1309.
- Johansen KL (2005). Exercise and chronic kidney disease: current recommendations. *Sports Med* **35**, 485–499.
- Johansen KL (2007). Exercise in the end-stage renal disease population. *J Am Soc Nephrol* **18**, 1845–1854.
- Johansen KL (2008). Exercise and dialysis. Hemodial Int 12, 290–300.
- John R, Webb M, Young A & Stevens PE (2004). Unreferred chronic kidney disease: a longitudinal study. *Am J Kidney Dis* **43**, 825–835.

- Kapasi ZF, Ouslander JG, Schnelle JF, Kutner M & Fahey JL (2003). Effects of an exercise intervention on immunologic parameters in frail elderly nursing home residents. J Gerontol A Biol Sci Med Sci 58, 636–643.
- Kasapis C & Thompson PD (2005). The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *J Am Coll Cardiol* **45**, 1563–1569.
- Kato S, Chmielewski M, Honda H, Pecoits-Filho R, Matsuo S, Yuzawa Y, Tranaeus A, Stenvinkel P & Lindholm B (2008). Aspects of immune dysfunction in end-stage renal disease. *Clin J Am Soc Nephrol* 3, 1526–1533.
- Keith DS, Nichols GA, Gullion CM, Brown JB & Smith DH (2004). Longitudinal follow-up and outcomes among a population with chronic kidney disease in a large managed care organization. Arch Intern Med 164, 659–663.
- Kellum JA, Song M & Li J (2004). Science review: extracellular acidosis and the immune response: clinical and physiologic implications. *Crit Care* **8**, 331–336.
- Kiechl S, Egger G, Mayr M, Wiedermann CJ, Bonora E, Oberhollenzer F, Muggeo M, Xu Q, Wick G, Poewe W & Willeit J (2001). Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study. *Circulation* **103**, 1064– 1070.
- Kim HW, Woo YS, Yang HN, Choi HM, Jo SK, Cho WY & Kim H-K (2011). Primed monocytes: putative culprits of chronic low-grade inflammation and impaired innate immune responses in patients on hemodialysis. *Clin Exp Nephrol* **15**, 258–263.
- Kimmel PL, Phillips TM, Simmens SJ, Peterson RA, Weihs KL, Alleyne S, Cruz I, Yanovski JA & Veis JH (1998). Immunologic function and survival in hemodialysis patients. *Kidney Int* 54, 236–244.
- Koc M, Toprak A, Arikan H, Odabasi Z, Elbir Y, Tulunay A, Asicioglu E, Eksioglu-Demiralp E, Glorieux G, Vanholder R & Akoglu E (2011). Toll-like receptor expression in monocytes in patients with chronic kidney disease and haemodialysis: relation with inflammation. Nephrol Dial Transplant 26, 955–963.
- Kohut ML & Senchina DS (2004). Reversing age-associated immunosenescence via exercise. *Exerc Immunol Rev* **10**, 6–41.
- Kopple JD, Kalantar-Zadeh K & Mehrotra R (2005). Risks of chronic metabolic acidosis in patients with chronic kidney disease. *Kidney Int Suppl* **95**, S21–S27.
- Kopple JD, Wang H, Casaburi R, Fournier M, Lewis MI, Taylor W & Storer TW (2007). Exercise in maintenance hemodialysis patients induces transcriptional changes in genes favoring anabolic muscle. J Am Soc Nephrol 18, 2975–2986.
- Kosmadakis GC, Bevington A, Smith AC, Clapp EL, Viana JL, Bishop NC & Feehally J (2010). Physical exercise in patients with severe kidney disease. *Nephron Clin Pract* **115**, c7–c16.
- Kosmadakis GC, John SG, Clapp EL, Viana JL, Smith AC, Bishop NC, Bevington A, Owen PJ, McIntyre CW & Feehally J (2011). Benefits of regular walking exercise in advanced predialysis chronic kidney disease. *Nephrol Dial Transplant*; DOI: 10.1093/ndt/gfr364.
- Kostka T & Praczko K (2007). Interrelationship between physical activity, symptomatology of upper respiratory tract infections, and depression in elderly people. *Gerontology* 53, 187– 193.
- Kostka T, Drygas W, Jegier A & Praczko K (2008). Physical activity and upper respiratory tract infections. *Int J Sports Med* **29**, 158–162.

- Koufaki P & Kouidi E (2010). Current best evidence recommendations on measurement and interpretation of physical function in patients with chronic kidney disease. Sports Med 40, 1055–1074.
- Kraut JA & Kurtz I (2005). Metabolic acidosis of CKD: diagnosis, clinical characteristics, and treatment. *Am J Kidney Dis* **45**, 978–993.
- Krummel MF, Sullivan TJ & Allison JP (1996). Superantigen responses and co-stimulation: CD28 and CTLA-4 have opposing effects on T cell expansion in vitro and in vivo. Int Immunol 8, 519–523.
- Kuroki Y, Tsuchida K, Go I, Aoyama M, Naganuma T, Takemoto Y & Nakatani T (2007). A study of innate immunity in patients with end-stage renal disease: special reference to toll-like receptor-2 and -4 expression in peripheral blood monocytes of hemodialysis patients. *Int J Mol Med* **19**, 783–790.
- Lacson E & Levin NW (2004). C-reactive protein and end-stage renal disease. *Semin Dial* **17**, 438–448.
- Lancaster GI, Khan Q, Drysdale P, Wallace F, Jeukendrup AE, Drayson MT & Gleeson M (2005). The physiological regulation of toll-like receptor expression and function in humans. J Physiol 563, 945–955.
- Landmann R (1992). Beta-adrenergic receptors in human leukocyte subpopulations. *Eur J Clin Invest* **22** Suppl 1, 30–36.
- Lardner A (2001). The effects of extracellular pH on immune function. *J Leukoc Biol* **69**, 522–530.
- Lee W, Senchina D, Martin A, Karandikar A, Konopka D & Kohut M (2004). Long-term Moderate Exercise Improves T-cell Proliferation In Older Adults (Abstract). *Med Sci Sports Exerc* 36, s228.
- Leehey DJ, Moinuddin I, Bast JP, Qureshi S, Jelinek CS, Cooper C, Edwards LC, Smith BM & Collins EG (2009). Aerobic exercise in obese diabetic patients with chronic kidney disease: a randomized and controlled pilot study. *Cardiovasc Diabetol* **8**, 62.
- Leveille SG, Gray S, LaCroix AZ, Ferrucci L, Black DJ & Guralnik JM (2000). Physical inactivity and smoking increase risk for serious infections in older women. *J Am Geriatr Soc* **48**, 1582–1588.
- Levey A, Greene T, Kusek J & Beck G (2000). A simplified equation to predict glomerular filtration rate from serum creatinine [Abstract]. *J Am Soc Nephrol* **11**, 155A.
- Levey AS & Coresh J (2011). Chronic kidney disease. *Lancet*; DOI: 10.1016/S0140-6736(11)60178-5.
- Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan GNational Kidney Foundation (2003). National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med* **139**, 137–147.
- Levey AS, Eckardt K-U, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N & Eknoyan G (2005). Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 67, 2089–2100.
- Levey AS, Atkins R, Coresh J, Cohen EP, Collins AJ, Eckardt K-U, Nahas ME, Jaber BL, Jadoul M, Levin A, Powe NR, Rossert J, Wheeler DC, Lameire N & Eknoyan G (2007). Chronic kidney disease as a global public health problem: approaches and initiatives a position statement from Kidney Disease Improving Global Outcomes. *Kidney Int* **72**, 247–259.

- Levey AS, de Jong PE, Coresh J, Nahas El M, Astor BC, Matsushita K, Gansevoort RT, Kasiske BL & Eckardt K-U (2011). The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int* 80, 17–28.
- Libby P (2002). Inflammation in atherosclerosis. Nature 420, 868-874.
- Libby P, Ridker PM, Hansson GKLeducq Transatlantic Network on Atherothrombosis (2009). Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol* **54**, 2129–2138.
- Libby P, Ridker PM & Hansson GK (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature* **473**, 317–325.
- Litjens NHR, van Druningen CJ & Betjes MGH (2006). Progressive loss of renal function is associated with activation and depletion of naive T lymphocytes. *Clin Immunol* **118**, 83–91.
- Mantuano E, Santi S, Filippi C, Manca-Rizza G, Paoletti S, Consani C, Giovannini L, Tramonti G, Carpi A & Panichi V (2007). Simvastatin and fluvastatin reduce interleukin-6 and interleukin-8 lipopolysaccharide (LPS) stimulated production by isolated human monocytes from chronic kidney disease patients. *Biomed Pharmacother* **61**, 360–365.
- Markovitch D, Tyrrell RM & Thompson D (2008). Acute moderate-intensity exercise in middleaged men has neither an anti- nor proinflammatory effect. *J Appl Physiol* **105**, 260–265.
- Martin SA, Pence BD & Woods JA (2009). Exercise and respiratory tract viral infections. *Exerc* Sport Sci Rev **37**, 157–164.
- Matthews CE, Ockene IS, Freedson PS, Rosal MC, Merriam PA & Hebert JR (2002). Moderate to vigorous physical activity and risk of upper-respiratory tract infection. *Med Sci Sports Exerc* **34**, 1242–1248.
- Maughan RJ (1982). A simple, rapid method for the determination of glucose, lactate, pyruvate, alanine, 3-hydroxybutyrate and acetoacetate on a single 20-mul blood sample. *Clin Chim Acta* **122**, 231–240.
- McCarthy DA & Dale MM (1988). The leucocytosis of exercise. A review and model. *Sports Med* **6**, 333–363.
- McCarthy DA, Grant M, Marbut M, Watling M, Wade AJ, Macdonald I, Nicholson S, Melsom RD & Perry JD (1991). Brief exercise induces an immediate and a delayed leucocytosis. *Br J Sports Med* 25, 191–195.
- McFarlin BK, Flynn MG, Campbell WW, Stewart LK & Timmerman KL (2004). TLR4 is lower in resistance-trained older women and related to inflammatory cytokines. *Med Sci Sports Exerc* 36, 1876–1883.
- McFarlin BK, Flynn MG, Campbell WW, Craig BA, Robinson JP, Stewart LK, Timmerman KL & Coen PM (2006). Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4. *J Gerontol A Biol Sci Med Sci* **61**, 388–393.
- Meier P, Fliedner von V, Markert M, van Melle G, Deppisch R & Wauters JP (2000). One-year immunological evaluation of chronic hemodialysis in end-stage renal disease patients. *Blood Purif* 18, 128–137.
- Meier P, Dayer E, Blanc E & Wauters J-P (2002). Early T cell activation correlates with expression of apoptosis markers in patients with end-stage renal disease. *J Am Soc Nephrol* **13**, 204–212.

- Meier P, Dayer E, Ronco P & Blanc E (2005). Dysregulation of IL-2/IL-2R system alters proliferation of early activated CD4+ T cell subset in patients with end-stage renal failure. *Clin Nephrol* **63**, 8–21.
- Meier P, Spertini F, Blanc E & Burnier M (2007). Oxidized low-density lipoproteins activate CD4+ T cell apoptosis in patients with end-stage renal disease through Fas engagement. *J Am Soc Nephrol* **18**, 331–342.
- Meier P, Meier R & Blanc E (2008). Influence of CD4+/CD25+ regulatory T cells on atherogenesis in patients with end-stage kidney disease. *Expert Rev Cardiovasc Ther* **6**, 987–997.
- Mendham AE, Donges CE, Liberts EA & Duffield R (2011). Effects of mode and intensity on the acute exercise-induced IL-6 and CRP responses in a sedentary, overweight population. *Eur J Appl Physiol* **111**, 1035–1045.
- Meuer SC, Hauer M, Kurz P, Meyer zum Büschenfelde KH & Köhler H (1987). Selective blockade of the antigen-receptor-mediated pathway of T cell activation in patients with impaired primary immune responses. *J Clin Invest* **80**, 743–749.
- Michishita R, Shono N, Inoue T, Tsuruta T & Node K (2010). Effect of exercise therapy on monocyte and neutrophil counts in overweight women. *Am J Med Sci* **339**, 152–156.
- Mittrücker HW, Shahinian A, Bouchard D, Kündig TM & Mak TW (1996). Induction of unresponsiveness and impaired T cell expansion by staphylococcal enterotoxin B in CD28-deficient mice. J Exp Med 183, 2481–2488.
- Moore KW, de Waal Malefyt R, Coffman RL & O'Garra A (2001). Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* **19**, 683–765.
- Morita Y, Yamamura M, Kashihara N & Makino H (1997). Increased production of interleukin-10 and inflammatory cytokines in blood monocytes of hemodialysis patients. *Res Commun Mol Pathol Pharmacol* **98**, 19–33.
- Moser B, Roth G, Brunner M, Lilaj T, Deicher R, Wolner E, Kovarik J, Boltz-Nitulescu G, Vychytil A & Ankersmit HJ (2003). Aberrant T cell activation and heightened apoptotic turnover in end-stage renal failure patients: a comparative evaluation between nondialysis, haemodialysis, and peritoneal dialysis. *Biochem Biophys Res Commun* 308, 581–585.
- Mustata S, Groeneveld S, Davidson W, Ford G, Kiland K & Manns B (2010). Effects of exercise training on physical impairment, arterial stiffness and health-related quality of life in patients with chronic kidney disease: a pilot study. *Int Urol Nephrol*; DOI: 10.1007/s11255-010-9823-7.
- Naqvi SB & Collins AJ (2006). Infectious complications in chronic kidney disease. *Adv Chronic Kidney Dis* **13**, 199–204.
- National Collaborating Centre for Chronic Conditions (2008). *Chronic kidney disease: national clinical guideline for early identification and management in adults in primary and secondary care.* Royal College of Physicians, London.
- National Kidney Foundation (2002). K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* **39** Suppl 1, S1–S266.
- Naudé PJW, Boer den JA, Luiten PGM & Eisel ULM (2011). Tumor necrosis factor receptor cross-talk. *FEBS J* 278, 888–898.
- Neuman MI, Willett WC & Curhan GC (2010). Physical activity and the risk of communityacquired pneumonia in US women. *Am J Med* **123**, 281.e7–281.e11.

- Nicklas BJ & Brinkley TE (2009). Exercise training as a treatment for chronic inflammation in the elderly. *Exerc Sport Sci Rev* **37**, 165–170.
- Nieman D (2011). Moderate Exercise Improves Immunity and Decreases Illnesss Rates. *Am J Lifestyle Med*; DOI: 10.1177/1559827610392876.
- Nieman DC, Miller AR, Henson DA, Warren BJ, Gusewitch G, Johnson RL, Davis JM, Butterworth DE, Herring JL & Nehlsen-Cannarella SL (1994). Effect of high- versus moderate-intensity exercise on lymphocyte subpopulations and proliferative response. *Int J Sports Med* **15**, 199–206.
- Nieman DC, Henson DA, Austin MD & Brown VA (2005). Immune response to a 30-minute walk. *Med Sci Sports Exerc* **37**, 57–62.
- Nieto FJ (2002). Infective agents and cardiovascular disease. Semin Vasc Med 2, 401-415.
- Nindl BC, Headley SA, Tuckow AP, Pandorf CE, Diamandi A, Khosravi MJ, Welles R, Jones M & Germain M (2004). IGF-I system responses during 12 weeks of resistance training in end-stage renal disease patients. *Growth Horm IGF Res* 14, 245–250.
- Ortega E, Bote ME, Giraldo E & García JJ (2010). Aquatic exercise improves the monocyte proand anti-inflammatory cytokine production balance in fibromyalgia patients. *Scand J Med Sci Sports*; DOI: 10.1111/j.1600-0838.2010.01132.x.
- Ostrowski K, Rohde T, Asp S, Schjerling P & Pedersen BK (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol* **515**, 287–291.
- Pachaly MA, do Nascimento MM, Suliman ME, Hayashi SY, Riella MC, Manfro RC, Stenvinkel P & Lindholm B (2008). Interleukin-6 is a better predictor of mortality as compared to Creactive protein, homocysteine, pentosidine and advanced oxidation protein products in hemodialysis patients. *Blood Purif* 26, 204–210.
- Painter P (2005). Physical functioning in end-stage renal disease patients: update 2005. *Hemodial Int* **9**, 218–235.
- Parfrey PS & Foley RN (1999). The clinical epidemiology of cardiac disease in chronic renal failure. *J Am Soc Nephrol* **10**, 1606–1615.
- Pawluczyk IZA, Yang B, Patel SR, Saleem MA & Topham PS (2011). Low-level C-reactive protein levels exert cytoprotective actions on human podocytes. *Nephrol Dial Transplant* 26, 2465–2475.
- Peake JM (2002). Exercise-induced alterations in neutrophil degranulation and respiratory burst activity: possible mechanisms of action. *Exerc Immunol Rev* **8**, 49–100.
- Pedersen BK (2009). The diseasome of physical inactivity--and the role of myokines in muscle-fat cross talk. *J Physiol* **587**, 5559–5568.
- Pedersen BK & Febbraio MA (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev* 88, 1379–1406.
- Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, Febbraio M & Saltin B (2003). Searching for the exercise factor: is IL-6 a candidate? *J Muscle Res Cell Motil* 24, 113–119.
- Pereira BJ, Shapiro L, King AJ, Falagas ME, Strom JA & Dinarello CA (1994). Plasma levels of IL-1 beta, TNF alpha and their specific inhibitors in undialyzed chronic renal failure, CAPD and hemodialysis patients. *Kidney Int* **45**, 890–896.

- Pereira R, Costa E, Gonçalves M, Miranda V, Faria MS, Quintanilha A, Belo L, Lima M & Santos-Silva A (2010). Neutrophil and monocyte activation in chronic kidney disease patients under hemodialysis and its relationship with resistance to recombinant human erythropoietin and to the hemodialysis procedure. *Hemodial Int* **14**, 295–301.
- Pesanti EL (2001). Immunologic defects and vaccination in patients with chronic renal failure. *Infect Dis Clin North Am* **15**, 813–832.
- Petersen AMW & Pedersen BK (2005). The anti-inflammatory effect of exercise. *J Appl Physiol* **98**, 1154–1162.
- Phillips MD, Flynn MG, McFarlin BK, Stewart LK & Timmerman KL (2010). Resistance training at eight-repetition maximum reduces the inflammatory milieu in elderly women. *Med Sci Sports Exerc* 42, 314–325.
- Plaisance EP & Grandjean PW (2006). Physical activity and high-sensitivity C-reactive protein. *Sports Med* **36**, 443–458.
- Ploeger HE, Takken T, de Greef MHG & Timmons BW (2009). The effects of acute and chronic exercise on inflammatory markers in children and adults with a chronic inflammatory disease: a systematic review. *Exerc Immunol Rev* **15**, 6–41.
- Polańska B, Augustyniak D, Makulska I, Niemczuk M, Zwolińska D & Jankowski A (2010). Elastase, α1-proteinase inhibitor, and interleukin-8 in pre-dialyzed and hemodialyzed patients with chronic kidney disease. *Pediatr Int* **52**, 735–743.
- Pyne DB (1994). Regulation of neutrophil function during exercise. Sports Med 17, 245–258.
- Raso V, Benard G, DA Silva Duarte AJ & Natale VM (2007). Effect of resistance training on immunological parameters of healthy elderly women. *Med Sci Sports Exerc* 39, 2152– 2159.
- Robson PJ, Blannin AK, Walsh NP, Castell LM & Gleeson M (1999). Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. *Int J Sports Med* **20**, 128–135.
- Rogacev KS, Seiler S, Zawada AM, Reichart B, Herath E, Roth D, Ulrich C, Fliser D & Heine GH (2011). CD14++CD16+ monocytes and cardiovascular outcome in patients with chronic kidney disease. *Eur Heart J* **32**, 84–92.
- Ross R & Bradshaw AJ (2009). The future of obesity reduction: beyond weight loss. *Nat Rev Endocrinol* **5**, 319–325.
- Rønsen O, Pedersen BK, Øritsland TR, Bahr R & Kjeldsen-Kragh J (2001). Leukocyte counts and lymphocyte responsiveness associated with repeated bouts of strenuous endurance exercise. J Appl Physiol 91, 425–434.
- Sansom DM (2000). CD28, CTLA-4 and their ligands: who does what and to whom? *Immunology* **101**, 169–177.
- Saran AM & DuBose TD (2008). Cardiovascular disease in chronic kidney disease. *Ther Adv Cardiovasc Dis* **2**, 425–434.
- Sardenberg C, Suassuna P, Watanabe R, Cruz Andreoli MC, Aparecida Dalboni M, Faria Seabra V, Draibe SA, Cendoroglo Neto M & Jaber B (2004). Balance between cytokine production by peripheral blood mononuclear cells and reactive oxygen species production by monocytes in patients with chronic kidney disease. *Ren Fail* 26, 673–681.
- Sarnak MJ & Jaber BL (2000). Mortality caused by sepsis in patients with end-stage renal disease compared with the general population. *Kidney Int* **58**, 1758–1764.

- Sarnak MJ & Jaber BL (2001). Pulmonary infectious mortality among patients with end-stage renal disease. *Chest* **120**, 1883–1887.
- Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW & American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention (2003). Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 108, 2154–2169.
- Schiavoni G, Di Pietro M, Ronco C, de Cal M, Cazzavillan S, Rassu M, Nicoletti M, Del Piano M
  & Sessa R (2010). Chlamydia pneumoniae infection as a risk factor for accelerated atherosclerosis in hemodialysis patients. *J Biol Regul Homeost Agents* 24, 367–375.
- Scott JPR, Sale C, Greeves JP, Casey A, Dutton J & Fraser WD (2011). Effect of exercise intensity in the cytokine response to an acute bout of running. *Med Sci Sports Exerc*; DOI: 10.1249/MSS.0b013e31822113a9.
- Segura-Ortí E (2010). [Exercise in haemodyalisis patients: a literature systematic review]. *Nefrologia* **30**, 236–246.
- Segura-Ortí E & Johansen KL (2010). Exercise in end-stage renal disease. Semin Dial 23, 422– 430.
- Sela S, Shurtz-Swirski R, Cohen-Mazor M, Mazor R, Chezar J, Shapiro G, Hassan K, Shkolnik G, Geron R & Kristal B (2005). Primed peripheral polymorphonuclear leukocyte: a culprit underlying chronic low-grade inflammation and systemic oxidative stress in chronic kidney disease. J Am Soc Nephrol 16, 2431–2438.
- Senchina DS & Kohut ML (2007). Immunological outcomes of exercise in older adults. *Clin Interv Aging* **2**, 3–16.
- Seyrek N, Karayaylali I, Balal M, Paydas S, Aikimbaev K, Cetiner S & Seydaoglu G (2005). Is there any relationship between serum levels of interleukin-10 and atherosclerosis in hemodialysis patients? *Scand J Urol Nephrol* **39**, 405–409.
- Sharpe AH & Freeman GJ (2002). The B7-CD28 superfamily. Nat Rev Immunol 2, 116–126.
- Shephard RJ (2003). Adhesion molecules, catecholamines and leucocyte redistribution during and following exercise. *Sports Med* **33**, 261–284.
- Shimizu K, Kimura F, Akimoto T, Akama T, Tanabe K, Nishijima T, Kuno S & Kono I (2008). Effect of moderate exercise training on T-helper cell subpopulations in elderly people. *Exerc Immunol Rev* **14**, 24–37.
- Simpson RJ & Guy K (2010). Coupling aging immunity with a sedentary lifestyle: has the damage already been done?--a mini-review. *Gerontology* **56**, 449–458.
- Simpson RJ, Florida-James GD, Cosgrove C, Whyte GP, Macrae S, Pircher H & Guy K (2007). High-intensity exercise elicits the mobilization of senescent T lymphocytes into the peripheral blood compartment in human subjects. J Appl Physiol **103**, 396–401.
- Sloan RP, Shapiro PA, Demeersman RE, McKinley PS, Tracey KJ, Slavov I, Fang Y & Flood PD (2007). Aerobic exercise attenuates inducible TNF production in humans. J Appl Physiol 103, 1007–1011.
- Smart N & Steele M (2011). Exercise Training in Hemodialysis Patients: A Systematic Review and Meta-Analysis. *Nephrology (Carlton)*; DOI: 10.1111/j.1440-1797.2011.01471.x.

- Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P & Vallance P (2004). Risk of myocardial infarction and stroke after acute infection or vaccination. N Engl J Med 351, 2611–2618.
- Smith JA, Gray AB, Pyne DB, Baker MS, Telford RD & Weidemann MJ (1996). Moderate exercise triggers both priming and activation of neutrophil subpopulations. *Am J Physiol* 270, R838–R845.
- Smith JK, Dykes R, Douglas JE, Krishnaswamy G & Berk S (1999). Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. *JAMA* **281**, 1722–1727.
- Stachowski J, Pollok M, Burrichter H & Baldamus CA (1991). Immunodeficiency in ESRDpatients is linked to altered IL-2 receptor density on T cell subsets. *J Clin Lab Immunol* 34, 171–177.
- Stachowski J, Pollok M, Barth C, Maciejewski J & Baldamus CA (1994). Non-responsiveness to hepatitis B vaccination in haemodialysis patients: association with impaired TCR/CD3 antigen receptor expression regulating co-stimulatory processes in antigen presentation and recognition. *Nephrol Dial Transplant* 9, 144–152.
- Starkie R, Ostrowski SR, Jauffred S, Febbraio M & Pedersen BK (2003). Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. *FASEB J* **17**, 884–886.
- Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B & Klarlund Pedersen B (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol* **529**, 237–242.
- Steensberg A, Fischer CP, Keller C, Møller K & Pedersen BK (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol Endocrinol Metab* **285**, E433–E437.
- Stenvinkel P (2010). Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. *J Intern Med* **268**, 456–467.
- Stenvinkel P, Heimburger O, Paultre F, Diczfalusy U, Wang T, Berglund L & Jogestrand T (1999). Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int* 55, 1899–1911.
- Stenvinkel P, Heimbürger O & Jogestrand T (2002a). Elevated interleukin-6 predicts progressive carotid artery atherosclerosis in dialysis patients: association with Chlamydia pneumoniae seropositivity. Am J Kidney Dis 39, 274–282.
- Stenvinkel P, Bárány P, Heimbürger O, Pecoits-Filho R & Lindholm B (2002*b*). Mortality, malnutrition, and atherosclerosis in ESRD: what is the role of interleukin-6? *Kidney Int Suppl* **80**, S103–S108.
- Stenvinkel P, Ketteler M, Johnson RJ, Lindholm B, Pecoits-Filho R, Riella M, Heimbürger O, Cederholm T & Girndt M (2005). IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia--the good, the bad, and the ugly. *Kidney Int* 67, 1216–1233.
- Stenvinkel P, Carrero JJ, Axelsson J, Lindholm B, Heimbürger O & Massy Z (2008). Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? *Clin J Am Soc Nephrol* **3**, 505–521.
- Stevens LA, Coresh J, Greene T & Levey AS (2006). Assessing kidney function--measured and estimated glomerular filtration rate. *N Engl J Med* **354**, 2473–2483.
- Stevens PE, O'donoghue DJ, de Lusignan S, van Vlymen J, Klebe B, Middleton R, Hague N, New J & Farmer CKT (2007). Chronic kidney disease management in the United Kingdom: NEOERICA project results. *Kidney Int* **72**, 92–99.

Stewart LK, Flynn MG, Campbell WW, Craig BA, Robinson JP, McFarlin BK, Timmerman KL, Coen PM, Felker J & Talbert E (2005). Influence of exercise training and age on CD14+ cell-surface expression of toll-like receptor 2 and 4. *Brain Behav Immun* **19**, 389–397.

Takeda K, Kaisho T & Akira S (2003). Toll-like receptors. Annu Rev Immunol 21, 335–376.

- Tanasescu M, Leitzmann MF, Rimm EB, Willett WC, Stampfer MJ & Hu FB (2002). Exercise type and intensity in relation to coronary heart disease in men. *JAMA* **288**, 1994–2000.
- Tilg H, Trehu E, Atkins MB, Dinarello CA & Mier JW (1994). Interleukin-6 (IL-6) as an antiinflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *Blood* **83**, 113–118.
- Timmerman KL, Flynn MG, Coen PM, Markofski MM & Pence BD (2008). Exercise traininginduced lowering of inflammatory (CD14+CD16+) monocytes: a role in the antiinflammatory influence of exercise? J Leukoc Biol 84, 1271–1278.
- Toft AD, Falahati A & Steensberg A (2011). Source and kinetics of interleukin-6 in humans during exercise demonstrated by a minimally invasive model. *Eur J Appl Physiol* **111**, 1351–1359.
- Tonelli M, Wiebe N, Culleton B, House A, Rabbat C, Fok M, McAlister F & Garg AX (2006). Chronic kidney disease and mortality risk: a systematic review. J Am Soc Nephrol 17, 2034–2047.
- Toussaint ND, Polkinghorne KR & Kerr PG (2008). Impact of intradialytic exercise on arterial compliance and B-type natriuretic peptide levels in hemodialysis patients. *Hemodial Int* 12, 254–263.
- Toyama K, Sugiyama S, Oka H, Sumida H & Ogawa H (2010). Exercise therapy correlates with improving renal function through modifying lipid metabolism in patients with cardiovascular disease and chronic kidney disease. *J Cardiol* **56**, 142–146.
- Tripepi G, Mallamaci F & Zoccali C (2005). Inflammation markers, adhesion molecules, and allcause and cardiovascular mortality in patients with ESRD: searching for the best risk marker by multivariate modeling. *J Am Soc Nephrol* **16**, S83–S88.
- UK Renal Registry (2010). UK Renal Registry 12th Annual Report (December 2009): Appendix G Coding: Ethnicity, EDTA Primary Renal Diagnoses, EDTA Causes of Death and Treatment Timeline Modality Codes. *Nephron Clin Pract* **115** Suppl 1, c358–c363.
- US Renal Data System (2010). USRDS 2010 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD.
- Vanholder R, Massy Z, Argiles A, Spasovski G, Verbeke F, Lameire NEuropean Uremic Toxin Work Group (2005). Chronic kidney disease as cause of cardiovascular morbidity and mortality. *Nephrol Dial Transplant* **20**, 1048–1056.
- Vermeulen M, Giordano M, Trevani AS, Sedlik C, Gamberale R, Fernández-Calotti P, Salamone G, Raiden S, Sanjurjo J & Geffner JR (2004). Acidosis improves uptake of antigens and MHC class I-restricted presentation by dendritic cells. *J Immunol* **172**, 3196–3204.
- Vider J, Lehtmaa J, Kullisaar T, Vihalemm T, Zilmer K, Kairane C, Landõr A, Karu T & Zilmer M (2001). Acute immune response in respect to exercise-induced oxidative stress. *Pathophysiology* **7**, 263–270.

- Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A & Simon P (2011).
   Position statement. Part one: Immune function and exercise. *Exerc Immunol Rev* 17, 6–63.
- Wang HE, Gamboa C, Warnock DG & Muntner P (2011). Chronic Kidney Disease and Risk of Death from Infection. *Am J Nephrol* **34**, 330–336.
- Wannamethee SG & Shaper AG (2001). Physical activity in the prevention of cardiovascular disease: an epidemiological perspective. *Sports Med* **31**, 101–114.
- Wanner C, Drechsler C & Krane V (2009). C-reactive protein and uremia. Semin Dial 22, 438– 441.
- Weiner DE, Tighiouart H, Stark PC, Amin MG, MacLeod B, Griffith JL, Salem DN, Levey AS & Sarnak MJ (2004). Kidney disease as a risk factor for recurrent cardiovascular disease and mortality. Am J Kidney Dis 44, 198–206.
- Wetmore JB, Lovett DH, Hung AM, Cook-Wiens G, Mahnken JD, Sen S & Johansen KL (2008). Associations of interleukin-6, C-reactive protein and serum amyloid A with mortality in haemodialysis patients. *Nephrology (Carlton)* **13**, 593–600.
- Wilund KR (2007). Is the anti-inflammatory effect of regular exercise responsible for reduced cardiovascular disease? *Clin Sci* **112**, 543–555.
- Wilund KR, Tomayko EJ, Wu P-T, Ryong Chung H, Vallurupalli S, Lakshminarayanan B & Fernhall B (2010). Intradialytic exercise training reduces oxidative stress and epicardial fat: a pilot study. *Nephrol Dial Transplant* **25**, 2695–2701.
- Woods JA, Lowder TW & Keylock KT (2002). Can exercise training improve immune function in the aged? *Ann N Y Acad Sci* **959**, 117–127.
- Yan H, Kuroiwa A, Tanaka H, Shindo M, Kiyonaga A & Nagayama A (2001). Effect of moderate exercise on immune senescence in men. *Eur J Appl Physiol* **86**, 105–111.
- Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H & Nordestgaard BG (2008). Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med* **359**, 1897–1908.
- Zawada AM, Rogacev KS, Rotter B, Winter P, Marell R-R, Fliser D & Heine GH (2011). SuperSAGE evidence for CD14++CD16+ monocytes as a third monocyte subset. *Blood*; DOI: 10.1182/blood-2011-01-326827.
- Załuska A, Załuska WT, Bednarek-Skublewska A & Ksiazek A (2002). Nutrition and hydration status improve with exercise training using stationary cycling during hemodialysis (HD) in patients with end-stage renal disease (ESRD). Ann Univ Mariae Curie Sklodowska Med 57, 342–346.
- Zhang L, Kao WHL, Berthier-Schaad Y, Plantinga L, Fink N, Smith MW & Coresh J (2007). C-Reactive protein haplotype predicts serum C-reactive protein levels but not cardiovascular disease risk in a dialysis cohort. *Am J Kidney Dis* **49**, 118–126.
- Zhang Q-L & Rothenbacher D (2008). Prevalence of chronic kidney disease in populationbased studies: systematic review. *BMC Public Health* **8**, 117.
- Ziegler-Heitbrock L (2007). The CD14+ CD16+ blood monocytes: their role in infection and inflammation. *J Leukoc Biol* **81**, 584–592.

- Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJM, Liu Y-J, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM & Lutz MB (2010). Nomenclature of monocytes and dendritic cells in blood. *Blood* **116**, e74–e80.
- Zoccali C, Mallamaci F, Tripepi G, Parlongo S, Cutrupi S, Benedetto FA, Bonanno G, Seminara G, Fatuzzo P, Rapisarda F & Malatino LS (2003). Chlamydia pneumoniae, overall and cardiovascular mortality in end-stage renal disease (ESRD). *Kidney Int* **64**, 579–584.
- Zoccali C, Tripepi G & Mallamaci F (2006). Dissecting inflammation in ESRD: do cytokines and C-reactive protein have a complementary prognostic value for mortality in dialysis patients? *J Am Soc Nephrol* **17**, S169–S173.