Intradialytic exercise training reduces oxidative stress and epicardial fat: a pilot study

Kenneth R. Wilund1,2, Emily J. Tomayko2, Pei-Tzu Wu1, Hae Ryong Chung1, Srikanth Vallurupalli4, Batlagundu Lakshminarayanan3 and Bo Fernhall1

1Department of Kinesiology and Community Health, 2Division of Nutritional Sciences, 3Department of Internal Medicine, University of Illinois, Urbana–Champaign, USA and 4Department of Medicine, Southern Illinois University School of Medicine, Springfield, USA

Correspondence and offprint requests to: Kenneth R. Wilund; E-mail: kwilund@illinois.edu

Abstract

Background. Cardiovascular disease (CVD) mortality rates are greatly elevated in chronic kidney disease patients receiving maintenance haemodialysis therapy. The purpose of this study was to evaluate the efficacy of intradialytic endurance exercise training on novel risk factors that may contribute to this excessive CVD risk.

Methods. Seventeen haemodialysis patients were randomized to either an intradialytic exercise training (cycling) group (EX; n = 8) or a non-exercising control group.
(CON; n = 9) for 4 months. At baseline and following the intervention, we measured serum parameters related to CVD risk and renal function, used echocardiography to measure variables related to cardiac structure and function and assessed physical performance by a validated shuttle walk test.

**Results.** Performance on the shuttle walk test increased by 17% in EX (P < 0.05), but did not change in CON. There was no change in serum lipids or inflammatory markers (C-reactive protein, interleukin-6) in either group. Serum thiobarbituric acid reactive substances, a marker of oxidative stress, were reduced by 38% in EX (P < 0.05), but did not change in CON. In addition, serum alkaline phosphatase (ALP), a putative risk factor for vascular calcification, was reduced by 27% in EX (P < 0.05), but did not change in CON. There was no change in left atrial volume, left ventricular mass or myocardial performance index in either group. However, the thickness of the epicardial fat layer was reduced by 11% in EX (P < 0.05), but did not change in CON. Furthermore, the change in physical performance was inversely correlated to the change in epicardial fat (r = −0.63; P = 0.03).

**Conclusions.** These results suggest that endurance exercise training may improve CVD risk in haemodialysis patients by decreasing novel risk factors including serum oxidative stress, ALP and epicardial fat.

**Keywords:** cardiovascular disease; exercise; haemodialysis; inflammation; oxidative stress

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**Introduction**

Patients with chronic kidney disease (CKD) receiving maintenance haemodialysis treatment suffer from a variety of comorbid diseases, many of which may be mechanistically linked. Muscle catabolism and wasting is especially common, and these lead to reduced muscle strength, declines in physical function and low levels of physical activity [1]. Physical inactivity exacerbates these functional declines and also promotes cardiovascular disease (CVD). This cycle of disease and disability greatly reduces the quality of life (QOL) and increases mortality rates in haemodialysis patients.

CKD is a chronic inflammatory condition, as reflected by elevated circulating levels of acute-phase proteins such as C-reactive protein (CRP) and pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) [2]. Excessive oxidative stress associated with uraemia is believed to play a critical role in the development of the chronic inflammation in CKD patients [3]. Oxidative stress contributes to inflammation in part by activating the nuclear transcription factor NF-κB, which mediates the expression of pro-inflammatory cytokines associated with immune and inflammatory responses [4]. Inflammation and oxidative stress both play a significant role in atherosclerosis development in CKD patients [2,5] and are also related to the pathogenesis of functional CVD outcomes, including arterial stiffness, increases in arterial wall intima–media thickness, left ventricular hypertrophy and declines in cardiac function [6,7]. Inflammation also reduces circulating levels of fetuin-A, a reverse acute-phase protein that acts as a systemic inhibitor of vascular calcification and arterial stiffness [8]. As a result of these abnormalities, cardiovascular events are 10 to 30 times greater in haemodialysis patients than in age- and sex-matched subjects in the general population [9].

Numerous studies have highlighted the importance of adipose tissue in relation to the inflammatory burden in CVD, describing the expression and secretion of both pro-inflammatory and protective factors, collectively termed adipocytokines [10,11]. More recently, research has begun to focus on the unique role of epicardial adipose tissue, a visceral fat depot surrounding the heart, on CVD risk. Like other white adipose tissue, epicardial fat functions as a lipid-storing depot, as an endocrine organ secreting hormones, and as an inflammatory tissue secreting cytokines. The close proximity of epicardial fat to the adventitia of the coronary arteries and the underlying myocardium suggests the possibility that it could play an especially important role in the pathogenesis of CVD [12]. Indeed, recent studies have shown that the expression and secretion of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) are increased in epicardial fat, relative to subcutaneous fat, in coronary artery disease patients [13]. This suggests that therapies that decrease the amount of epicardial fat may reduce CVD risk.

It is well established that endurance exercise training helps reduce fat mass, but there is significant controversy regarding whether or not it promotes a reduction in visceral fat over other fat depots [14]. Recently, epicardial fat was shown to be reduced in young, healthy, obese men after 12 weeks of endurance exercise training (jogging) [15]. However, functional limitations and other factors may limit the intensity at which many haemodialysis patients can exercise [1], and it is not known if similar benefits would be realized with moderate intensity intradialytic cycling. While numerous studies have shown that intradialytic exercise improves physical function and QOL in haemodialysis patients [1,16–18], surprisingly little is known about the effects of endurance exercise training on CVD risk in this population [1]. As a result, the objective of this study was to evaluate the efficacy of intradialytic endurance exercise training (cycling) on factors related to the excessive CVD risk in haemodialysis patients, specifically, markers of systemic inflammation (CRP, IL-6 and fetuin-A), oxidative stress [lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) assay], cardiac structure and function [left ventricular mass (LVM), relative wall thickness, left atrial volume and myocardial performance index (MPI)] and epicardial fat thickness.

**Materials and methods**

**Subjects**

Seventeen patients on maintenance haemodialysis (nine females, eight males) were recruited from the Champaign–Urbana Dialysis Clinic (Champaign, IL). Patients were screened for eligibility using a health and medical history questionnaire. All participants gave written informed consent and this study was approved by the University of Illinois Institutional Review Board. Inclusion/exclusion criteria for patients included the
Intradialytic exercise training reduces oxidative stress and epicardial fat following: (i) age ≥ 30–70 years; (ii) non-smoking; (iii) body mass index (BMI) < 35 kg/m²; (iv) no orthopaedic problems that prevented cycling during dialysis; (v) no chronic obstructive pulmonary disease (COPD), coronary heart failure or cardiovascular surgery (e.g. coronary bypass, valve replacement or angioplasty) in the past 6 months; (vi) medical clearance from a primary care physician; (vii) no participation in intradialytic exercise training for 6 months prior to recruitment in the study.

Study design
Following recruitment, screening and baseline testing, eligible subjects were randomly assigned to one of two groups: (i) usual care/control (CON; n = 9); (ii) intradialytic exercise training (EX; n = 8).

Exercise training intervention
Subjects in the EX group underwent a 4-month intradialytic endurance exercise training programme. This programme consisted of cycling 3 days/week on specialized cycle ergometers (Champ-Cycle™, Champion Manufacturing, Inc., Elkhart, IN) placed in front of each subject's dialysis chair. Subjects started the training programme by cycling at a tolerable pace for 5 min during their first exercise session. The duration of exercise increased by 5 to 10 min per session, depending on each subject's individual tolerance, until they were able to cycle continuously for a total of 45 min per session at a rating of perceived exertion of 12–14 (‘somewhat hard’ [19]). The subjects reached this level of exercise in 1.8 ± 0.6 weeks. Subjects maintained this duration and intensity of work for the remainder of the 4-month intervention. All exercise sessions were attended by study staff to encourage the subjects and monitor their response to exercise (e.g. heart rate and blood pressure). Compliance with the exercise protocol was measured as the percentage of exercise sessions successfully completed. To count as complete, a minimum of 75% of the goal exercise time for that session had to be performed. Subjects in the CON group were not given access to cycle ergometers during their dialysis sessions.

Clinical testing and measurements
At baseline and immediately following the 4-month intervention (final testing), all patients underwent a series of tests described below to evaluate the effects of the intradialytic exercise programme on our primary outcomes. All testing sessions were conducted on a 'non-dialysis day', 18–24 h following a dialysis treatment. All tests were analysed by study personnel blinded to the subject's group assignment.

Incremental shuttle walk test
Physical performance was measured by distance walked during an incremental shuttle walk test (ISWT). The ISWT is a progressive test in which patients walk back and forth continuously on a 10-m course. The walking speed is paced by a series of beeps that signal when the subject should have completed the 10-m walk. The pace is progressively increased so that the walking speed at the end of each successive minute is greater than or equal to 1.12, 1.54, 1.88, 2.26, 2.64, 3.02, 3.34 and 3.78 mph. The test was terminated when the subject was unable to complete the 10-m course before the subsequent beep. The ISWTS were performed on non-dialysis days, 18–24 h after a previous dialysis session, and post-intervention evaluations were performed at least 36–48 h after any previous exercise bout.

Blood chemistry
Blood was collected from patients in a non-fasted state from their dialysis lines during regularly scheduled (monthly) blood collection times at the clinic, minimum of 48–72 h after any scheduled exercise bout. Plasma was collected from blood samples by centrifugation, aliquoted and stored at −80°C until analysed. To control for variation between runs, baseline and final test samples from each subject were analysed simultaneously. Plasma total cholesterol levels were measured using a commercial enzymatic kit (Wako Inc., Richmond, VA). CRP, IL-6 and fetuin-A were measured in triplicate using commercially available ELISA kits (InCiPR ELISA #1668Z, Diagnostic Automation Inc., CA; Quantakine HS Human IL-6 #HS600B, R&D Systems, MN; Human Fetuin-A ELISA #RD1815, BioVendor, NC). Serum lipid peroxidation, a marker of oxidative stress, was measured by the TBARS assay, as described [20]. In brief, serum samples were diluted with PBS and incubated with or without 100 mM of 2,2'-azobis,2-amidinopropane hydrochloride (AAPH), a free radical generator, for 2 h at 37°C. Lipid peroxidation was calculated by subtracting values obtained in the presence and absence of AAPH.

Serum potassium, phosphate, calcium, alkaline phosphatase (ALP), calcium–phosphorus product (Ca × P product), blood urea nitrogen (BUN) and albumin were measured using an autoanalyser (Olympus, Inc.) by Spectra Labs (Rockleigh, NJ).

Blood pressure
Brachial blood pressure was measured using an automatic digital blood pressure monitor (Omron IntelliSense HEM-907XL, IL). Subjects were seated for 10 min prior to the first reading. Two measurements, 2 min apart, were performed. If these two measurements were within 10% of each other, the average of the two measurements was taken as the final recorded blood pressure. If not, a third measure was taken, and the two closest measures were averaged.

Echocardiography
Echocardiography was performed using a multi-frequency (1.5–4.25 MHz) transtracheal transducer (Acuson Sequoia C512, Mountain View, CA) to assess parameters related to cardiac structure and function. To minimize the effect of variations in fluid volume in haemodialysis patients, studies were performed 18–24 h after a haemodialysis session. LVM was measured by M-mode echocardiography, as described previously [21]. LVM index was measured according to the formula LVM index = LVM/body surface area. Relative wall thickness was measured as RWT = 2 × (PWTd/L VEDD) where PWTd is the posterior wall thickness at end-diastole and LVEDD is the left ventricular dimension at end-diastole. The MPI reflects both systolic and diastolic function of the heart and was measured as MCOT = LVET/LVET where MCOT is the mitral valve closure opening time and LVET is the left ventricular ejection time. Left atrial volume was calculated using the biplane area = length method at end ventricular systole with precaution taken to avoid foreshortening. The left atrial volume index was indexed to body surface area to derive the left atrial volume index. The thickness of the epicardial fat layer was also measured by echocardiography, as previously described [22]. In brief, standard parasternal and apical views were obtained with subjects in the left lateral decubitus position. Epicardial fat was identified as the echo-free space between the outer wall of the myocardium and the visceral layer of the pericardium and its thickness was measured perpendicularly on the free wall of the right ventricle at end-systole in three cardiac cycles [22]. Maximum epicardial fat thickness was measured at the point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aorta annulus. The average value of three cardiac cycles for each echocardiographic view was considered as the epicardial fat thickness. The intra- and interobserver coefficient of variation for the epicardial fat measurement in our laboratory is <5%, which is similar to what has been reported in other studies [23].

Statistical analysis
All statistical analyses were performed using the SPSS software and significance was based on a two-tailed alpha value of 0.05. Distribution statistics for the residuals were calculated to determine whether assumptions of normality were met (i.e. skewness and kurtosis < 2.0). Repeated-measures analysis of variance (group × time) was used to assess group differences in our major outcomes. Main effects were only considered when interactions were not significant, as a significant interaction indicates that the effect of one independent variable depends on the value of the other. When significant interactions were detected, paired sample t-tests were conducted to determine if values between time points differed significantly within each activity group. Correlation analysis was used to identify relationships between selected variables of interest.

Results
Subject characteristics at baseline and final testing are shown in Table 1. The aetiology of each patient's renal fail-
ure and the presence of underlying CVD are described in Table 2. A total of 17 patients were recruited for the study, nine in CON and eight in EX. One subject in EX withdrew due to a hip fracture that was unrelated to the exercise intervention, one subject in CON withdrew due to moving out of the area and a second subject in CON did not complete the baseline shuttle walk test due to a scheduling error.

At baseline, the two groups did not differ significantly regarding age, body weight, height, BMI, months on dialysis, frequency of dialysis sessions, blood pressure, physical performance, cardiac function or haematological variables (Table 1).

There were no interactive or main effects of activity group and time on BMI, systolic blood pressure or diastolic blood pressure (Table 1).

There was a significant interaction (P < 0.05) between activity group and time for serum TBARS and ALP activity, as they were reduced by 38 and 27%, respectively, in EX, but did not change in CON (Table 1).

**Table 2. Aetiology of CKD and underlying CVD in study patients**

<table>
<thead>
<tr>
<th>Aetiology of CKD</th>
<th>CON</th>
<th>EX</th>
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<tbody>
<tr>
<td>Hypertension</td>
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<td>3</td>
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<tr>
<td>Diabetes</td>
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<td>4</td>
</tr>
<tr>
<td>Focal glomerulosclerosis</td>
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<td></td>
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<tr>
<td>Membranous nephropathy</td>
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<td>1</td>
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<tr>
<td>Scleroderma</td>
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<tr>
<td>Amyloidosis</td>
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<table>
<thead>
<tr>
<th>Cardiac disease</th>
<th>CON</th>
<th>EX</th>
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<tbody>
<tr>
<td>Congestive heart failure</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>3</td>
<td>0</td>
</tr>
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</table>

Data are reported by means ± SEM. *P < 0.05 for an interaction effect between time and physical activity group, **P < 0.05 compared to baseline measure within a physical activity group. Ca × P product, calcium–phosphorus product; CRP, C-reactive protein; IL-6, interleukin 6; TBARS, thio-barbituric acid reactive substances; PON, paraoxonase; ALP, alkaline phosphatase; SBP and DBP, systolic and diastolic blood pressure, respectively; LV MI, left ventricular mass index; LA VI, left atrial volume index; MPI, myocardial performance index; h/week, hours per week undergoing dialysis treatment.
There was a significant interaction between activity group and time for ISWT performance, as the distance walked during this test increased by 15% in EX (P = 0.03), but did not change in CON (Figure 1).

There were no interactive or main effects of activity group and time on MPI, LVM index, relative wall thickness or left atrial volume index. However, there was a significant interaction between time and activity group for epicardial fat thickness, as it was significantly reduced in EX (−9.8%, P = 0.03), but did not change in CON (0.03%, P = 0.96) (Figure 2). Furthermore, when data from both groups were combined, the change in performance on the ISWT was inversely correlated to the change in epicardial fat (r = −0.66, P = 0.01) (Figure 3) and the change in serum ALP levels (r = −0.60, P = 0.02).

Discussion

The primary findings in this paper were that 4 months of intradialytic exercise training at a moderate intensity increased physical performance and reduced serum TBARS, serum ALP, and the thickness of the epicardial fat layer. Furthermore, the change in epicardial fat levels was inversely correlated to the change in physical performance. To the best of our knowledge, this is the first time that intradialytic exercise has been shown to reduce levels of these novel CVD risk factors. These benefits occurred in response to a rather modest amount of exercise: intradialytic cycling 3 days/week for 45 min at a moderate intensity—an exercise dose that is easily achievable by most dialysis patients.

We also believe this is the first time that the ISWT has been used to measure changes in physical performance in dialysis patients in response to intradialytic exercise. Shuttle walk tests are appropriate to assess function in older individuals and people with disease since functional limitations often prevent these individuals from achieving standard criteria of more objective tests (e.g. VO2max testing) [1]. The average change in distance walked during the ISWT in the EX group was 45 ± 16 m, which appears to be marginally less than what has been reported following rehabilitation programs in COPD patients and other populations, but is still in the range of what is thought to be clinically significant [24].

Epicardial fat is a highly inflammatory fat depot surrounding the heart that has been recently shown to be a marker for the presence and severity of CVD [13,25]. The close proximity of epicardial fat to the adventitia of the coronary arteries and the underlying myocardium suggests the possibility that it could play an especially important role in the development of CVD [10,12]. Evidence indicates that epicardial fat may locally modulate cardiovascular morphology and function. Iacobellis et al. showed that epicardial fat is positively correlated with abdominal visceral adiposity [23], atherosclerosis [26] and cardiometabolic risk [22,27]. As a result, interventions that reduce the extent of epicardial fat may have clinical benefit. Recently, Kim et al. [15] showed that 12 weeks of running at a moderate intensity reduced epicardial fat thickness in middle-aged obese men by 8.6%, which was similar to the 9.8% reduction in epicardial fat we found in the current study. However, the study by Kim et al. did not include a non-exercising control group. Our study confirms and extends the findings of Kim et al. by showing that moderate intensity exercise training significantly reduces epicardial fat compared to a non-exercising control group. Importantly, the reduction in epicardial fat was not correlated with weight change, suggesting that epicardial fat may be a very responsive adipose tissue that can be reduced by relatively modest amounts of exercise in the absence of weight loss.

Previous studies in patients undergoing coronary artery bypass grafting have shown that epicardial fat is highly correlated with inflammation [13,28], and coronary artery disease patients have significantly higher levels of inflammatory cytokines (IL-1β, IL-6 and TNF-α) released from epicardial fat than that from subcutaneous fat [13,29]. Despite this, we did not see changes in any marker of systemic inflammation in response to the change in epicardial fat, including traditional inflammatory markers such as CRP and IL-6, or markers of inflammation especially important in CKD patients, including albumin and fetuin-A. This is somewhat surprising, given that many, though not all, pre-
vious studies have shown that exercise training reduces systemic markers of inflammation [30,31]. However, plasma concentrations of circulating inflammatory cytokines may not be correlated to inflammation in tissues [13], so it is possible that the reductions in epicardial fat affected artery wall inflammation, but this was not captured by measuring systemic levels of inflammatory markers.

Our data are consistent with the cross-sectional study of Hung et al. [32], which showed no evidence for a correlation between TNF-α and physical activity levels in haemodialysis patients. Castaneda et al. reported that 12 weeks of resistance exercise reduced CRP and IL-6 levels in patients with moderate CKD [33], but we are unaware of any studies that have demonstrated an anti-inflammatory effect of endurance exercise training in haemodialysis patients. We also hypothesized that the levels of fetuin-A would increase after the exercise intervention, based in part on our previous work showing that VO_{2}\text{max} in older men [34]. Fetuin-A is a reverse acute-phase protein, which is reduced with inflammation, and a systemic inhibitor of vascular calcification [8]. However, fetuin-A levels did not change in either group in this study, possibly because there was no change in other markers of inflammation. Taken together, these data suggests that the modest anti-inflammatory effects of exercise training seen in other populations may be insufficient to have significant effects on systemic inflammatory markers in dialysis patients, a population with extreme inflammatory stresses.

Excessive oxidative stress is believed to be partly responsible for the increased inflammation and CVD burden in haemodialysis patients [3,35,36]. The increased oxidative stress in haemodialysis patients is mainly attributed to the retention of oxidized solute by the loss of kidney function [3]. Growing evidence indicates that endurance exercise training results in reduced O_{2}^{-} production and up-regulating antioxidant enzymes activity [1]. Our finding of decreased TBARS levels in the exercise group suggests a reduction in the burden of oxidative stress due to an increase in serum antioxidant activity, which may help reduce CVD risk in haemodialysis patients.

The 27% reduction in serum ALP in EX (Table 1) may be clinically significant, as serum ALP levels are inversely associated with bone mineral density in haemodialysis patients [37] and positively associated with coronary artery calcification [38], a complex disorder associated with abnormal mineral metabolism [39]. In healthy populations, exercise training has been shown to increase serum ALP levels [40]. By contrast, our data coincides with data from Yurtkuran et al. [41] indicating that physical activity reduces ALP levels in haemodialysis patients. This discrepancy may be due to abnormalities in mineral metabolism in the haemodialysis population. Regardless, the reduction in ALP suggests a potential mechanism by which intradialytic exercise improves CVD risk and reduces bone disorders in haemodialysis patients.

Elevated serum phosphate levels also are believed to contribute to vascular calcification. Vaithilingam et al. previously showed that 1 week of intradialytic cycling reduced serum phosphate levels, possibly by improving the perfusion of skeletal muscle during dialysis [42]. However, we did not find changes in phosphorous levels after our 4-month exercise intervention and are uncertain of the reasons for our discrepant findings.

There were several limitations to our study. First, our sample size was relatively small, which may have limited our ability to detect differences in our primary outcomes due to low statistical power. The limited sample size also did not allow us to control for many factors that may have impacted the results, including diabetes status, medications, gender and race. Furthermore, the 4-month intervention may have been too short to improve other CVD risk factors, including markers of inflammation, and metrics related to cardiovascular and renal function. Additional research with larger study populations and longer interventions will be needed to more thoroughly assess the cardiovascular benefits associated with intradialytic exercise training. We also cannot rule out the possibility that the improvements seen in the EX group may be partially attributed to the extra individual attention and social interaction they received while exercising, despite our best attempts to socialize equally with patients in the CON group. Finally, we collected medication lists at baseline only, so any changes in medications (e.g. addition of immunosuppressive medications) that occurred after this time may have affected our results.

Conclusion

In summary, we demonstrated that 4 months of intradialytic endurance exercise improved physical performance and reduced serum oxidative stress (TBARS), ALP and epicardial fat levels. These data suggest novel mechanisms by which intradialytic exercise training may reduce CVD risk. Furthermore, this study adds to the considerable evidence providing support for the adoption of intradialytic exercise as a standard component of care for haemodialysis patients.

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Conflict of interest statement. None declared.

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