Endothelial dysfunction and cellular repair in heart failure with preserved ejection fraction : response to a single maximal exercise bout

Endothelial dysfunction and cellular repair in heart failure with preserved ejection fraction: response to acute exercise

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Total word count: 3181

Abstract

Aims - Endothelial dysfunction contributes to exercise intolerance in heart failure with preserved ejection fraction (HFpEF). Endothelial progenitor cells (EPC) and angiogenic T lymphocytes (TA) participate in endothelial repair. Recruitment of EPC or TA with exercise might explain the vascular benefits of exercise training. We studied baseline endothelial function, EPC and TA numbers in HFpEF patients and the effects of a single exercise bout.

Methods and Results - HFpEF patients (n=26) and age- and sex-matched healthy volunteers (HV, n=26) performed a maximal exercise test. Before and 10 minutes after exercise, EPC and TA were quantified by flow cytometry and endothelial function was assessed by reactive hyperaemia index. HFpEF patients had endothelial dysfunction at baseline (p=0.036). Worse endothelial function predicted worse diastolic function in a multiple linear regression (E/e' ratio: ß=-7.5 p=0.001). EPC and TA counts were lower in HFpEF in comparison with HV (p=0.025 and p=0.047 respectively). Lower TA levels predicted higher New York Heart Association functional class (ß=$-1.1 \cdot 10^{-4}$, p=0.042). Acute exercise did not further worsen endothelial function in HFpEF patients (p=1.0), in contrast with HV (p=0.003). TA increased with exercise in both groups (HV p=0.047, HFpEF p=0.047).

Conclusions - We conclude that HFpEF patients have microvascular endothelial dysfunction. Lower EPC and TA suggest deficient endothelial repair in HFpEF. Exercise increases TA in HV, and this recruitment is intact in HFpEF patients. How repeated exercise impacts HFpEF pathophysiology through endothelial function and repair requires further study.

Keywords

Heart failure, HFpEF, endothelium, endothelial progenitor cell, angiogenic T cell, exercise

Introduction

The burden of heart failure with preserved ejection fraction (HFpEF) is increasing and mortality remains high despite decades of research.[1] Exercise intolerance is the hallmark clinical symptom, and in current guidelines, exercise training is recommended to improve symptoms and quality of life in HFpEF patients.[2] A pharmacological therapy that improves prognosis is lacking in these patients, which is at least partly due to an insufficient understanding of HFpEF pathophysiology.[3]

Recent evidence implicates the endothelium as a central mediator in the development of HFpEF: typical comorbidities such as hypertension, diabetes mellitus, chronic kidney disease and obesity induce an inflammatory state, which reduces bioavailability of the main endothelial effector molecule nitric oxide (NO).[4] This NO shortage, clinically translated into endothelial dysfunction, modulates diastolic function and cardiomyocyte stiffness and reduces peripheral vasodilatory capacity.[5,6] Animal studies have indeed shown endothelial inflammation and endothelial dysfunction in different HFpEF models.[7,8] Data in HFpEF patients is conflicting: most studies agree brachial artery endothelial function is not different from controls, but microvascular function is reduced.[9] Repair of deficient endothelium is possible through endothelial progenitor cells (EPC), circulating bone-marrow derived cells that mobilize to sites of injury or ischemia, secrete vascular growth factors and are able to integrate into the endothelial layer.[10] Angiogenic T cells (TA), a subpopulation of T lymphocytes with high angiogenic properties have been shown to help proliferate EPC and mature endothelial cells in vitro.[11]

Although exercise training increases aerobic capacity and reduces symptoms in HFpEF patients,[12] the mechanisms underlying these improvements are largely unknown. Exercise training is known to promote EPC and TA mobilization, providing a possible clue to the vascular benefits of training.[13] In patients with HFrEF, levels of circulating EPC and TA are reduced at baseline, and TA are recruited acutely by exercise.[14] EPC and TA levels and effects of acute exercise are yet unknown in patients with HFpEF.

We hypothesized that HFpEF patients have (i) microvascular endothelial dysfunction at rest, (ii) lower levels of circulating EPC and TA, (iii) further endothelial dysfunction after acute exercise, and (iv) intact recruitment of TA with acute exercise.

Materials and methods

Study design and participants

Twenty-six ambulatory and clinically stable HFpEF patients were recruited in the Antwerp University Hospital between September 2015 and May 2017. Inclusion criteria were (i) signs or symptoms of heart failure, New York Heart Association (NYHA) class II or III; (ii) left ventricular (LV) ejection fraction ≥50%; (iii) echocardiographic E/e' ratio >15 or E/e' 8-15 and plasma brain natriuretic peptide (BNP) >80 pg/mL; (iv) structured exercise <2 x 30 minutes per week. Exclusion criteria were (i) other cardiac causes for heart failure symptoms (severe valvular disease, untreated coronary artery disease, uncontrolled hypertension or arrhythmias, primary cardiomyopathy), (ii) significant pulmonary disease (forced expiratory volume <50% predicted), (iii) any comorbidity that may influence one-year prognosis and (iv) inability to exercise.

Additionally, 26 age- and sex-matched healthy volunteers (HV) were recruited. Volunteers were required to be sedentary (structured exercise <2 x 30 minutes per week), asymptomatic, free of cardiovascular disease, diabetes and hypertension, and not taking drugs with a cardiovascular effect (including statins). Cardiac structural or functional abnormalities were excluded by electrocardiogram and transthoracic echocardiography.

Subjects were called in after an overnight fast for blood sampling. After a light breakfast (sandwiches, no tea or coffee), endothelial function was assessed and a symptom-limited maximal cardiopulmonary exercise test (CPET) was performed. Immediately after peak exercise, blood was drawn from an antecubital vein. The first 3 ml of blood were discarded in order to prevent contamination with circulating endothelial cells. Endothelial function was re-assessed within 10 minutes after the end of the test. All participants provided written informed consent. This study abides to the Declaration of Helsinki and was approved by the ethics committee of the Antwerp University Hospital.

Cardiopulmonary exercise test

Exercise capacity was assessed by a symptom-limited maximal CPET using a ramp protocol of 20W + 10W/min on a bicycle ergometer (Ergoline-Schiller). Gas exchange was measured breath-by-breath throughout the test, starting with a 4-minute resting measurement and ending with a 5-minute recovery measurement. Ventilation (VE), oxygen uptake (VO_2) and carbon dioxide production (VCO_2) data was averaged per 10-second period. Peak VO_2 was calculated as the mean VO_2 during the final 30 seconds of exercise. The anaerobic threshold, assessed by the V-slope method and the respiratory exchange ratio (RER) were recorded. Predicted peak VO_2 was calculated by the Jones formulas.

Assessment of endothelial function

Endothelial function was assessed by peripheral arterial tonometry (PAT) at the fingertip (EndoPAT, Itamar Medical) as described previously.[15] In short, measurement was performed supine in a quiet temperature-controlled room (21-24°C). Patients were instructed to abstain from caffeine, alcohol and exercise during 24 hours before the measurement. After 5 minutes of baseline measurement, a blood pressure cuff was inflated at the forearm during 5 minutes to 100 mmHg above systolic blood pressure, and subsequently released causing an endothelium-dependent reactive hyperaemia. The PAT ratio was calculated by dividing the fingertip signal at the cuffed arm with the fingertip signal at the non-cuffed arm. The reactive hyperaemia index (RHI), the PAT ratio at 90-150s after cuff release corrected for baseline amplitude, was calculated by dedicated software (Itamar Medical). A RHI below the median has been described to predict a worse prognosis in HFpEF patients. [16]

Echocardiography

Echocardiography was performed within 7 days of other tests. Left atrial volume was calculated by the area-length method and indexed for body surface area. End-expiratory E (early filling) and A (atrial filling) waves were obtained from a pulse wave Doppler sample volume at the tips of the mitral valve leaflets. E' (early relaxation) wave was assessed by a Tissue Doppler measurement at the level of the septal mitral annulus. In patients with atrial fibrillation, measurements were averaged across at least

5 cardiac cycles. All echocardiograms were performed and analysed by one of two experienced operators (ABG and CMVDH).

Quantification of EPC and TA

Flow cytometry for EPC and TA was performed on a FACSCanto II flow cytometer (BD Biosciences). Fluorescence-minus-one and nonreactive isotype-matched antibodies were used as controls. All gating was done using FACSDiva software v6.1.2 (BD Biosciences) by a single operator (ABG). Counting of peripheral blood leukocytes, monocytes and lymphocytes was performed on an Advia 2120 cytometer (Siemens).

Endothelial progenitor cells

EPC were defined as CD34+KDR+CD45^{dim} cells.[17] Analysis and gating strategy have been published previously.[18] Briefly, whole blood was fixated (TransFix, Caltag Medsystems) and processed 1 to 4 days after sampling.[19] After lysis of red blood cells with ammonium chloride and addition of Fc receptor blocking reagent (Miltenyi Biotec), samples were stained with fluorochrome-conjugated antibodies for 30 minutes. The following antibodies were used: anti-CD34 PE-Cy7, anti-CD45 APC-H7 (BD Biosciences) and KDR-APC (R&D Systems). Before analysis, Syto13 (ThermoFisher) was added to exclude non-nucleated cells and debris. A minimum of one million events was recorded. Mononuclear cells were identified on a forward scatter-side scatter plot and a gate was set on CD45^{dim} cells. Secondary gates for CD34 and KDR were then joined. EPC were expressed per million mononuclear cells (/10^6 MNC). Absolute EPC count was calculated as CD34+KDR+CD45dim cells per CD45+ cells multiplied by leukocyte count.

Angiogenic T cells

TA were defined as CD3+CD31+CD184+ cells.[11] Analysis and gating strategy have been published previously.[18] Briefly, whole blood was fixated (TransFix, Caltag Medsystems) and processed 1 to 4 days after sampling.[19] After lysis of red blood cells with ammonium chloride and addition of Fc receptor blocking reagent (Miltenyi Biotec), samples were stained with fluorochrome-conjugated antibodies for 30 minutes. The following antibodies were used: anti-CD31 FITC, anti-CD3 PerCP, anti-

CD184 APC (BD Biosciences). A minimum of 500000 events was recorded. Mononuclear cells were identified on a forward scatter-side scatter plot and a primary gate was set on CD3+ cells. Next, CD31 positivity was gated using a histogram. Then, on a CD31 versus CD184 plot the double positive population of TA was identified. Both CD3+CD31+ and CD3+CD31+CD184+ cell counts were recorded, as CD3+CD31+ cells possess angiogenic capability themselves and CD184 expression may be transient.[20] TA were expressed /10^6 MNC. Absolute TA count was calculated as the percentage of TA relative to total flowcytometer lymphocyte count multiplied by Advia cytometer lymphocyte count.

Statistical analysis

Continuous variables are expressed as mean ± SD. Variables with skewed distribution (Shapiro-Wilk test) are expressed as median (interquartile range). Baseline comparisons were performed using Welch two-sample t-test (continuous variables), Wilcoxon rank sum test (skewed continuous variables) and Pearson's Chi-squared test with Yates' continuity correction (categorical variables). Spearman coefficients (rho) were used for correlations. Linear mixed models were used to analyse repeated measures data. First, an uncorrected model was constructed using time and group as fixed effects and patient ID as random effect. Then, covariates were added in a single block, and subsequently removed when not significantly contributing to the analysis. Diagnostics included empirical Bayes estimates and residuals distributions, standardized residuals vs. fitted values plots and quantile-quantile plots. If anomalies were noted on diagnostics, analysis was repeated after log transformation of the outcome variable. Holm correction was used for post-hoc multiple comparisons. A multiple linear regression model was used to assess independent determinants of peak VO_2, NYHA class and E/e' ratio. Nonlinear regression was used to fit a third-degree polynomial curve to PAT ratio values over time. Between groups, curves were compared with an extra sum-of-squares F test. Covariates for multivariate (linear or mixed) models were selected based on experience and previous literature. For RHI, EPC and TA covariates were age, gender and body mass index (BMI). Covariates for peak VO_2 were age, gender, BMI and rest heart rate; for E/e' ratio age, gender, rest heart rate and systolic blood pressure; and for NYHA class age and BMI. A two-sided p-value < 0.05 was considered significant. All data was analysed using R v3.4.3 (R Foundation for Statistical Computing) with packages *nlme* and *multcomp*.

Results

Demographics and clinical characteristics

Healthy volunteers and HFpEF patients were well matched for age and gender (Table 1). Several comorbidities were more prevalent in HFpEF patients, including hypertension, hyperlipidaemia, obesity, diabetes and chronic kidney disease (Table 1). HFpEF patients were characterized by diastolic dysfunction (increased E/e' ratio), structural cardiac changes (increased left atrial volume) and increased BNP levels. Cholesterol levels and rest heart rate were lower in patients, owing to statin and beta blocker treatment. CPET performance was worse in HFpEF patients, who achieved a lower peak VO_2, peak heart rate and peak workload and steeper slope of the VE/VCO_2 relationship compared to HV (Table 1).

Baseline endothelial function and cell numbers

Endothelial function values and cell counts are presented in Table 2. Endothelial function was impaired in HFpEF patients: the PAT ratio was consistently lower in HFpEF patients after cuff release, and RHI (adjusting PAT measurements for systemic effects and baseline variation) was significantly reduced ($p=0.036$, Table 2 and Fig. 1A-B). Also, the baseline amount of circulating EPC and TA was significantly lower in HFpEF compared to HV (EPC $p=0.025$; TA $p=0.047$, Table 2, Fig. 1C-D). This was also true for absolute EPC and TA numbers (EPC $p=0.035$; TA $p=0.014$, Table 2). Baseline numbers of circulating leukocytes were comparable between HV and HFpEF patients ($p=0.350$, Table 2).

Effect of acute exercise on endothelial function and cell numbers

A single exercise bout decreased RHI in HV, while it did not aggravate the pre-existent endothelial dysfunction in HFpEF patients (HV $p=0.003$, HFpEF $p=1.00$, Table 2 and Fig. 2A-B). No exercise-induced changes were seen in EPC, neither relative to mononuclear cells or in absolute count (all $p>0.10$, Table 2 and Fig. 2C). Relative and absolute numbers of circulating TA significantly increased with exercise in both groups (Relative: HV $p=0.047$, HFpEF $p=0.047$, Absolute: HV $p<0.001$, HFpEF $p<0.001$, Table 2 and Fig. 2D). Acute exercise recruited leukocytes in both groups (HV $p<0.001$, HFpEF $p<0.001$, Table 2).

Associations between outcome variables and clinical characteristics

Table 3 and Fig. 3 show the associations between endothelial function, cellular repair mechanisms and clinical HFpEF characteristics. RHI showed an inverse relation with E/e' ratio: better endothelial function predicted better diastolic function (ß=-7.53, adjusted R^2=0.20, p=0.001, multiple linear regression corrected for age, Fig 3A). Endothelial function was also modestly related to NYHA class and peak VO_2 (rho=-0.381, p=0.007 and rho=0.349, p=0.015 respectively) but these relations were lost in multiple linear regression (correcting for age and BMI respectively). Higher baseline TA predicted less severe symptoms assessed by NYHA class (ß=$-1.12 \cdot 10^{-4}$, adjusted R^2=0.23, p=0.042, multiple linear regression corrected for BMI, Fig 3B). Neither EPC nor TA were related to endothelial function (all p>0.10).

Discussion

Our results confirm a microvascular endothelial dysfunction in HFpEF patients. We are the first to report lower EPC and TA counts in HFpEF, suggesting deficient endothelial repair. Patients with higher remaining TA have less symptoms. Acute exercise does not further reduce endothelial function in HFpEF patients. Exercise-induced recruitment of TA is intact in HFpEF.

Endothelial dysfunction in HFpEF

The detection of microvascular endothelial dysfunction in HFpEF is highly relevant from a pathophysiological point of view. By downregulating protein kinase G, reduced NO bioavailability in the coronary microvasculature is hypothesized to be a central mediator in the development of stiffness and hypertrophy in adjacent cardiomyocytes, which can lead to HFpEF.[4] Also, peripheral endothelial dysfunction can contribute to exercise intolerance in HFpEF by limiting blood supply to exercising muscles.[21] Although endothelial dysfunction in HFpEF has been reported previously, its presence is controversial because of conflicting results of several large studies (reviewed in [9]). Early studies have found no significant differences in *macrovascular* function with appropriately matched controls using flow-mediated dilation of the brachial artery measured by ultrasound.[22] In contrast, all studies using the operator-independent EndoPAT to measure *microvascular* endothelial function, as in our current study,

have reported a lower RHI in HFpEF patients.[5,16] Indeed, Lee et al showed that while flow-mediated dilation was not different between HFpEF patients and controls, simultaneously measured microvascular function was significantly impaired.[23] Our study confirms a relevant microvascular endothelial dysfunction in HFpEF patients.

Effect of acute exercise on the endothelium

It is well established that a single bout of exhaustive exercise has primarily negative physiologic effects. This contrasts with the important beneficial impact of repeated exercise training.[24] Acute exercise causes increased reactive oxygen species production and initiates an acute inflammatory response. At the level of the endothelium, this scavenges NO and uncouples endothelial NO synthase, causing a transient endothelial dysfunction.[25] In healthy, untrained subjects, we could confirm this finding. However, HFpEF patients did not exhibit a further decline in endothelial function after acute exercise. It is possible that HFpEF patients might not have reached the same level of maximal exercise compared to HV during CPET, causing less endothelial oxidative stress and inflammation. Although exercise effort as measured by peak RER was significantly higher in HV, 81% of HFpEF patients had peak RER >1.1 (Table 1). When including peak RER as a factor in our statistical model, differences in endothelial function remain significant (p-group=0.045, p-time=0.706, p-interaction=0.034, model corrected for age and BMI). Also, when repeating statistical analysis excluding patients with peak RER <1.1, differences in endothelial function remain significant (p-group=0.019, p-time=0.701, p-interaction=0.035, model corrected for age and BMI). We can conclude that differences in CPET effort do not explain the absence of a deterioration of endothelial function in HFpEF. HFpEF patients are known to exhibit increased oxidative stress and inflammatory cytokines at rest.[26] We suggest that NO availability is already maximally reduced at baseline, such that acute exercise cannot further impair reactive hyperaemia.

Deficient endothelial repair in HFpEF and effect of acute exercise

We are the first to report reduced circulating EPC and TA, indicating deficient endothelial repair, in HFpEF patients. Acute exercise reversed this deficiency by recruiting TA. Patients with higher

remaining TA had fewer symptoms. These results suggest a beneficial role for TA in HFpEF pathophysiology. EPC, on the other hand, were not recruited by an exercise bout. Possibly, the increased oxidative stress in HFpEF inhibits an exercise-induced increase in EPC.[27]

Whereas EPC and TA numbers were not directly correlated to endothelial function in this study, nor in previous reports,[18,28] their role in endothelial repair is well known.[29,30] As such, therapies directed at increasing the numbers of circulating TA could be tested in HFpEF patients. One such therapy is exercise training: our group and others have shown improvement in circulating angiogenic cell function in heart failure with reduced ejection fraction (HFrEF) patients.[31,32] Additionally, it is well-established that exercise training improves endothelial function both in healthy and heart failure populations.[24] Concerning HFpEF, several exercise training interventions have succeeded in improving symptoms and peak VO_2 in HFpEF patients.[12] While training is able to improve endothelial function in HFpEF animals,[8] this has failed to translate to human patients.[33]

In contrast to earlier studies from our group and others,[13,34–36] we did not see a recruitment of EPC in healthy subjects. Ross et al have described an age-related decline in the mobilization of EPC and TA in response to exercise,[13] which could help explain this finding in our comparatively old population. Also, evolutions in gating protocols for EPC could contribute to differences with older studies.[37]

Limitations

Finally, we would like to address the following limitations of this study. First, definitive associations cannot be concluded from our study due to the cross-sectional design. Second, while our HV are well matched for age and sex, we did not match for comorbidities. Rest heart rate, diastolic blood pressure and total cholesterol were higher in HV than in HFpEF (Table 1). This is due to the higher use of antihypertensive, heart rate reducing and cholesterol reducing drugs in the HFpEF group. However, the ratio of total to HDL cholesterol is comparable between groups, and cholesterol did not show any correlation with outcome variables. Third, one could argue that the elderly HV recruited in our study do not reflect the general population, as elderly people without cardiovascular risk factors or medication use are relatively rare in a Western population. However, allowing risk factors such as

hypertension and/or dyslipidaemia in the control group could confound further analysis. We consider it appropriate to compare HFpEF patients to a 'healthy aging' population.

Conclusions

We report reduced baseline numbers of circulating endothelium-repairing cells parallel to microvascular endothelial dysfunction in HFpEF patients. Acute exercise recruited angiogenic TA in HV, and this mobilization is intact in HFpEF patients. Higher TA predicted fewer symptoms in HFpEF patients. Endothelial function did not worsen after acute exercise in HFpEF patients, which suggests NO is already maximally depleted at baseline. The effect of exercise training on endothelial function and repair in HFpEF remains to be determined, but hopes are high that unravelling the beneficial effects of exercise will eventually lead to efficient endothelium-targeting therapies in HFpEF patients.

Acknowledgements

We are grateful to Nadine Possemiers and Kurt Wuyts for their excellent assistance during CPET; Sam C. Latet and Katrijn Van Ackeren for their expertise in flow cytometry; and all cardiologists at the Antwerp University Hospital for their efforts in recruiting patients. Research groups Cardiovascular Diseases and Physiopharmacology are part of the Infla-Med Research Centre of Excellence. Last, we sincerely thank all healthy volunteers and their recruiters.

Funding

EMVC is supported by the Flanders Research Funds (FWO) as senior clinical investigator.

Conflict of interest

The authors report no conflicts of interest relevant to this research.

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421

Figure legends

Figure 1: Endothelial function and cellular repair at baseline. Green: healthy volunteers, red: HFpEF patients. **A:** Curves showing vasodilatory response after cuff ischemia, expressed as PAT ratio measured by the EndoPAT device. Healthy volunteers consistently show better vasodilatory response than HFpEF patients. Mean ± standard error, extra sum of squares F test. **B:** Reactive hyperaemia index is lower in HFpEF patients at baseline. Adjusted p value from linear mixed models analysis. **C:** The number of circulating EPC is reduced in HFpEF patients at baseline. Adjusted p value from linear mixed models analysis. **D:** The number of circulating TA is reduced in HFpEF patients at baseline. Adjusted p value from linear mixed models analysis. EPC = endothelial progenitor cells, HFpEF = heart failure with preserved ejection fraction, MNC = mononuclear cells, PAT = peripheral arterial tonometry, TA = angiogenic T cells.

Figure 2: Endothelial function and cellular repair before and after exercise. Green: healthy volunteers, red: HFpEF patients. **A:** Curves showing vasodilatory response after cuff ischemia, expressed as PAT ratio measured by the EndoPAT device. After exercise, the response in HFpEF patients remains comparable with the measurement before exercise (see Fig. 1A). The response in healthy volunteers decreases to a level similar to HFpEF patients. Mean ± standard error, extra sum of squares F test. **B:** Reactive hyperaemia index is lower in HFpEF patients before exercise. After exercise, no changes occur in HFpEF patients. In healthy volunteers reactive hyperaemia decreases to the level of HFpEF patients. Median ± interquartile range, adjusted p values from linear mixed models analysis. **C:** EPC count is lower at baseline in HFpEF patients. No significant changes occur with exercise. Median ± interquartile range, adjusted p values from linear mixed models analysis. **D:** TA count is lower at baseline in HFpEF patients. TA count increases after exercise, both in healthy volunteers and HFpEF patients. Median ± interquartile range, adjusted p values from linear mixed models analysis. EPC = endothelial progenitor cells, HFpEF = heart failure with preserved ejection fraction, MNC = mononuclear cells, PAT = peripheral arterial tonometry, TA = angiogenic T cells.

Figure 3: Associations of endothelial function and cellular repair with clinical characteristics. Green: healthy volunteers, red: HFpEF patients. **A:** Correlation plot of endothelial function (expressed as reactive hyperaemia index) and diastolic function (E/e' ratio). Subjects with better endothelial function had better diastolic function. ß coefficient, p value and regression line from multiple linear regression adjusting for age. **B:** Box plot of angiogenic T cells stratified per NYHA class, showing a significant inverse relation. ß coefficient, p value and regression line from multiple linear regression adjusting for body mass index. HFpEF = heart failure with preserved ejection fraction, MNC = mononuclear cells, NYHA = New York Heart Association, peak VO_2 = peak oxygen uptake, TA = angiogenic T cells.

Tables

Table 1: Baseline clinical characteristics

	Healthy (n=26)		HFpEF (n=26)		P value
Age (years)	73	± 6	74	± 7	0.622
Gender (*n*, % female)	16	(61.5)	16	(61.5)	1.000
Heart rate (bpm)	69	± 8	63	± 9	**0.013**
Systolic blood pressure (mmHg)	133	± 15	130	± 19	0.620
Diastolic blood pressure (mmHg)	75	± 8	65	± 9	**0.004**
Body mass index (kg/m^2)	26.3	(23.1 – 28.0)	29.1	(26.8 – 32.9)	**0.002**
NYHA class					
I (*n*, %)	26	(100)	0	(0)	**<0.001**
II (*n*, %)	0	(0)	16	(61.5)	
III (*n*, %)	0	(0)	10	(38.5)	
Comorbidities					
Atrial fibrillation (*n*, %)	0	(0)	8	(30.8)	**0.024**
Coronary artery disease (*n*, %)	0	(0)	11	(42.3)	**0.001**
Diabetes mellitus (*n*, %)	0	(0)	8	(30.8)	**0.007**
Family history of cardiovascular disease (*n*, %)	7	(26.9)	8	(30.8)	1.000
Hyperlipidaemia (*n*, %)	2	(7.7)	20	(76.9)	**<0.001**
Hypertension (*n*, %)	0	(0)	22	(84.6)	**<0.001**
Obesity (*n*, %)	4	(15.4)	12	(46.2)	**0.035**
Sleep apnoea (*n*, %)	0	(0)	9	(34.6)	**0.003**
Previous smoker (*n*, %)	20	(76.9)	16	(61.5)	0.367
Medication use					
Angiotensin conversion enzyme inhibitor or angiotensin receptor blocker (*n*, %)	0	(0)	15	(57.7)	**<0.001**
Acetylsalicic acid or antiplatelet (*n*, %)	2	(7.7)	14	(53.8)	**<0.001**
Antiarrhythmic therapy (*n*, %)	0	(0)	3	(11.5)	0.234
Anticoagulant therapy (*n*, %)	0	(0)	10	(38.5)	**0.002**
Aldosterone antagonist (*n*, %)	0	(0)	2	(7.7)	0.471
Beta blocker (*n*, %)	0	(0)	17	(65.4)	**<0.001**
Calcium antagonist (*n*, %)	0	(0)	10	(38.5)	**0.002**
Diuretic (*n*, %)	0	(0)	15	(57.7)	**<0.001**
Nitrate (*n*, %)	0	(0)	7	(26.9)	**0.015**
Statin (*n*, %)	0	(0)	15	(57.7)	**<0.001**
Laboratory measurements					
Brain natriuretic peptide (pg/mL)	/		157	(112 – 231) *n=22*	/
Cholesterol total (mg/dL)	211	(195 – 241)	171	(149 – 204)	**0.003**
Cholesterol HDL (mg/dL)	68	(56 – 80)	53	(46 – 61)	**<0.001**
Cholesterol LDL (mg/dL)	132	(109 – 146)	105	(82 – 140)	**0.062**
Cholesterol ratio total/HDL	3.28	(2.86 – 3.69)	3.37	(3.04 – 4.17)	0.440

Creatinine (mg/dL)	0.79	(0.69 – 0.93)	0.92	(0.79 – 1.19)	**0.014**
Glomerular filtration rate* (mL/min/1.73m^2)	82.0	(73.8 – 86.8)	63.2	(50.4 – 83.0)	**0.003**
Haemoglobin (g/dL)	14.2	(13.8 – 14.8)	13.3	(12.1 – 13.9)	**<0.001**
High sensitivity C-reactive protein (mg/dL)	1.20	(0.86 – 2.20)	2.40	(0.83 – 5.88)	0.131
Echocardiography					
Left ventricular ejection fraction (%)	58.6	(54.6 – 62.8)	58.0	(55.0 – 64.0)	0.843
E/A	0.81	(0.74 – 0.91)	1.0	(0.82 – 1.26) *n=23*	**0.013**
E/e' septal	10.1	(9.3 – 11.7)	16.5	(14.0 – 20.0)	**<0.001**
Estimated systolic pulmonary artery pressure (mmHg)	27.3	± 7.6 *n=22*	32.2	± 8.4 *n=17*	0.069
Left atrial volume index (mL/m^2)	22.9	(17.3 – 27.5) *n=22*	43.2	(33.5 – 49.4) *n=22*	**<0.001**
Diastolic function					
Normal	20	(76.9)	0	(0)	
Grade 1 diastolic dysfunction	2	(7.7)	7	(26.9)	**<0.001**
Grade 2 diastolic dysfunction	0	(0)	11	(42.3)	
Grade 3 diastolic dysfunction	0	(0)	2	(7.7)	
Indeterminate	4	(15.4)	6	(23.1)	
Cardiopulmonary exercise test					
Peak heart rate (bpm)	154	(144 – 162)	109	(100 – 130)	**<0.001**
Peak respiratory exchange ratio	1.24	(1.17 – 1.27)	1.17	(1.11 – 1.25)	**0.039**
VE/VCO_2 slope	23.7	(22.7 – 26.0)	31.5	(28.8 – 34.1)	**<0.001**
Peak VO_2 (mL/kg/min)	23.3	(21.6 – 29.0)	17.5	(13.6 – 19.1)	**<0.001**
Percent predicted VO_2 (%)	123.9	(94.3 – 145.3)	83.6	(70.8 – 93.0)	**<0.001**
Peak workload (W)	100	(90 – 127.5)	70	(70 – 100)	**<0.001**

Normally distributed variables: mean ± SD, Welch two-sample t-test. Skewed variables: median (interquartile range), Wilcoxon rank sum test. Categorical variables: Pearson's chi-square test with Yates' continuity correction. HDL = high density lipoprotein, HFpEF = heart failure with preserved ejection fraction, LDL = low density lipoprotein, VE = ventilatory equivalent, VCO_2 = carbon dioxide uptake, VO_2 = oxygen uptake. * Estimated by the Chronic Kidney Disease Epidemiology Collaboration formula.

Table 2: Endothelial function and cell numbers before and after exercise

	Before exercise				After exercise				P value			Model corrected for
	Healthy		HFpEF		Healthy		HFpEF		Group	Time	Inter-action	
Endothelial function												
Reactive hyperaemia index	2.74	(2.09–3.41)	1.89 *	(1.62–2.64)	1.93 †	(1.60–2.88)	2.16	(1.65–2.61)	**0.048**	0.733	**0.013**	Age, BMI
Endothelial repair - relative												
EPC ‡ (/10^6 MNC)	63	(42–83)	39 *	(25 – 64)	76	(51–97)	31 *	(16–68)	**0.043**	0.953	0.190	/
TA ‡ (/10^6 MNC)	2168	(533–4961)	720 *	(324–1592)	4431 †	(1388–6082)	1053 *†	(405–3280)	**0.019**	**0.016**	0.981	/
CD3+CD31+ ‡ (/10^6 MNC)	11392	(8890-15668)	8075 *	(4069-11693)	15385	(12179-18067)	13791 †	(6782-17677)	**0.002**	**<0.001**	0.072	/
Endothelial repair - absolute												
EPC ‡ (cells/mL)	406	(237-561)	196 *	(132-462)	657	(459-976)	282 *	(151-620)	**0.022**	0.125	0.124	/
TA ‡ (cells/mL)	7085	(1580-14048)	1974 *	(946-5984)	21858 †	(757-29067)	3261 *†	(1920-14863)	**0.017**	**<0.001**	0.441	/
CD3+CD31+ ‡ (cells/mL)	39812	(27382-51246)	22214 *	(11728-32298)	74138 †	(59286-97602)	59668 *†	(24441-82400)	**0.004**	**<0.001**	0.495	/
Leukocytes - absolute												
Leukocyte count (10^3/mL)	5605	(5020–6340)	6000	(5265–6900)	8140 †	(7288–8464)	8120 †	(7335–8932)	0.744	**<0.001**	0.071	Gender

Median (interquartile range), linear mixed models analysis. EPC = endothelial progenitor cells, HFpEF = heart failure with preserved ejection fraction, MNC = mononuclear cells, TA = angiogenic T cells. * $p<0.05$ vs. Healthy, † $p<0.05$ vs. before exercise, p value is adjusted for multiple comparisons. ‡ Variable was log-transformed for analysis.

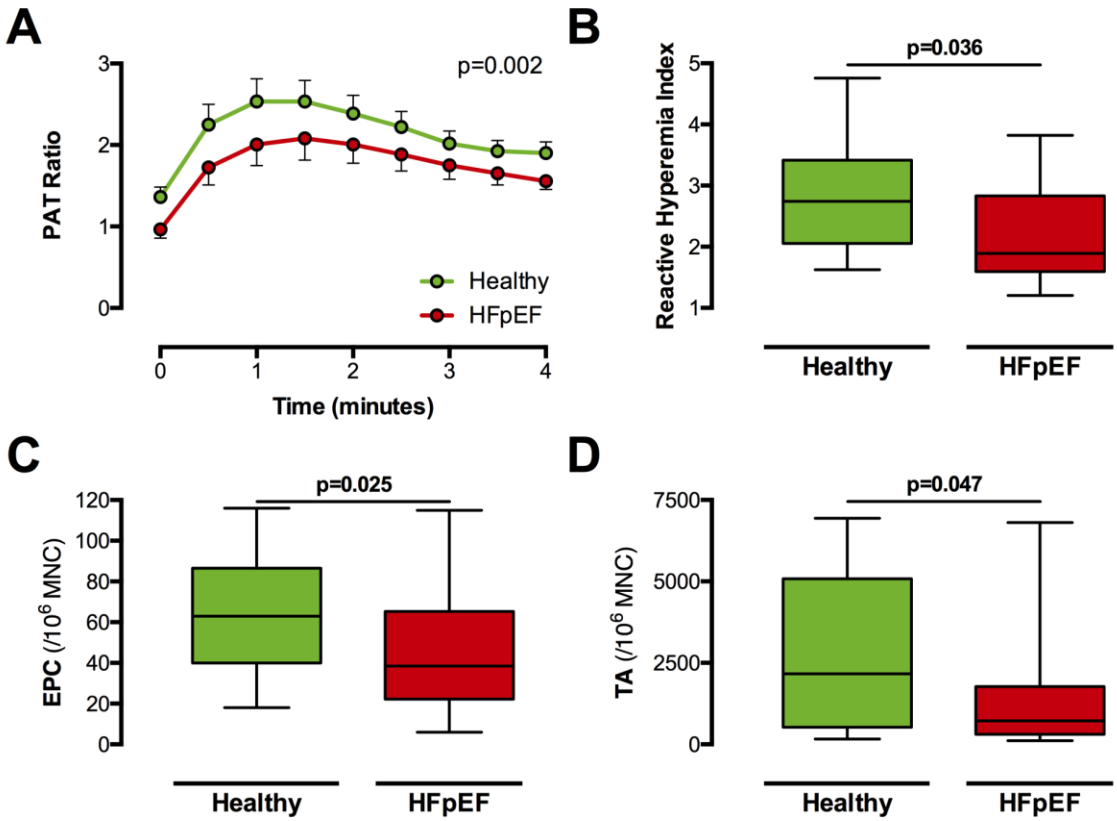
Table 3: Correlation and linear regression analysis between clinical characteristics and endothelial function and repair

	Correlation analysis		**Multiple linear regression analysis**			
	Spearman rho	P value	Regression coefficient ß (95% confidence interval)	Adjusted R^2	Model corrected for significant influence of	P value
E/e' ratio						
Baseline RHI *	-0.411	**0.004**	-7.53 (-11.87 – -3.19)	0.202	Age	**0.001**
Baseline EPC	-0.141	0.323	/	/	/	/
Baseline TA	-0.233	0.103	/	/	/	/
New York Heart Association class						
Baseline RHI *	-0.381	**0.007**	-0.40 (-1.12 – 0.33)	0.179	BMI	0.278
Baseline EPC	-0.260	0.066	/	/	/	/
Baseline TA	-0.348	**0.013**	$-1.12 \cdot 10^{-4}$ ($-2.19 \cdot 10^{-4}$ – $-0.04 \cdot 10^{-4}$)	0.230	BMI	**0.042**
Peak VO_2						
Baseline RHI *	0.349	**0.015**	3.48 (-1.80 – 8.76)	0.358	Age, BMI, gender	0.191
Baseline EPC	0.243	0.086	/	/	/	/
Baseline TA	0.222	0.121	/	/	/	/

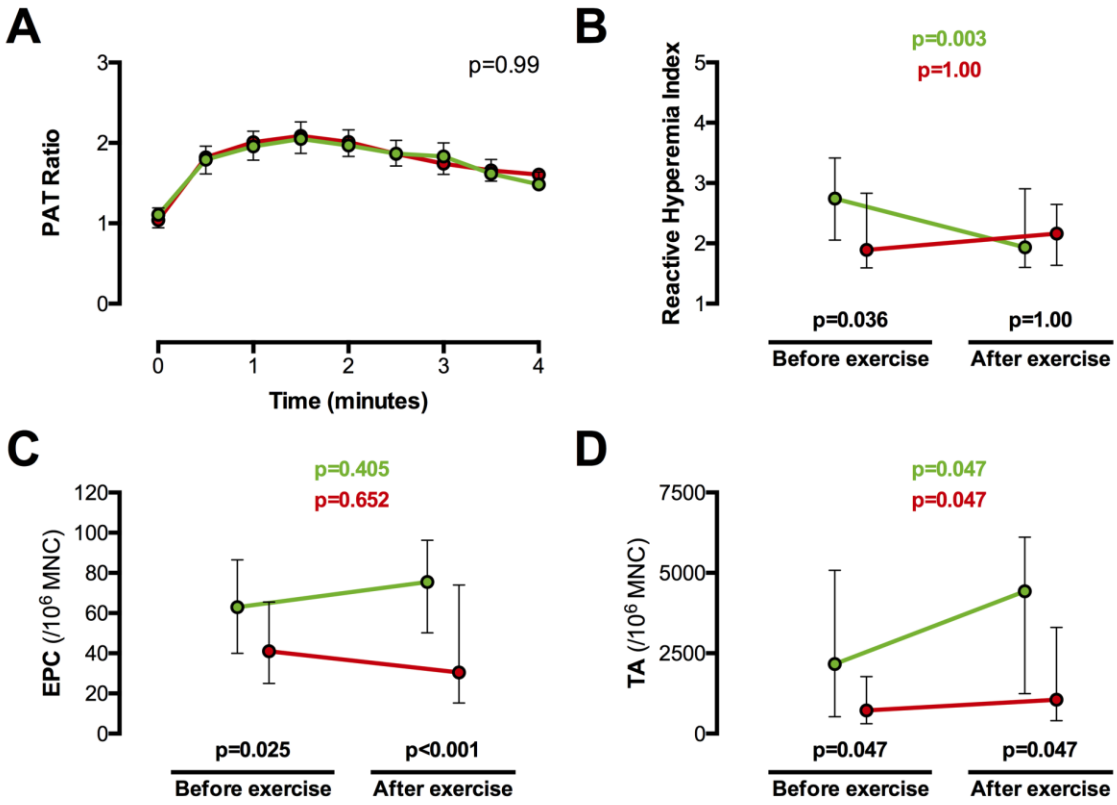
BMI = body mass index, EPC = endothelial progenitor cells (relative to 10^6 mononuclear cells), RHI = reactive hyperaemia index, TA = angiogenic T cells (relative to 10^6 mononuclear cells), VO_2 = oxygen uptake. * Variable was log-transformed before linear regression analysis

Figures

Figure 1



482 Figure 2



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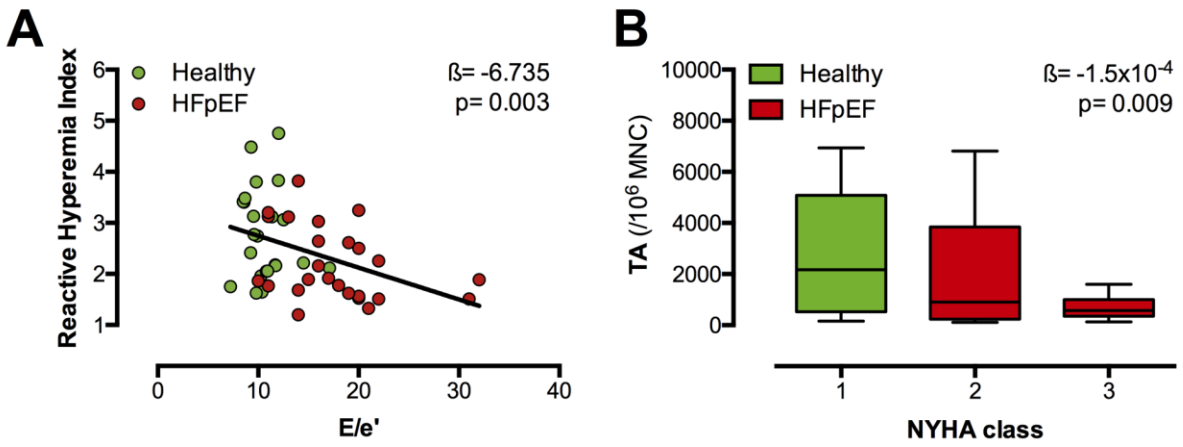
485 Figure 3



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