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Effect of 6 weeks of high-intensity one-legged cycling on functional sympatholysis and ATP signaling in patients with heart failure

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ABSTRACT

Breathlessness during daily activities is the primary symptom in patients with heart failure (HF).

Poor correlation between the hemodynamic parameters of left ventricular performance and perceived symptoms suggests that other factors such as skeletal muscle function plays a role in determining exercise capacity. We investigated the effect of six weeks of high-intensity one-legged cycling (HIC; 8x4 at 90% one-legged cycling max) on: 1) the ability to override sympathetic vasoconstriction (arterial infusion of tyramine) during one-legged knee-extensor exercise (KEE); 2) vascular function (arterial infusion of ACh, SNP, tyramine and ATP); 3) exercise capacity in HF patients with reduced ejection fraction (n=8) compared with healthy individuals (n=6). Arterial tyramine infusion lowered leg blood flow and leg vascular conductance at rest and during KEE before the training intervention in both groups (P<0.05), but not during KEE after the training intervention. There was no difference between groups. The peak vasodilatory response to ATP was blunted in the HF patients. (P<0.05), whereas there was no difference in ACh- and SNP- induced vasodilation between HF patients and healthy individuals. ACh induced vasodilation increased in the HF patients after the training intervention (P<0.05). HIC improved aerobic capacity in both groups (P<0.05), whereas only the HF patients improved six min walking distance (P<0.05). These results suggest that exercise hyperemia and functional sympatholysis is not altered in HF patients, and that functional sympatholysis is improved with HIC in both HF patients and healthy individuals. Moreover, these results suggest that the peak vasodilatory response to ATP is blunted in HF.

New & Noteworthy

The ability to override sympathetic vasoconstrictor activity (by arterial tyramine infusion) during exercise is not different between heart failure patients and healthy individuals and is improved by
high-intensity one-legged cycling training. The peak vasodilatory response to ATP is reduced in heart failure patients.
INTRODUCTION

Chronic heart failure (HF) is associated with exercise intolerance primarily because of breathlessness and muscle fatigue (54, 60). These symptoms often lead to reduced physical activity, which progressively worsen the exercise intolerance. Exercise intolerance does not seem to be related to left ventricular performance (12), indicating that other factors play a major role.

Abnormal skeletal muscle perfusion and metabolism, as well as abnormal autonomic reflexes originating in the skeletal muscles have been observed (4, 21, 45, 46). When exercising with a small muscle mass, and thereby removing any central hemodynamic limitation to skeletal muscle perfusion (1), HF patients are able to achieve equally high peak muscle perfusion rates as healthy individuals (11, 31, 32, 59). Therefore, single leg training might be optimal for improving exercise capacity in HF patients (8, 31, 58), but the underlying mechanisms of how improved muscle function reduce symptoms of HF are still unclear.

Skeletal muscle blood flow is regulated by a complex interaction between vasodilator and vasoconstrictor substances (16), including nitric oxide (NO) and prostacyclin (3, 36, 39). HF is associated with endothelial NO dysfunction (10, 26, 27) and exercise training has been shown to improve endothelial NO function both locally and systemically (14, 23, 28, 62). In recent years, ATP has been proposed to contribute to exercise hyperemia by inducing local vasodilation and overriding sympathetic vasoconstrictor activity (25, 50, 51). However, it remains unknown if ATP signaling is altered in HF patients.

In healthy humans, the vasoconstrictor effect of increased sympathetic nerve activity (SNA) during muscle contraction is blunted, termed “functional sympatholysis” (49) and this mechanism is thought to play an important role in securing adequate delivery of O₂ to contracting muscle. HF is associated with increased levels of SNA (31, 34, 47, 53), and an impaired functional sympatholysis could therefore accelerate vasoconstrictor activity in these patients. Whether functional
sympatholysis is impaired in HF patients remains unknown. The ability for functional sympatholysis is heavily dependent on training status of the exercising muscle (35, 37, 56) and exercise training could therefore improve functional sympatholysis in HF.

The aim of the study was to evaluate the effect of high-intensity one-legged cycling (HIC) training on leg hemodynamics and oxygen uptake, functional sympatholysis and endothelial function. To investigate this, we determined endothelial function and leg hemodynamics during arterial ATP infusion and one-legged knee extensor exercise (KEE), with and without co-infusion of tyramine before and after six weeks of HIC in heart failure patients with reduced ejection fraction and healthy individuals.
METHODS

Fourteen subjects, 8 heart failure (HF) patients New York Heart Association Classification (NYHA-class II) with mildly reduced ejection fraction and 6 healthy individuals (Table 1) were studied before and after six weeks of HIC. Patients were recruited from Hospitals in the Region of Copenhagen whereas the healthy individuals were recruited from advertisements. All subjects were screened with a medical history interview, physical examination, blood chemistry analysis, and resting 12-lead electrocardiogram. Stable HF patients (with a reduced ejection fraction 37±2%, at the time of diagnosis) in stable pharmacological treatment (ACE-inhibitors and beta-blockers) with and without implantable cardioverter defibrillator were recruited. Patients with intermittent claudication, aneurysm in a. femoralis, moderate to severe heart valve disease and arrhythmias were excluded. Other exclusion criteria were chronic obstructive pulmonary disease (forced expiratory volume in 1 sec <60%) and renal failure (creatinine >2.5 mg dL⁻¹). The healthy individuals were non-smokers and none of the subjects had been diagnosed with cardiovascular disease, renal dysfunction, insulin resistance, diabetes, or hypercholesterolaemia. Before the start of the training intervention standard echocardiography was performed (Vivid 9, GE Healthcare, Pittsburgh, PA, USA).

The study was approved by the Ethics Committee of the Capital Region of Denmark (H-3-2013-048) and conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all subjects before enrolment into the study.

Initial testing

Before the experimental day all subjects visited the laboratory to be accustomed to the KEE (1). On the same day the subjects performed: (1) an incremental bicycle test on a bicycle ergometer (Monark 839E; Monark, Varberg, Sweden) to determine peak pulmonary oxygen uptake (l/min,
VO$_2$peak) (Cosmed CPET, Rome, Italy) and peak workload (Watt$_{peak}$), (2) an incremental KEE test, to determine peak workload, and (3) distance during a six minute walk (6MWD) test (54). Each test was separated by >30 min of rest. On the same day body composition was assessed by whole-body dual-energy X-ray absorptiometry scanning (Lunar Prodigy Advance; GE Healthcare, Madison, WI).

**Experimental protocol**

All subjects refrained from caffeine, alcohol and exercise 24 hours before the experimental day. Medication was gradually withdrawn from the HF patients (ACE-inhibitors were withdrawn 48 hours prior to the experimental day, whereas beta-blockers, anti-platelet and diuretic drugs were withdrawn 24 hours before the experimental day). On the experimental day all subjects arrived at the laboratory after a light breakfast. After local anesthesia (Lidocaine (20 mg dL$^{-1}$)), catheters were placed in the femoral artery and vein of the experimental leg and in the left brachial artery. In the experimental leg, two microdialysis probes (63 MD Catheter, M Dialysis AB, Stockholm, Sweden) membrane length of 30 mm and a cut-off at 20 KDa were inserted into m. vastus lateralis. After insertion of catheters the experimental leg was passively moved for ~5 min in supine position, with the purpose of minimizing the tissue response to insertion trauma.

Following 30-60 min of rest, the subjects received a femoral arterial infusion of: 1) Tyramine 1µmol/min/kg leg mass (Sigma Aldrich, St Louis MO); 2) sodium nitroprusside (SNP) 2 µg/min/kg leg mass (Nitropress, Hospira, Lake Forrest. IL); 3) ACh 100 µg/min/kg leg mass$^{-1}$ (Miochol-E, Bausch + Lomb, Berlin, Germany); 4) ATP 0.05 (ATP1) µmol/min/kg leg mass (Sigma Aldrich) with and without co-infusion of tyramine 1µmol/min/kg leg mass; 5) ATP 0.3 (ATP2) and 3.0 (ATP3) µmol/min/kg leg mass. Each dose was infused for 2.5 min and measurements were obtained after 2.0 min. All infusions were separated by ~20 min. After additional 60-90 min of supine rest
the subjects completed two exercise bouts separated by ~20 min: 1) 2 min one-legged passive leg movement (PLM); 2) 11 min of KEE at an absolute workload of 10 watt. During the 11 min of KEE, tyramine (1µmol/min/kg leg mass) was infused for 3 minutes (5-8 mins of exercise). Leg blood flow was measured and arterial and venous blood samples (1-5 ml) were drawn simultaneously before and during (after: 1 min during PLM; and 3.5, 7 and, 10 during the 11 min of KEE) each trial.

Exercise training

The subjects performed supervised HIC on a cycle ergometer (Monark 839E) modified with a fixed flywheel. The subjects completed six weeks of training with three training sessions per week. Each training session consisted of 10 min two-legged warmup at 50 watts where after 8x4 min intervals at ~90% of one-legged Watt\textsubscript{max} were completed (four intervals with each leg). Each interval was separated by 1.5-2 min rest. The workload during the intervals was determined from a graded one-legged cycling test during the first training session. Heart rate was monitored during each training session (Polar WearLink, Polar Electro Denmark Aps, Denmark) and intervals were rated from the Borg scale.

Microdialysis

Microdialysate was collected for ~10 min during resting conditions, during infusion of tyramine, ACh, ATP+tyramine and ATP and KEE. After collection of samples, the microdialysate was weighed, and the actual flow rate was calculated to estimate any loss of fluid or abnormal decrease in perfusion rate. The microdialysis probes were perfused with isotonic saline (9 g NaCl/l) at a rate of 5 µl min\textsuperscript{-1} and to determine the relative exchange of substances, a small amount (2.7 nM) of [2.8-\textsuperscript{3}H] ATP (<0.1 µCi/ml) was added to the perfusate for calculation of probe recovery. The molecular
probe recovery (PR) was calculated as $PR = (dpm_{\text{infusat}} - dpm_{\text{dialysate}}) / dpm_{\text{infusat}}$, where dpm denotes disintegrations per min (22, 52). The $[2.8-^3\text{H}]$ ATP activity (in dpm) was measured on a liquid scintillation counter (Tri-Carb 2910 TR, Perkin Elmer) after an addition of the infusate and dialysate (5 µl each) were added to 3 ml Ultima Gold scintillation liquid (Perkin Elmer, MA).

Measurements and calculations

Femoral arterial blood flow was measured with ultrasound Doppler (Logic E9, GE Healthcare, Pittsburgh, PA) equipped with a linear probe operating an imaging frequency of 9MHz and Doppler frequency of 4.2–5.0MHz. All recordings were assessed using an insonation angle $\leq 60^\circ$. The sample volume was maximized according to the width of the vessel, and kept clear of the vessel walls. A low-velocity filter (velocities $<1.8\text{ms}^{-1}$) rejected noises caused by turbulence at the vascular wall. Doppler tracings and B-mode images were recorded continuously and Doppler tracings were averaged over 8 heart cycles at the time of blood sampling. Arterial diameter was determined during the systole from arterial B-mode images under a perpendicular insonation angle at supine and seated rest, assuming negligible changes in vessel-diameter during exercise.

Intra-arterial (brachial artery) and venous pressures were monitored with transducers (Pressure Monitoring Kit, Baxter, IL) positioned at the level of the experimental leg. Leg mass was calculated from the whole-body dual energy x-ray absorptiometry scanning. Blood gasses, hemoglobin, and lactate concentrations were measured using ABL825 flex analyzer (Radiometer, Copenhagen Denmark). Leg vascular conductance (LVC) was calculated as leg blood flow (LBF)/(mean arterial pressure (MAP) – femoral venous pressure (FVP)). Functional sympatholysis was calculated as percentage change in LBF and LVC after 2 min of arterial tyramine infusion during exercise compared to exercise without tyramine infusion (1.5 min before tyramine infusion and 2 min after...
the stop of tyramine infusion). The hemodynamic measurements in the healthy individuals before the training have been published (18).

**Analysis of nitrite, nitrate, prostacyclin, endothelin-1 and norepinephrine**

Stable metabolites of NO, NO2- and NO3 – (NOx) were measured using colorimetric assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). The stable metabolite of prostacyclin (PGI$_2$) 6-keto prostaglandin F$_{1\alpha}$, was measured using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) following manufacturer’s instructions. Plasma endothelin-1 was measure using immunoassay (QuantiGlo, R&D systems, Minneapolis, MI) following manufacturer’s instructions. Plasma [NE] was determined with a radioimmunoassay (LDN, Nordhorn, Germany). Dialysate K$^+$ was measured by flame photometry (FLM3, Radiometer, Denmark) using lithium as internal standard. Dialysate lactate was measured with an enzymatic–spectrophotometric method using a Cobas Mira analyser (Roche, Branchburg, NJ).

**Statistical analysis**

One-way analysis of variance (ANOVA) was used to compare between-group baseline characteristics, and within groups for changes in interstitial variables and plasma [NA]. A two-way repeated measures analysis of variance (ANOVA) was performed to test significance within and between trials and before and after the training intervention within the two groups. A two-way ANOVA was performed to test significance between the two groups. Following a significant F test, all pair-wise differences were identified using Tukey’s honestly significant difference (HSD) post hoc procedure. A significant interaction indicates that the outcome variable is different within or between groups before and after the training intervention. All data are presented as means ± SEM. All analyses were conducted using SIGMAPLOT (13) and statistical significance was accepted.
when $P<0.05$. For technical reasons and/or catheter displacement during the post test, infusion and blood samples could not be obtained in two HF patients.
RESULTS

Baseline characteristics

Table 1 shows baseline characteristics for HF and the healthy individuals. There were no differences in age, sex or anthropometric measurements between groups. The ejection fraction was lower in the HF patients compared to the healthy individuals (P<0.05). We found no difference in resting leg blood flow, MAP, or LVC between groups.

Leg hemodynamic responses to SNP infusion

Arterial infusion of SNP increased LBF and LVC (P<0.05), whereas MAP decreased in both groups (P<0.05). There was no difference in LBF, LVC or MAP before and after the training intervention in both groups, and no difference between groups (Figure 1).

Leg hemodynamic responses to ACh infusion

In the healthy individuals, arterial infusion of ACh increased LBF and LVC (P<0.05), and lowered MAP (P<0.05) similarly before and after the training intervention (Figure 1). ACh increased LBF and LVC in the HF patients both before and after the training intervention (P<0.05), but the increase in LBF and LVC was higher after the training intervention (P<0.05). After the training intervention, MAP was lower during infusion of ACh in the HF patients (P<0.05). There was no difference between groups in LBF, LVC and MAP, before and after the training intervention (Figure 1).

Leg hemodynamic responses to tyramine, and ATP with and without co-infusion of tyramine

ATP: Both groups increased LBF and LVC in response to ATP (P<0.05; Figure 2), but in the HF patients the effect was blunted at the highest dose (ATP3). At the highest infusion rate, LBF tended
(P=0.06) to be higher before the training intervention and both LBF and LVC were higher (P<0.05) after the training intervention in the healthy individuals. Within the healthy individuals LBF tended (P=0.055) to be, and LVC (P<0.05) was higher after the training intervention during the highest ATP infusion (ATP3). **Tyramine:** In both groups, LBF and LVC decreased similarly during tyramine infusion (P<0.05) before and after the training intervention (Figure 3) and there was no difference between groups. **ATP+tyramine:** Both before and after the training intervention, LBF and LVC decreased in both groups (P<0.05) when tyramine was co-infused with ATP (Figure 3), whereas MAP increased in both groups (P<0.05). The decrease in LBF was greater before (P<0.05) and with a tendency (P=0.083) to be greater after the training intervention in the healthy individuals when compared to the HF patients. The decrease in LVC tended to be greater both before and after training (Pre: P=0.053; Post: P=0.096) in the healthy individuals when compared to the HF patients (Figure 3).

**Leg hemodynamic responses during exercise with and without infusion of tyramine**

LBF, LVC and MAP increased (P<0.05) in both groups during exercise (10 W) and there was no difference between groups. Before the training intervention, LBF and LVC was lowered in both groups when tyramine was infused during exercise (P<0.05) and increased after the termination of tyramine infusion (P<0.05; Figure 4). After the training intervention, LBF, LVC and MAP remained similar before, during, and after tyramine infusion.

**Leg (a-v) O\(_2\)** and leg VO\(_2\) were similar with and without tyramine in both groups before and after the training intervention, this despite a higher O\(_2\) extraction in the HF patients reflected by a lower femoral venous O\(_2\) saturation (P<0.05; Table 2). Before the training intervention, leg VO\(_2\) increased in both groups when tyramine infusion was terminated (P<0.05), but not after the training intervention. In the healthy individuals leg VO\(_2\) was lower after the training intervention when
tyramine infusion was terminated (P<0.05). Between groups, leg VO\textsubscript{2} was higher in the HF patients after the training intervention after tyramine infusion (P<0.05).

**Exercise capacity**

Six weeks of HIC increased absolute and relative VO\textsubscript{2peak} in both groups (P<0.05), with no difference between groups. Training increased cycling Watt\textsubscript{peak} only in the healthy individuals (P<0.05), whereas Watt\textsubscript{peak} during KEE only increased in the HF patients (P<0.05). There was no difference in Watt\textsubscript{peak} between groups during both cycling and KEE before and after the training intervention. 6 MWD was longer in the healthy individuals before the training intervention. Exercise training increased 6 MWD in the HF patients only (P<0.05), and there was no difference in 6 MWD after the training intervention between groups. Variables are listed in table 3.

**Plasma norepinephrine at rest and during exercise**

Before and after the training intervention resting plasma [NE] was not different between groups (Healthy: pre 0.7±0.2 mmol/l vs post 1.0±0.2 mmol/l; HF: pre 1.3±0.5 mmol/l vs post 1.1±0.5 mmol/l, respectively). In both groups, [NE] increased during tyramine infusion (P<0.05), and within the healthy group, [NE] was higher during tyramine infusion after the training (P<0.05) (Healthy: pre 1.3±0.2 mmol/l vs post 2.2±0.5 mmol/l; HF: 1.9±0.7 mmol/l vs post 3.0±1.2 mmol/l, respectively). Both before and after the training intervention, infusion of tyramine during exercise increased [NE] similarly in both groups (P<0.05; Figure 5).
Plasma endothelin-1

There was no difference in [ET-1] between the two groups before and after training (Healthy rest: pre 1.1±0.2 vs post 1.4±0.4; HF rest: pre 1.3±0.2 vs post 1.6±0.2). In the healthy individuals resting [ET-1] was higher (P<0.05) after training.

Plasma NOx at rest, during arterial ACh infusion, and during PLM and KEE

Plasma [NOx] was not different within or between groups at baseline and during ACh infusion both before and after the training intervention. Before and after the training intervention, plasma NOx was unaltered during PLM and KEE and there was no difference between groups.

Interstitial variables during rest and exercise

Before the training intervention, interstitial [lactate] levels increased with exercise in both groups (P<0.05), and in the HF group it tended (P=0.055) to increase during exercise after the training intervention (Table 4). In the HF patients, [lactate] was higher (P<0.05) before compared to after the training intervention. Before the training intervention, [PGI₂] tended to be lower at rest (P=0.08) and during exercise (P=0.07) in the HF patients compared to the healthy individuals. No difference in interstitial [potassium] was observed before and after the training intervention in either group or between groups.

Muscle interstitial NOx at rest, during arterial ACh infusion, and during PLM and KEE

Muscle interstitial [NOx] was not different within or between groups at baseline and during arterial infusion of ACh. In the healthy individuals, [NOx] increased during PLM only after the training intervention (P<0.05), and was higher compared to before the training intervention (P<0.05). In the
HF patients, [NOx] increased during PLM only before the training intervention (P<0.05). [NOx] did not increase during exercise when compared to baseline (Table 4).
DISCUSSION

We evaluated the effect of six weeks of high-intensity one-legged cycling (HIC) in patients with mild heart failure (HF) and healthy individuals. The major findings were that: a) HIC increased aerobic capacity in parallel with improved ability to override sympathetic vasoconstriction (arterial infusion of tyramine) during small muscle mass exercise in both groups; b) the peak vasodilatory responsiveness to arterial ATP infusion was lower in HF patients; c) HIC increased ACh induced vasodilation in the HF patients. These results suggest that exercise hyperemia and the ability for functional sympatholysis during exercise is maintained in HF and that functional sympatholysis is improved with high intensity training in both healthy individuals and HF patients. Moreover, the peak vasodilatory response to ATP appears to be reduced in patients with HF.

Blood flow and oxygen delivery

We found that the leg hemodynamic responses to KEE did not differ between the HF patients and healthy individuals when evaluated at the same workload, and that it was unaltered by exercise training. Despite a higher oxygen extraction, the metabolic demand was not different between groups. These findings agree with observations showing an unaltered blood flow response in HF patients during exercise with a small active muscle (2, 11, 31, 33). However, our findings are in contrast to observations during whole body exercise, where HF patients show an attenuated exercise hyperemia when compared to healthy individuals (31, 54, 55, 61). This discrepancy is most likely explained by the differences in the active muscle mass. The present results may therefore not reflect exercise engaging a large muscle mass with higher levels of sympathetic nerve activity (20, 31, 55). In agreement with the similar exercise hyperemia in the HF patients and healthy individuals, plasma and interstitial NOx, a stable metabolite of NO production, PGI_2, as well as plasma endothelin-1 were not different between the two groups. Surprisingly, endothelin-1 levels were increased after
the training intervention in the healthy group, which contrast with previous observations that have demonstrated similar or reduced levels after a period of exercise training (29, 43). Notably, exercise training has been found to reduce the vasoconstrictor effect of endothelin (9), thereby counteracting the effect of increased levels.

**α-adrenergic responsiveness and functional sympatholysis**

The tyramine induced increase in plasma [NE] and the reduction in LBF and LVC was similar in the two groups indicating that α-adrenergic vasoconstrictor responsiveness is unaltered in HF, and unaffected by training. In support of the present findings, forearm vascular responsiveness to phenylephrine, a selective α1-adrenoceptor agonist, was similar in HF patients and healthy individuals (19), and 5 weeks of training did not alter LVC or [NE] in young healthy individuals (37). However, the present data contrasts recent observations in our lab showing reduced postjunctional α-adrenergic vasoconstrictor responsiveness in norm- and hypertensive individuals after training (38). A possible explanation for the different observations may be related to a shorter training intervention and weakly training volume in present study, as altered responsiveness in Mortensen et al., 2014 (38), was observed after eight weeks of training (with three-four training sessions per week).

The reduction in LBF and LVC in both groups during exercise, with similar tyramine-induced increase in [NE], indicates that functional sympatholysis is unaltered in HF during exercise with an isolated muscle mass. However, the unaltered exercise hyperemia when tyramine was infused during exercise after training indicates that HIC improves the ability to override SNA (functional sympatholysis) in the leg during exercise in both healthy individuals and HF patients (Figure 6). In sedentary, functional sympatholysis is attenuated in the forearm (7) and in the leg (40, 42), but
improved in the trained state (37, 40). The present data supports and extends these observations by demonstrating that training improves functional sympatholysis in HF patients when exercising with a small muscle mass. In contrast to the literature (15, 31) we observed similar [NE] during resting conditions and during exercise in the two groups both before and after the training intervention. Whether improved functional sympatholysis during exercise with a local muscle mass can be extrapolated to exercise with large muscle mass remains unclear, as HF patients show an exaggerated sympathetic vasoconstriction when exercising with a large muscle mass (15). However, our observations suggest that impaired functional sympatholysis may be more related to physical state rather than age or diseased state. Therefore, the exercise intolerance often observed in HF patients may not be related to the failing heart.

**ATP response**

Arterial ATP infusion resulted in a dose dependent vasodilatory response in the healthy individuals, whereas LBF and LVC were blunted at the highest dose in the HF patients. The similar vasodilatory response as the healthy individuals at the two lowest doses of ATP indicate that downstream signaling pathway is preserved in HF. The failure to increase LVC at the highest dose therefore appears to be more related to the vasodilatory capacity of the vasculature than ATP signaling per se. ATP is a potent vasoactive substance and observations in young healthy individuals have shown peak hemodynamic responses similar to maximal exercise at infusion rates similar to the rates in present study (50). It remains unknown whether the lower ejection fraction in the HF patients is responsible for the lower peak hyperemic response or vice versa. However, in healthy individuals stroke volume is higher at peak vasodilation during ATP infusion when compared to peak vasodilation during exercise, leading the increase in cardiac output observed during ATP infusion
Training does not seem to alter the peak vasodilatory response to ATP in HF patients in contrast to healthy individuals who are able to increase their peak vasodilatory dose response. The improved vasodilatory response in the healthy individuals is in contrast to previous observations from our lab showing unaltered or even reduced ATP responsiveness with training (37, 40).

A surprising observation was that ATP tended to be more sympatholytic with HF compared to healthy individuals despite a diminished vasodilatory response to ATP. In addition, training, or at least HIC, seems not to alter the sympatholytic effect of ATP, despite of the improved peak vasodilatory response observed in the healthy group (Figure 3). The sympatholytic properties of ATP are likely caused by interfering of the post-junctional vascular adrenergic receptors and/or intracellular signaling pathways in the smooth muscle cells (57). A possibility is that the relative short withdrawal period of medication affected the higher sympatholytic properties in HF especially given that certain medications have been shown to improve the ability for functional sympatholysis in hypertensive individuals (48). It has previously been reported that the vasodilatory effect of ATP is maintained in both the forearm and in the leg in elderly healthy individuals conflicting with our observations, whereas our results agree that the sympatholytic properties of ATP is not related to training status (24, 40). Thus, other factors than ATP appear to be important in the ability to abolish SNA, and the sympatholytic properties of ATP seems not to be related to the physical state.

**Endothelial function**

HIC improved endothelial-dependent vasodilator response to arterial infusion of ACh in HF patients but not in healthy individuals. However there was no difference in the vasodilator response to ACh between groups before and after the training intervention. Exercise training has previously shown to improve endothelium-dependent vasodilation in HF patients with both local (14, 23) and systemic
effects (28), and also improved endothelium-dependent vasodilation is observed healthy individuals (5). The improved vasodilator response to ACh in the HF patients was not associated with increased [NOx] in the interstitial space or in plasma.

**Exercise capacity**

Six weeks of HIC improved VO$_2$peak by 9% and Watt$_{peak}$ by 13% in the healthy individuals and VO$_2$peak by 12% and 6 MWD by 8% in the HF patients. This indicates that HIC is beneficial for both healthy individuals and HF patients with mildly reduced ejection fraction. Before the training intervention, 6 MWD was lower in the HF patients when compared to the healthy individuals, whereas no difference was observed after training. HIC therefore not only increases cycling capacity, but also increase functionality which is of great clinical relevance. Our findings are in agreement with previous observations showing that single leg training improved maximal exercise capacity in HF patients and was well tolerated (8, 30, 58).

**Experimental considerations**

This study has led to some experimental considerations in regards to the observed vascular function and exercise hyperemia: a) the HF patients were pharmacological stable and recreational physical active, which might have influenced the level of exercise hyperemia and the response to ACh and; b) the medication could have lowered the vasoconstricting neurohormens and influenced the response to ACh in the HF patients. The patients were gradually withdrawn from their medication prior to the experimental day (ACE-inhibitors 48 hours; beta-blockers, anti-platelet and diuretic drugs 24 hours), and in regards to half-life of the medication (44) the ACE-inhibiting medication might have lowered the release of vasoconstricting neurohormens and improved the endothelial-dependent vasodilation (6, 41) as the NO bioavailability is increased with ACE-inhibitors (17). However, the gradually withdrawn from medication was similar before the experimental day before
and after training, indicating that training and not the medical treatment resulted in the improved endothelial function in the HF patients. The majority of the patients had only mildly reduced ejection fraction and low NYHA classification and thus do not represent patients with more severe HF where the neurohormonal activation is increased in parallel with severity of stage.

Conclusion

The ability for functional sympatholysis during KEE is closely coupled to the physical state, and does not appear to be altered in mild stage HF. ATP signaling appears to be preserved in HF, but the peak vasodilatory capacity of ATP is reduced.
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Author contributions

Conception and design (GWM and SPM), acquisition, analysis, or interpretation (GWM, UWI, JR, BKP, SPM), drafting the manuscript (GWM and SPM), revising the manuscript critically for important intellectual content (UWI, JR, BKP).

All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Table 1. Baseline characteristics before and after six weeks of training.

Values are means ± SEM. P-values at baseline characteristics refer to 1-way ANOVA between groups (Healthy n = 8; Heart failure n = 6). P-values within and between the 2 groups before and after training (Healthy n = 8; Heart failure n = 6) refer to 2-way repeated measures ANOVA.* P<0.05 different from before training within groups. #P<0.05 difference between groups. Blood pressures, femoral arterial blood flow and diameter were obtained after catheterization, but before the beginning of the experimental protocol.

<table>
<thead>
<tr>
<th></th>
<th>Healthy individuals</th>
<th>Heart failure patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (male/female)</td>
<td>5/1</td>
<td>7/1</td>
</tr>
<tr>
<td>NYHA class II</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Age</td>
<td>66±2</td>
<td>58±4</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>59±1#</td>
<td>45±5</td>
</tr>
<tr>
<td>Etiology (Isch/Non-isch)</td>
<td>0/0</td>
<td>7/1</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Diuretics</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Antiplatelet drugs</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Statins</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.2±0.8</td>
<td>3.5±0.8</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.54±0.3</td>
<td>1.25±0.3</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.19±0.7</td>
<td>1.91±0.9</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.32±0.8</td>
<td>1.39±0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Before training</th>
<th>After training</th>
<th>Before training</th>
<th>After training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>176±3</td>
<td>176±2</td>
<td>176±4</td>
<td>176±5</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>77±5</td>
<td>77±5</td>
<td>89±6</td>
<td>88±6</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>25±2</td>
<td>25±2</td>
<td>29±2</td>
<td>29±2</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>25±4</td>
<td>26±5</td>
<td>32±3</td>
<td>31±3</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>54±3</td>
<td>54±3</td>
<td>58±4</td>
<td>58±4</td>
</tr>
<tr>
<td>Heart rate (beats min⁻¹)</td>
<td>52±2</td>
<td>56±3</td>
<td>63±3</td>
<td>61±3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>141±7</td>
<td>140±5</td>
<td>138±5</td>
<td>141±4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>66±2</td>
<td>68±3</td>
<td>73±4</td>
<td>74±3</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>92±5</td>
<td>92±3</td>
<td>96±5</td>
<td>97±4</td>
</tr>
<tr>
<td>Experimental leg mass (kg)</td>
<td>11.7±0.7</td>
<td>11.7±0.8</td>
<td>12.4±0.7</td>
<td>12.3±1.0</td>
</tr>
<tr>
<td>Leg blood flow (ml/min/kg leg mass)</td>
<td>18±2</td>
<td>22±3</td>
<td>16±2</td>
<td>17±2</td>
</tr>
</tbody>
</table>
Table 2. Blood variables during rest and one-legged knee-extensor exercise with and without co-infusion of tyramine

a. femoral arterial. v. femoral venous. Values are means ± SEM. P-values refer to 2-way repeated measures ANOVA within and between the 2 groups (Healthy n = 6; Heart failure n = 6). * P<0.05 different from baseline #P<0.05 difference between groups.

<table>
<thead>
<tr>
<th>Blood variable</th>
<th>Before training</th>
<th>After training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Heart failure</td>
</tr>
<tr>
<td></td>
<td>individuals</td>
<td>patients</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>After training</td>
</tr>
<tr>
<td></td>
<td>10 W</td>
<td>10 W +</td>
</tr>
<tr>
<td></td>
<td>10 W +</td>
<td>tyramine</td>
</tr>
<tr>
<td></td>
<td>tyramine</td>
<td>10 W</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>88±5</td>
<td>94±4</td>
</tr>
<tr>
<td>v</td>
<td>35±4</td>
<td>26±2*</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>14.1±0.5</td>
<td>14.3±0.5</td>
</tr>
<tr>
<td>v</td>
<td>13.8±0.5</td>
<td>14.3±0.4</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>97±0</td>
<td>97±0</td>
</tr>
<tr>
<td>v</td>
<td>62±5</td>
<td>40±5*</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1.2±0.2</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>v</td>
<td>1.4±0.2</td>
<td>2.7±0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Before training</th>
<th>After training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>10 W</td>
</tr>
<tr>
<td></td>
<td>10 W + tyramine</td>
<td>10 W</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>86±4</td>
<td>91±5</td>
</tr>
<tr>
<td>v</td>
<td>30±3</td>
<td>21±1*#</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>14.1±0.5</td>
<td>14.2±0.5</td>
</tr>
<tr>
<td>v</td>
<td>13.5±0.5</td>
<td>14.2±0.4</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>97±0</td>
<td>97±0</td>
</tr>
<tr>
<td>v</td>
<td>51±6</td>
<td>27±2*#</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1.2±0.1</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>v</td>
<td>1.3±0.2</td>
<td>2.1±0.6</td>
</tr>
</tbody>
</table>
Table 3. Exercise capacity before and after the training intervention

Values are means ± SEM. P-values refer to 2-way repeated measures ANOVA within and between the 2 groups (Healthy n = 8; Heart failure n = 6). * P<0.05 different from before training within groups. #P<0.05 difference between groups.

<table>
<thead>
<tr>
<th>Blood variable</th>
<th>Healthy individuals</th>
<th>Heart failure patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before training</td>
<td>After training</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before training</td>
</tr>
<tr>
<td>VO_{peak} (l/min)</td>
<td>2.4±0.2</td>
<td>2.6±0.2*</td>
</tr>
<tr>
<td>VO_{peak} relative (ml/kg/min)</td>
<td>31±2</td>
<td>34±3*</td>
</tr>
<tr>
<td>Watt_{peak} 2-legged cycling</td>
<td>158±10</td>
<td>178±12*</td>
</tr>
<tr>
<td>Watt_{peak} 1-legged knee-extensor exercise</td>
<td>39±4</td>
<td>54±5</td>
</tr>
<tr>
<td>6 minute walk test</td>
<td>663±23#</td>
<td>677±32</td>
</tr>
<tr>
<td>Borg scale during training</td>
<td></td>
<td>15±1</td>
</tr>
</tbody>
</table>

Table 4. Interstitial variables from the vastus lateralis during rest and one-legged knee-extensor exercise

Values are means ± SEM. P-values refer to 1-way ANOVA within groups (Healthy n = 6; Heart failure n = 6). * P<0.05 different from rest. # P<0.05 different from before training

<table>
<thead>
<tr>
<th>Blood variable</th>
<th>Healthy individuals</th>
<th>Heart failure patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before training</td>
<td>After training</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before training</td>
</tr>
<tr>
<td>NOx (µmol/l)</td>
<td>18±3</td>
<td>27±4</td>
</tr>
<tr>
<td>6-keto PGF$_{1a}$</td>
<td>394±176</td>
<td>273±93</td>
</tr>
<tr>
<td>Potassium (mM)</td>
<td>23.2±6.9</td>
<td>24.9±15.1</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>1.1±0.2</td>
<td>2.1±0.2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Before training</td>
<td>After training</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before training</td>
</tr>
<tr>
<td>NOx (µmol/l)</td>
<td>13±3</td>
<td>19±5</td>
</tr>
<tr>
<td>6-keto PGF$_{1a}$</td>
<td>191±95</td>
<td>157±86</td>
</tr>
<tr>
<td>Potassium (mM)</td>
<td>12.3±3.2</td>
<td>16.1±8.2</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>0.7±0.1</td>
<td>2.1±0.4*</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Leg vascular conductance during arterial SNP and ACh infusion. P-values refer to 2-way repeated measures ANOVA within and between the 2 groups (Healthy n = 6; Heart failure n = 6). *P<0.05 different from baseline. $ P<0.05 different from before training.

Figure 2. Leg blood flow and leg vascular conductance during arterial ATP infusion. P-values refer to 2-way repeated measures ANOVA within and between the 2 groups (Healthy n = 6; Heart failure n = 6). *P<0.05 different between infusion rates. $ P<0.05 different from before training. # P<0.05 difference between groups.

Figure 3. Changes in leg blood flow and leg vascular conductance during arterial tyramine infusion and during arterial co-infusion of ATP and tyramine. P-values refer to 2-way repeated measures ANOVA within and between the 2 groups (Healthy n = 6; Heart failure n = 6). * P<0.05 different from baseline. $ P<0.05 different from ATP infusion. # P<0.05 difference between groups.

Figure 4. Leg hemodynamics during exercise with and without arterial infusion of tyramine. P-values refer to 2-way repeated measures ANOVA within and between the 2 groups (Healthy n = 6; Heart failure n = 6). * P<0.05 different from exercise without infusion of tyramine. $ P<0.05 different from before training.

Figure 5. Change in femoral venous norepinephrine during exercise with infusion of tyramine. P-values refer to 1-way ANOVA within groups (Healthy n = 6; Heart failure n = 6). *P<0.05 different from without infusion of tyramine.

Figure 6. Relative changes in leg blood flow and leg vascular conductance during tyramine infusion, tyramine and ATP infusion, and exercise and tyramine infusion. P-values refer to 2-way repeated measures ANOVA within and between the 2 groups (Healthy n = 6; Heart failure n = 6). $ P<0.05 different from before training. # P<0.05 difference between groups.
Reference list


Figure 1.
Figure 2.
Figure 3.
Figure 4.

Legend:
- Healthy pre
- Healthy post
- Heart failure pre
- Heart failure post

Leg blood flow (l/min)

Leg (a-v) O2 difference (ml/l)

Leg vascular conductance (ml/min/mmHg)

Leg oxygen uptake (ml/min)

10 Watt 10 Watt + tyramine infusion

10 Watt 10 Watt + tyramine infusion

Key:
- * Significantly different from Healthy pre
- ** Significantly different from Healthy post
- $ Significantly different from Heart failure pre
- $ Significantly different from Heart failure post
Figure 5:

Tyramine infusion during exercise

Tyramine induced change in femoral venous norepinephrine (mmol/l)

Healthy pre
Healthy post
Heart failure pre
Heart failure post

* * *
Figure 6:

Leg blood flow (% change)

Healthy pre
Healthy post
Heart failure pre
Heart failure post

Leg vascular conductance (% change)

Tyramine
ATP+ Tyramine
Exercise+ Tyramine

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