Regular exercise during haemodialysis promotes an anti-inflammatory leukocyte profile

Maurice Dungey PhD 1, 2
Hannah ML Young MSc 2
Darren R Churchward, MRes 2
James O Burton DM 2
Alice C Smith PhD 2
Nicolette C Bishop PhD 1

Affiliations:
1 School of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire, United Kingdom
2 Leicester Kidney Exercise Team, John Walls Renal Unit, University Hospitals of Leicester and Department of Infection, Immunity and Inflammation, University of Leicester, Leicestershire, United Kingdom

Running title: Intradialytic exercise and inflammation

Corresponding author:
Dr Nicolette C Bishop,
School of Sport, Exercise and Health Sciences
Loughborough University,
Loughborough,
Leicestershire
LE11 3TU
United Kingdom
Tel. 01509 226385 Email. N.C.Bishop@lboro.ac.uk
Abstract

Cardiovascular disease (CVD) is the most common cause of mortality in haemodialysis (HD) patients and is highly predicted by markers of chronic inflammation. Regular exercise may have beneficial anti-inflammatory effects, but this is unclear in HD patients. This study assessed the effect of regular intradialytic exercise on soluble inflammatory factors and inflammatory leukocyte phenotypes.

Methods

Twenty-two HD patients from a centre where intradialytic cycling was offered thrice-weekly and 16 HD patients receiving usual care volunteered. Exercising patients aimed to cycle for 30 min at RPE of “somewhat hard”. Baseline characteristic were compared with 16 healthy age-matched individuals. Physical function, soluble inflammatory markers and leukocyte phenotypes were assessed again after 6 months of regular exercise.

Results

Patients were less active than their healthy counterparts and had significant elevations in measures of inflammation (IL-6, CRP, TNF-α, intermediate and non-classical monocytes; all P<0.001). Six months of regular intradialytic exercise improved physical function (sit-to-stand 60). After 6 months the proportion of intermediate monocytes in the exercising patients reduced compared to non-exercisers (7.58±1.68 to 6.38±1.81% vs. 6.86±1.45 to 7.88±1.66%; P<0.01). Numbers (but not proportion) of regulatory T cells decreased in the non-exercising patients only (P<0.05). Training had no significant effect on circulating IL-6, CRP or TNF-α concentrations.

Conclusions
These findings suggest regular intradialytic exercise is associated with an anti-inflammatory effect at a circulating cellular level but not in circulating cytokines. This may be protective against the increased risk of CVD and mortality that is associated with chronic inflammation and elevated numbers of intermediate monocytes.

**Key words:** cytokines; exercise; haemodialysis; inflammation; monocytes,
Introduction

Haemodialysis (HD) patients have a vastly elevated risk of all-cause and, specifically, cardiovascular mortality (1), which is the most common cause of death in these patients. This increased risk of cardiovascular disease (CVD) morbidity and mortality is multifactorial but includes factors relating directly to kidney disease (e.g. the malnutrition-inflammation-complex syndrome) and to the process of HD itself (2). HD patients have chronically elevated circulating soluble markers of inflammation (including IL-6, CRP and TNF-α) which strongly associate with mortality and cardiovascular events (3). Altered inflammatory immune cell populations and phenotypes are also evident in HD patients, including a shift toward the ‘pro-inflammatory’ CD16⁺ monocyte subpopulations (4,5) which are also strongly associated with increased cardiovascular mortality in chronic kidney disease (CKD) (6).

Regular exercise is associated with reduced mortality and morbidity in various chronic diseases and is associated with a plethora of cardioprotective benefits (7). The so-called ‘anti-inflammatory’ effect of regular exercise may play a key role in this through a number of different mechanisms. These include altering the balance of inflammatory immune cell phenotypes, reducing visceral adipose tissue mass and inflammatory cytokine release and, at higher intensities, through release of a potent anti-inflammatory cascade stimulated by muscle-derived cytokines (8,9). The importance of promoting an active lifestyle in the management of kidney disease is recognised in national and federal guidelines and in the scientific literature, as evidenced by a recent Cochrane Review (10), but the incorporation of exercise into clinical service has been slow and lags behind other long-term conditions. Within the HD population, patients are frequently inactive and the greater levels of inactivity are associated with an increased risk of mortality (11,12). However, despite reports in the literature of apparent anti-inflammatory effects of exercise in other patient populations (8,9),
including earlier stage CKD (13), the impact of regular exercise in HD patients on inflammatory markers, such as CRP, IL-6 and TNF-α, remains unclear (14). Furthermore, no studies in HD patients have determined the effect of regular exercise on inflammatory leukocyte phenotypes known to be central to chronic systemic inflammatory responses. This study aimed to determine the impact of a pragmatic 6-month intradialytic exercise programme on circulating soluble and cellular markers of chronic systemic inflammation.
Subjects and Methods

Participants

The study was approved by the East Midlands Research Ethics Committee (Northampton; 11/EM/0149). Additional ethical approval for the healthy cohort was granted by the Loughborough University Ethical Advisory Committee. All participants gave written informed consent.

Patients were recruited from two satellite HD units within the same renal network; one offered an intradialytic exercise programme as part of clinical care and one did not. An independent Consultant Nephrologist verified the medical suitability of all patients to exercise. Exclusion criteria for the study were: <18 years; established contraindications to exercise (15); lower limb vascular access; recent clinically overt infection; already taking part in exercise programme; or an insufficient command of English to consent.

Following recruitment of the patient group, age-matched healthy participants were recruited from the local area. Healthy individuals completed a health screen and were ineligible if they had any history of kidney disease, diabetes, recent infection, cardiovascular disease, smoking, or took any drugs known to affect digestion or metabolism. Healthy individuals donated a one-off, serum blood sample and completed the same outcome measures as the HD patients (described below) within a laboratory setting.

Exercise programme

The exercise group participated in a progressive intradialytic exercise programme for 6 months; the non-exercising control group continued HD treatment as per their usual care.

Exercise was provided in the form of a specially designed recumbent cycle (Letto series;
Motomed, Reck, Germany). Patients aimed to cycle at a “somewhat hard” rating of perceived exertion (RPE 12-14) (16) with supervised intradialytic exercise offered thrice-weekly. The exercise programme was designed with a pragmatic approach, more details are provided in the supplementary material.

**Randomisation**

A HD unit providing exercise and a unit not providing exercise were selected based on practicality and therefore the study was not randomised. All non-exercising patients were eligible to exercise if such a programme were available at their unit. This design reduced the sampling bias of using patients unable to, or not interested in exercise as controls and ‘exercise-contamination’ of control patients that occurs when control and exercise patients share shifts.

**Outcome measures**

Blood samples were collected prior to HD at baseline and after 6 months (at least 48 h after previous exercise session); eGFR was calculated using the simplified MDRD equation (17). Medical details were extracted from the patients’ clinical notes.

Plasma concentrations of CRP (IBL International GmbH, Hamburg, Germany), IL-6 and TNF-α (R&D systems, Abingdon, Oxfordshire, UK) were determined using commercially available high-sensitivity ELISAs. Assessment of monocyte phenotypes (classical: CD14++CD16-, intermediate: CD14++CD16+ and non-classical: CD14−CD16++) and
regulatory T cells (Tregs: CD4+CD25+CD127low/-) via flow cytometry are described in the supplementary material.

Activity levels were determined using a tri-axial accelerometer (SenseWear, BodyMedia Inc., Pittsburgh, USA). The participant wore the accelerometer for a single 7-day period collecting minute-by-minute data. Physical function was assessed using the sit-to-stand 60 (STS 60) test that is validated against more detailed and lengthy assessment methods (18).

**Omitted samples**

Patients on immunosuppressive therapy were excluded from analysis of inflammatory factors (Figure 1). Four exercise patients were omitted from analyses of monocyte phenotype due to unclear fluorescence patterns. Some patients declined physical function and activity outcomes; numbers for each outcome are presented in the results.

**Sample size**

Sample size for this study was pragmatic: all eligible patients in the two dialysis units were invited to participate (Figure 1).

**Statistics**

Data is presented as mean ± standard deviation or median (25th-75th percentiles) unless otherwise stated. Baseline comparisons between groups were completed using independent t-tests, non-parametric Mann-Whitney tests or Chi-square where applicable. Two-factor
mixed-measures ANOVA was used to analyse the effect of exercise: group (exercise vs. control) x time (baseline vs. 6 months). Where a significant group x time interaction was found post hoc analysis explored the differences using paired t-tests within groups and independent t-tests for between-group differences in change from baseline at 6 months. Comparisons between treatment and non-treatment day activity levels were assessed using Wilcoxon signed-rank test. Effect sizes (ES) were calculated using Cohen’s D (19).

All statistical analysis was completed on Statistical Package for Social Sciences (SPSS v.21, IBM, New York, USA). Statistical significance was accepted at $P<0.05$. 

Results

Recruitment and Participant flow
A total of 38 HD patients participated in the study (Figure 1). Twenty-two patients were recruited into the exercise group of which 16 completed the 6-month study period (27.3% attrition rate). Sixteen non-exercising control HD patients volunteered, of which 15 completed the study. Sixteen healthy participants volunteered and blood samples were obtained from 15.

Implementation of intervention
The exercising patients participated in 38 ± 12 exercise sessions. The mean session duration was 35 (30-42) min with patients exercising at 63 ± 8 rpm and perceiving the exercise to be “somewhat hard” (RPE: 12 (11-13)). Mean power output was 16 ± 7 W.

Participant characteristics
The age and sex of the HD patients and healthy participants were similar (Table 1). The age of the exercise and non-exercising HD groups were different (57.0 ± 10.5 vs. 70.2 ± 13.7 years; P=0.005), with more South Asian patients in the exercise group (7/16 vs. 1/15; P=0.02), which reflected the demographic of the centres where recruitment took place. However, there were no differences in circulating or cellular inflammatory markers between the exercising and non-exercising groups.
Outcomes

Inflammatory markers

HD patients had significantly higher concentrations of circulating IL-6 (ES=1.32), TNF-α (ES=1.37) and CRP (ES=1.35) than the healthy participants (Table 2).

Circulating concentrations of IL-6, CRP and TNF-α did not differ between the exercising and non-exercising patients at baseline (Table 2). ANOVA found no interaction or main effects in response to the 6-month exercise programme (Figure 2a-c).

Monocytes

The proportion and number of intermediate (ES=1.23 and ES=1.17 respectively) and non-classical monocytes (ES=1.56 and ES=1.31) were significantly greater in HD patients compared to healthy participants (Table 2).

There were no significant baseline differences between the exercise and non-exercising HD patients in the proportion of classical, intermediate, or non-classical monocytes (Table 2). ANOVA revealed a significant group x time interaction in the proportion (F=10.4, P=0.004) and number (F=4.91, P=0.04) of intermediate monocytes between the exercise and non-exercising HD patients, whereas classical and non-classical monocytes did not show any significant effects (Figure 2d-f). Post hoc tests showed a trend for reduced proportion of intermediate monocytes in the exercising patients (7.58 ± 1.68 to 6.38 ± 1.81 %; P=0.08, ES=0.68), and an increase in non-exercising HD patients (6.86 ± 1.45 to 7.88 ± 1.66 %; P=0.02, ES=0.65). The magnitude of change in the proportion of intermediate monocytes observed in the exercising patients was significantly different from the non-exercising patients (P=0.004).
Regulatory T cells

The number of circulating CD4+ lymphocytes were higher in the HD patients compared to the healthy participants (ES=0.71, Table 2). However, the proportion of circulating CD4+ lymphocytes that were Tregs was lower in the HD patients (ES=0.72, Table 2). The number of Tregs did not differ between groups.

At baseline, there were no significant differences between the exercising and non-exercising HD patients in the proportion or number of Tregs or CD4+ lymphocytes (Table 2).

ANOVA revealed group x time interactions in the proportion and number of CD4+ lymphocytes (F=11.3, *P*=0.002, and F=12.7, *P*=0.001; Figure 2h). In both cases post hoc paired t-tests revealed a significant decrease in the non-exercising patients from baseline to 6 months (*P*<0.001, ES=0.67, and *P*=0.001, ES=0.61 respectively) with no significant change in the exercise group.

ANOVA also revealed a group x time interaction in the number of Tregs (F=9.89, *P*=0.004). Post hoc paired t-tests revealed a trend for an increase in exercising patients (*P*=0.10, ES=0.23), and a decrease in non-exercising patients (*P*=0.003, ES=0.50; Figure 2g). The magnitude of change in the number of Tregs observed over the 6 months in the exercising patients was significantly different than that seen in the non-exercising patients (*P*=0.02).

There was no change in the proportion of CD4+ lymphocytes that were Tregs (interaction: *P*=0.74, Figure 2i).

Physical function

Compared to healthy participants, HD patients had significantly lower levels of physical function, as measured by the STS 60 test (ES=1.28; Table 3).
At baseline there were no significant differences between the exercising and non-exercising HD groups ($P=0.88$). Change in physical function was different between groups ($F=28.1$, $P<0.001$; Figure 3), with increases in the exercise group ($P<0.001$, ES=1.47), but not the non-exercising group ($P=0.21$, ES=0.18).

**Activity levels**

Twenty-four HD patients and 16 healthy participants provided usable accelerometer data. Habitual activity levels were lower in the HD patients than the healthy cohort (Table 3). The number of steps completed each day (ES=1.14) and the time spent physically active were greater in the healthy group (ES=0.50). There were no differences between HD subgroups (Table 3).

Patients’ activity levels were significantly reduced on HD treatment days compared to non-treatment days; including number of steps (2100 (1643-3015) vs. 3279 (2350-5055) steps/day; $P<0.001$, ES=0.38), and non-sedentary time (52.4 (15.7-95-5) vs. 57.2 (27.5-106.0) min/day; $P=0.04$, ES=0.05).

**Anthropometric and clinical parameters**

Regular exercise did not induce changes in weight, BMI, resting blood pressures, medications, haematology; all $P>0.05$. 


**Discussion**

This study is the first to demonstrate that completion of a 6-month intradialytic exercise programme promotes a shift away from the pro-inflammatory and pro-atherogenic intermediate monocyte phenotype towards an anti-inflammatory circulating leukocyte profile, as evidenced by an increase in the number of the anti-inflammatory Treg phenotype, compared to non-exercising patients. We also confirm elevated circulating and cellular markers of systemic inflammation in HD patients compared to an age-matched healthy cohort: CRP, IL-6 and TNF-α were significantly elevated in the HD cohort, the proportion of Tregs were reduced, and monocyte populations shifted toward intermediate and non-classical phenotypes. Physical activity levels and physical function were also lower in HD patients.

This is the first study in any clinical population to show that exercise training selectively diminishes the proportion of monocytes within the inflammatory intermediate subset.

Elsewhere, in an inactive elderly, but healthy group, 12 weeks of thrice-weekly endurance and resistance exercise training significantly reduced the percentage of CD14⁺CD16⁺ monocytes and decreased the TNF-α response to LPS stimulation (20). However, CD16⁺ monocytes were not analysed separately as intermediate and non-classical subsets, as this is a relatively new classification system (21). Whether the present findings are applicable to populations other than HD patients is not known, but may explain these previous observed changes in ‘CD16⁺’ monocytes.

Elevations in circulating intermediate monocytes are associated with an increased cardiovascular risk in non-CKD, CKD and HD patients (4,6,22) and have been described as a promising therapeutic target for CVD in CKD (23). In agreement with the present study, the
number and proportion of intermediate monocytes are increased in HD patients compared to healthy cohorts (4,5). Intermediate monocytes secrete pro-inflammatory cytokines (TNF-α, IL-1β and IL-6), express toll-like receptor 4 and adhesion molecules (CCR5 and CX3CR1), and exhibit spontaneous reactive oxygen species production (24-26), all considered mechanisms for cardiovascular dysfunction. The reduction in intermediate monocytes that we report here is encouraging as it may be a mechanism by which exercise protects against systemic inflammation and associated cardiovascular disorders in HD patients.

In the present study, the proportion of CD4⁺ lymphocytes that were Tregs was lower in HD patients than in the healthy group. Reduced Treg capacity has been reported previously in HD patients (27,28). Abnormalities in Treg activity are harmful; deficiencies are associated with autoimmunity, inflammation and allergy whereas over-activation is associated with increased risk of chronic infections and tumour growth (29). The diminished Treg capacity in HD patients may contribute to chronic immune activation and inflammation typically exhibited in this population.

The favourable change in the number of Tregs in the exercise group after 6 months compared to the non-exercising group may represent enhanced anti-inflammatory capacity. Sedentary but otherwise healthy individuals have demonstrated lower numbers of Tregs than physically active groups and antigen-stimulated IL-10 release correlates with this cell population (30). However, as we found no change in the proportion of CD4⁺ lymphocytes that were Tregs the increase we observed may simply relate to differences in overall CD4⁺ numbers. This is further supported by the observation that the number of CD4⁺ lymphocytes decreased significantly in the non-exercising group but not in the exercise group. Therefore it is not
clear whether the enhanced Treg population in the exercise group actually represents a true anti-inflammatory adaptation.

Circulating CRP, IL-6 and TNF-α were not improved after 6 months of regular intradialytic exercise in this study. Examination of the literature to date reveals that no well-controlled trials have reported significant meaningful reductions in these soluble inflammatory markers after exercise training in HD patients (14). Well-designed studies have reported no change in CRP after 3-4 months of intradialytic cycling or extradialytic resistance training (31,32), or with maintained CRP but no improvement in IL-6 or TNF-α (33,34). In pre-dialysis CKD cohorts resistance exercise that increases muscle mass (35) and regular walking exercise have been shown to favourably alter circulating inflammatory factors (13).

In a large cohort of healthy individuals ($n=4,289$), physical activity was associated with reduced CRP and IL-6, and increasing activity levels reduced markers of inflammation over a 10-year follow-up (36). Therefore, for these soluble inflammatory markers, a large cohort or a long exercise intervention may be required to detect meaningful change. This is likely exacerbated in HD patients by the large intra- and inter-individual variation of IL-6, CRP and TNF-α. The day-to-day variation (i.e. due to fluid overload, recurrent infections, HD itself) in this population may limit the use of CRP and circulating cytokines in isolation to detect long-term change.

Previous studies have demonstrated sedentary behaviour in HD patients (11,37) but the extent of inactivity is worth highlighting. Significantly, the least active HD patients have a 62% increased mortality risk (12) and those with the lowest physical function have higher hospitalisation rates and all-cause mortality (38,39). A recent Cochrane review concludes
there are various benefits of increasing physical activity for health and wellbeing in CKD (10) and the present study adds to this body of evidence. Despite the seemingly low power output the exercise group improved physical function. This improvement was probably augmented by low baseline values giving greater potential for improvement. Increasing physical function has a positive impact on quality of life and is valued very highly by patients; therefore, promotion of this key outcome is likely to be more effective in encouraging continued participation in physical activity.

This exercise programme was limited to one mode and prescription of exercise and was not standardised per each patient; different exercise modalities, at different times or intensities may have different results. Nonetheless, this study makes a major contribution because it was pragmatic in design thereby ascertaining a realistic response to an achievable intradialytic program per se in a naturally heterogeneous HD cohort. There was, therefore, expected variation in the absolute exercise intensity achieved in this study (comparable to other studies (31,40)), however, subjective intensity remained quite consistent, as evidenced by RPE.

For reasons of practicality and to avoid sampling bias and ‘exercise-contamination’ of control patients that occurs when control and exercise patients share shifts this study was not randomised. This led to different demographics between the exercise and non-exercising groups, including ethnicity and age, but did not confound the key outcome measures. Likewise, haemoglobin was different between groups at baseline, but as this did not change over time, it is unlikely to have influenced the findings. Finally, the small sample size is due to the practicalities of running such a study despite approaching all eligible patients. This pragmatic evaluation of intradialytic exercise provides important novel evidence into anti-
inflammatory adaptations that provides a primary point of reference for future investigations into the therapeutic benefits of exercise in this population.

Conclusions

HD patients are sedentary and demonstrate high levels of chronic inflammation, yet we show for the first time that undertaking regular exercise during HD has an anti-inflammatory effect at a circulating cellular level. The reduction in intermediate monocytes may be protective against the substantially increased risk of cardiovascular morbidity and mortality and further supports the therapeutic potential of regular exercise in these patients.
Acknowledgements

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Research idea and study design: NCB and ACS; data acquisition: MD, HMLY, DRC; data analysis/interpretation: MD; supervision or mentorship: NCB, ACS, JOB. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Conflict of Interest

Outside the submitted work Reck UK (manufacturers of the exercise bike) funded MD, HMLY and JOB to attend the 2012 BMJ Awards. There are no other financial conflicts of interest.

The results of this study have been reported honestly, accurately, and without fabrication, falsification, or inappropriate data manipulation.
References


18) McIntyre CW, Selby NM, Sigrist M, Pearce LE, Mercer TH, Naish PF. Patients receiving maintenance dialysis have more severe functionally significant skeletal


### Table 1 - Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>Healthy group (n=16)</th>
<th>Combined HD patients (n=31)</th>
<th>P value</th>
<th>Exercise HD patients (n=16)</th>
<th>Non-Exercising HD patients (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.5 ± 10.9</td>
<td>63.4 ± 13.7</td>
<td>0.63</td>
<td>57.0 ± 10.5</td>
<td>70.2 ± 13.7</td>
<td>0.005*</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>8/8</td>
<td>18/13</td>
<td>0.37</td>
<td>8/8</td>
<td>10/5</td>
<td>0.35</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.3 ± 16.6</td>
<td>75.9 ± 23.1</td>
<td>0.92</td>
<td>71.0 ± 24.9</td>
<td>81.2 ± 20.6</td>
<td>0.23</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 5.0</td>
<td>27.1 ± 6.4</td>
<td>0.58</td>
<td>25.9 ± 6.7</td>
<td>28.4 ± 6.1</td>
<td>0.29</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>129 ± 13</td>
<td>136 ± 21</td>
<td>0.16</td>
<td>135 ± 24</td>
<td>138 ± 19</td>
<td>0.68</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>102 ± 22</td>
<td>#</td>
<td>-</td>
<td>#</td>
<td>#</td>
<td>-</td>
</tr>
<tr>
<td>Number of co-morbidities</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>0.57</td>
</tr>
</tbody>
</table>

BMI, body mass index; eGFR, estimated glomerular filtration rate; HD, haemodialysis
# All HD patients had established end-stage renal disease and had received haemodialysis treatment for at least 3 months.
* denotes a significant difference between exercising HD patient and non-exercising HD patient groups.
Data is presented as mean ± SD.
Table 2 - Comparisons in markers of inflammation and haematology at baseline between a healthy group and HD patients, and between the exercise and non-exercising HD groups.

<table>
<thead>
<tr>
<th>Markers of inflammation (n=15 and n=28)</th>
<th>Healthy group</th>
<th>HD group</th>
<th>P value</th>
<th>Exercise HD group</th>
<th>Non-exercising HD group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.81 (0.43-1.76)</td>
<td>4.63 (2.73-7.09)</td>
<td>&lt;0.001*</td>
<td>5.39 ± 3.05</td>
<td>4.92 ± 2.79</td>
<td>0.68</td>
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<tr>
<td>TNF-α (pg/mL)</td>
<td>0.96 (0.60-1.73)</td>
<td>3.21 (2.66-4.62)</td>
<td>&lt;0.001*</td>
<td>3.22 (2.72-4.24)</td>
<td>3.20 (2.64-4.67)</td>
<td>0.77</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.83 (0.21-1.90)</td>
<td>4.61 (2.68-9.78)</td>
<td>&lt;0.001*</td>
<td>3.99 (2.34-6.86)</td>
<td>7.89 (3.39-10.4)</td>
<td>0.37</td>
</tr>
<tr>
<td>Classical monocytes (%) n1</td>
<td>89.5 (87.0-92.1)</td>
<td>80.3 (72.6-82.2)</td>
<td>&lt;0.001*</td>
<td>80.5 (75.4-82.3)</td>
<td>79.8 (72.6-80.9)</td>
<td>0.53</td>
</tr>
<tr>
<td>Classical monocytes (cells/μL)n1</td>
<td>506 ± 130</td>
<td>516 ± 112</td>
<td>0.78</td>
<td>435 (410-525)</td>
<td>580 (558-599)</td>
<td>0.09</td>
</tr>
<tr>
<td>Intermediate monocytes (%) n1</td>
<td>4.34 (3.74-4.95)</td>
<td>6.77 (5.98-8.47)</td>
<td>&lt;0.001*</td>
<td>7.58 ± 1.68</td>
<td>6.86 ± 1.45</td>
<td>0.27</td>
</tr>
<tr>
<td>Intermediate monocytes (cells/μL)n1</td>
<td>21.8 (19.4-29.1)</td>
<td>45.9 (40.5-52.3)</td>
<td>&lt;0.001*</td>
<td>46.3 ± 14.7</td>
<td>49.4 ± 14.2</td>
<td>0.61</td>
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<tr>
<td>Non-classical monocytes (%) n1</td>
<td>6.45 (4.2-8.5)</td>
<td>13.6 (11.2-17.7)</td>
<td>&lt;0.001*</td>
<td>12.4 (10.8-16.4)</td>
<td>13.8 (12.7-17.5)</td>
<td>0.36</td>
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<tr>
<td>Non-classical monocytes (cells/μL)n1</td>
<td>30.0 (23-50)</td>
<td>90.9 (72.2-124.5)</td>
<td>&lt;0.001*</td>
<td>75.9 (59.9-105.7)</td>
<td>96.9 (89.0-139.2)</td>
<td>0.11</td>
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<tr>
<td>Regulatory T cells (%) n2</td>
<td>7.67 ± 0.98</td>
<td>6.75 ± 1.53</td>
<td>0.02</td>
<td>6.93 ± 1.77</td>
<td>6.55 ± 1.24</td>
<td>0.53</td>
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<tr>
<td>Regulatory T cell (cells/μL) n2</td>
<td>20.1 (18.6-25.0)</td>
<td>23.7 (17.3-36.0)</td>
<td>0.53</td>
<td>23.6 (16.0-38.0)</td>
<td>23.8 (19.1-32.4)</td>
<td>0.57</td>
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<tr>
<td>CD4+ lymphocytes (%) n2</td>
<td>20.2 (17.4-23.9)</td>
<td>24.1 (20.1-28.9)</td>
<td>0.18</td>
<td>23.9 ± 8.0</td>
<td>24.7 ± 7.2</td>
<td>0.79</td>
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<tr>
<td>CD4+ lymphocytes (cells/μL)n2</td>
<td>264 (227-369)</td>
<td>366 (269-493)</td>
<td>0.05*</td>
<td>317 (253-496)</td>
<td>369 (301-483)</td>
<td>0.53</td>
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<table>
<thead>
<tr>
<th>Haematology (n=15 and n=31)</th>
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<tbody>
<tr>
<td>Haemoglobin (g/L)</td>
<td>134 ± 17</td>
<td>118 ± 12</td>
<td>0.003*</td>
<td>126 (118-131)</td>
<td>105 (102-110)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Red blood cells (x10¹²/L)</td>
<td>4.49 ± 0.44</td>
<td>3.67 ± 0.48</td>
<td>&lt;0.001*</td>
<td>3.81 ± 0.58</td>
<td>3.55</td>
<td>0.14</td>
</tr>
<tr>
<td>White blood cells (x10⁹/L)</td>
<td>5.14 ± 0.95</td>
<td>7.05 ± 2.56</td>
<td>0.001*</td>
<td>6.64 ± 2.44</td>
<td>7.47</td>
<td>0.38</td>
</tr>
<tr>
<td>Neutrophils (x10⁹/L)</td>
<td>2.8 (2.4-3.5)</td>
<td>3.9 (3.0-5.6)</td>
<td>0.005*</td>
<td>3.6 (2.9-5.6)</td>
<td>4.3 (3.7-5.9)</td>
<td>0.32</td>
</tr>
<tr>
<td>Monocytes (x10⁹/L)</td>
<td>0.5 (0.5-0.7)</td>
<td>0.7 (0.5-0.8)</td>
<td>0.24</td>
<td>0.6 (0.5-0.7)</td>
<td>0.7 (0.6-0.8)</td>
<td>0.054</td>
</tr>
<tr>
<td>Lymphocytes (x10⁹/L)</td>
<td>1.5 ± 0.4</td>
<td>1.6 ± 0.6</td>
<td>0.45</td>
<td>1.58 ± 0.66</td>
<td>1.68</td>
<td>0.67</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; HD, haemodialysis; IL-6, interleukin-6; TNF, tumour necrosis factor.

Classical monocytes refer to CD14⁺CD16⁻, intermediate monocytes, CD14⁺CD16⁺, non-classical monocytes, CD14⁻CD16⁺⁺.

Regulatory T cells (%) and (cells/μL) refer to the percentage and number of CD4⁺ lymphocytes that are CD25⁺CD127low⁻.

n1, for monocytes n=24 for HD group; n=11 for Exercise HD group, n=13 for Non-exercising HD group.

n2, for % regulatory T cells and % CD4⁺ lymphocytes n=16 for healthy group.

* denotes a significant difference between healthy and HD groups, † denotes a significant difference between exercise and non-exercise groups.

P value derived from independent t-tests or Mann-Whitney test where applicable.

Data is presented as mean ± SD or median (25th-75th percentiles).
Table 3 - Comparisons in physical activity and function between a healthy group and HD patients, and between the exercise and non-exercising HD groups.

<table>
<thead>
<tr>
<th>Physical activity and function</th>
<th>Healthy group</th>
<th>HD group</th>
<th>P value</th>
<th>Exercise HD group</th>
<th>Non-exercising HD group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Expenditure (kJ/day)</td>
<td>(n=16)</td>
<td>(n=24)</td>
<td>0.12</td>
<td>(n=10)</td>
<td>(n=14)</td>
<td>0.82</td>
</tr>
<tr>
<td>Steps (per day)</td>
<td>7594 (5847-9539)</td>
<td>2934 (2058-4460)</td>
<td>&lt;0.001*</td>
<td>2237 (1995-3902)</td>
<td>3234 (2527-4462)</td>
<td>0.47</td>
</tr>
<tr>
<td>Time physically active (min/day)</td>
<td>105 (86-142)</td>
<td>55 (26-98)</td>
<td>0.009*</td>
<td>63 (49-102)</td>
<td>51 (16-87)</td>
<td>0.26</td>
</tr>
<tr>
<td>Time sedentary (min/day)</td>
<td>1335 (1295-1354)</td>
<td>1385 (1342-1413)</td>
<td>0.009*</td>
<td>1389 (1353-1424)</td>
<td>1376 (1338-1390)</td>
<td>0.26</td>
</tr>
<tr>
<td>STS 60 (reps)</td>
<td>28 ± 13</td>
<td>13 ± 9</td>
<td>0.001*</td>
<td>13 ± 10</td>
<td>13 ± 9</td>
<td>0.84</td>
</tr>
</tbody>
</table>

* denotes a significant difference between healthy and HD groups.  
P value derived from independent t-tests or Mann-Whitney test where applicable.  
Data is presented as mean ± SD or median (25<sup>th</sup>-75<sup>th</sup> percentiles).  

n, for STS 60 n=23 for HD group; and n=11 for Exercise HD group and n=12 for Non-exercising HD group.
Figure legends

**Figure 1** – Consolidated Standards of Reporting Trials (CONSORT) diagram. HD, haemodialysis.

**Figure 2** – Changes in indices of systemic inflammation after 6 months in the exercise and non-exercise HD patients. Showing a) IL-6; b) CRP; c) TNF-α; the proportion of monocytes that are d) classical, e) intermediate, f) non-classical phenotype; g) number of regulatory T cells, h) number of CD4+ lymphocytes, i) proportion of CD4+ lymphocytes that are regulatory T cells. Each black square and circle represent an exercise and non-exercising patient; bars show mean ± SEM. * denotes a significant difference in change between exercise and non-exercising patients (P<0.05). Exercising patients: n=15, (except for monocytes where n=11), Non-exercising patients: n=13.

**Figure 3** – Sit-to-stand 60 score at baseline and 6 months in exercising and non-exercising HD patients. Lines represent individual patients; grey bars show the mean. * denotes a significant change from baseline (P<0.001). Exercise group: n=11, Non-exercising group: n=12.
Enrollment and Allocation

Patients at exercising HD unit
Assessed for eligibility (n=104)
- Excluded (n=82)
  - Not meeting inclusion criteria (n=79)
  - Declined to participate (n=3)

Allocated to intervention (n=22)
- Lost to follow-up (n=6)
  - Transplant (n=1)
  - Death (n=1)
  - Insufficient exercise (n=1)
  - Infection / injury (n=2)
  - Other morbidity (n=1)

Analysed (n=16)
- Excluded from analysis of inflammatory markers (n=1) – immunosuppressive therapy

Patients at non-exercising HD unit
Assessed for eligibility (n=78)
- Excluded (n=62)
  - Not meeting inclusion criteria (n=58)
  - Declined to participate (n=4)

Allocated to intervention (n=16)
- Lost to follow-up (n=1)
  - Transplant (n=1)

Analysed (n=15)
- Excluded from analysis of inflammatory markers (n=2) – immunosuppressive therapy