Benda, NMM, Seeger, JPH, van Lier, DPT, Bellersen, L, van Dijk, APJ, Hopman, MTE and Thijssen, DHJ

Heart failure patients demonstrate impaired changes in brachial artery blood flow and shear rate pattern during moderate-intensity cycle exercise

HEART FAILURE PATIENTS DEMONSTRATE IMPAIRED CHANGES IN BRACHIAL ARTERY BLOOD FLOW AND SHEAR RATE PATTERN DURING MODERATE-INTENSITY CYCLE EXERCISE

NATHALIE M.M. BENDA[1]
JOOST P.H. SEEGER[1,3]
DIRK P.T. VAN LIER[1]
LOUISE BELLERSEN[2]
ARIE P.J. VAN DIJK[2]
MARIA T.E. HOPMAN[1]
DICK H.J. THIJSSEN[1,3]

Radboud university medical center, Radboud Institute for Health Sciences, *Departments of* [1]*Physiology*, [2]*Cardiology*, Nijmegen, the Netherlands
Liverpool John Moores University, [3]*Research Institute for Sport and Exercise Sciences*, Liverpool, United Kingdom

Short title: Blood flow responses to endurance exercise in heart failure

KEY WORDS: Shear stress, vascular adaptation, endurance exercise
WORD COUNT: 3433
ABSTRACT WORD COUNT: 249
NUMBER OF REFERENCES: 47
FIGURES: 4
TABLES: 4

Author for correspondence:
Dr. Dick HJ Thijssen, Department of Physiology, Radboud university medical center, Radboud Institute for Health Sciences, Philips van Leydenlaan 15, 6525 EX, Nijmegen, the Netherlands. Email: dick.thijssen@radboudumc.nl, Tel: +31243614222

NEW FINDINGS

-We explored whether heart failure (HF) patients demonstrate different exercise-induced brachial artery shear rate patterns compared to controls.

-Moderate-intensity cycle exercise in HF patients is associated with an attenuated increase in brachial artery antegrade and mean shear rate as well as skin temperature compared to controls.

-Differences between HF patients and controls cannot be fully explained by differences in workload.

-HF patients therefore demonstrate a less favourable shear rate pattern during cycle exercise compared to controls.

-The exact consequences for vascular adaptation to exercise training should be further explored.

ABSTRACT

Introduction. Repeated elevations in shear rate (SR) in conduit arteries, such as present during exercise, represent a key stimulus to improve vascular function. We examined whether heart failure (HF) patients demonstrate distinct changes in SR in response to moderate-intensity cycle exercise compared to healthy controls.

Methods. We examined brachial artery SR during 40 minutes of cycle exercise at a work rate equivalent to 65% peak oxygen uptake in 14 HF patients (65±7 yrs, 13:1 male:female) and 14 controls (61±5 yrs, 12:2 male:female). Brachial artery diameter, SR and oscillatory shear index (OSI) were assessed using ultrasound at baseline and during exercise.

Results. HF patients demonstrated an attenuated increase in mean and antegrade brachial artery SR during exercise compared to controls ('time*group'-interaction: P=0.003 and P<0.001, respectively). Retrograde SR increased at the onset of exercise and remained increased throughout the exercise period in both groups ('time*group'-interaction: P=0.11). In controls, the immediate increase in OSI during exercise ('time': P<0.001) is normalized after 35 minutes of cycling. In contrast, the increase in OSI after the onset of exercise did not normalize in HF ('time*group'-interaction: P=0.029). Subgroup analysis of 5 HF patients and 5 controls with comparable workload (97±13 *versus* 90±22 Watt, P=0.59) confirmed the presence of distinct changes in mean SR during exercise ('time*group'-interaction: P=0.030). Between-group differences in antegrade/retrograde SR or OSI did not reach statistical significance ('time*group'-interactions: P>0.05).

Conclusion. HF patients demonstrate a less favourable SR pattern during cycle exercise than controls, characterized by an attenuated mean and antegrade SR, and increased OSI.

INTRODUCTION

Patients with heart failure (HF) are characterized by reduced myocardial function and impaired peripheral vascular function (Drexler *et al.*, 1993; Drexler, 1995; Brubaker, 1997). Exercise training has potent effects on symptoms and prognosis of HF (Hambrecht *et al.*, 1998; Maiorana *et al.*, 2000a; Maiorana *et al.*, 2000b; Wisloff *et al.*, 2007), which are, at least partly, mediated through direct improvement of peripheral vascular function and structure (Green *et al.*, 2008). Previous studies demonstrated that (repeated) elevations in shear rate (SR) represent a key stimulus for these beneficial vascular adaptations (Tinken *et al.*, 2009; Tinken *et al.*, 2010). Recent studies in healthy humans have confirmed that repeated elevations in shear contribute to vascular adaptation in active (Tinken *et al.*, 2010) and non-active (Birk *et al.*, 2012) vascular beds. Under resting conditions, SR pattern in peripheral vessels, such as the brachial artery, varies across the cardiac cycle, with a large antegrade component during systole being followed by a retrograde component in early diastole (Blackshear *et al.*, 1979). Previous studies have related repeated exposure to elevations in antegrade SR to improvement in vascular function, whilst elevations in the retrograde component exert a proatherogenic effect on the endothelium (Laughlin *et al.*, 2008; Thijssen *et al.*, 2009b).

Immediately after the onset of lower limb cycle exercise, an increased retrograde flow or SR is present in the brachial artery (Green *et al.*, 2002; Thijssen *et al.*, 2009a), probably due to an increase in vascular resistance in the inactive upper limbs that is mediated through sympathetic vasoconstriction (Padilla *et al.*, 2010). This SR pattern at the onset of exercise alters when exercise continues, represented by an attenuation of the retrograde SR and simultaneous increase in mean and antegrade SR (Simmons *et al.*, 2011). Simmons and colleagues demonstrated that normalization of retrograde SR is partly related to

thermoregulatory responses (Simmons *et al.*, 2011). More specifically, exercise caused an increase in core body and skin temperatures as well as a decrease in peripheral vascular tone, which subsequently resulted in a normalization of the retrograde SR during prolonged exercise in healthy volunteers (Simmons *et al.*, 2011).

HF patients are known to have peripheral vascular abnormalities (Packer, 1988; Drexler *et al.*, 1993; Drexler, 1995; Poelzl *et al.*, 2005) and/or altered thermoregulatory responses to exercise (Griffin *et al.*, 1993; Cui *et al.*, 2005; Green *et al.*, 2006). More specifically, HF patients have a diminished endothelial function (Drexler *et al.*, 1993) and demonstrate an attenuated forearm blood flow response to handgrip exercise (Takeshita *et al.*, 1996). Moreover, (short-term) exercise causes a decrease in core body and skin temperature in HF patients (Shellock *et al.*, 1983; Griffin *et al.*, 1993). These abnormalities could affect SR pattern during exercise in HF patients. To our knowledge, no previous study examined SR patterns during exercise in HF patients. Therefore, the primary purpose of our study was to compare the changes in brachial artery SR pattern during lower limb exercise between HF patients and healthy controls. We hypothesize that HF patients have different brachial artery SR responses to lower limb cycle exercise compared to controls. Specifically, we expect HF patients to have 1) an attenuated exercise-mediated increase in mean and antegrade SR and 2) prolonged retrograde SR compared to healthy controls.

METHODS

Ethical approval

This study was approved by the Medical Ethical Committee of the Radboud university medical center (CMO Regio Arnhem-Nijmegen) and complies with the Declaration of

Helsinki. Written informed consent was obtained from each participant before inclusion in this study.

Participants

Fourteen patients (65±7 yrs, 13:1 male:female) with HF New York Heart Association class I-III and a left ventricular ejection fraction lower than 45% were recruited from the Departments of Cardiology of the Radboud university medical center and Canisius Wilhelmina Hospital (Nijmegen, The Netherlands) (Table 1). Furthermore, we recruited 14 healthy controls (61±5 yrs, 12:2 male:female) (Table 1). All patients were in a pharmacologically and clinically stable situation for at least one month. One patient increased the dosage of fosinopril one week prior to the measurements. Control participants were free of overt cardiovascular diseases and did not use medication affecting the cardiovascular system. None of the participants were diagnosed with diabetes mellitus.

Experimental protocol

Participants reported to the laboratory twice and were instructed to continue their medication (e.g. β-blockers), with the exception of diuretics for practical reasons, prior to all measurements. On day 1, a medical screening was performed after which participants underwent a maximal incremental cycling test to determine physical fitness. Prior to day 2, participants refrained from consuming coffee, tea, chocolate, vitamin C and alcohol for 18 hours prior to testing. Participants were instructed to avoid any strenuous physical activity within the 24 hours before testing, and to consume a light meal at least two hours before testing. The measurements were performed in a temperature-controlled room (21.9 ± 0.8 ºC). After instrumentation, participants rested in the supine position for 10 minutes, followed by measurement of blood pressure. Subsequently, the participants were positioned on the cycle

ergometer for a 30-minute moderate-intensity exercise, preceded by a 10-minute warm-up. We continuously measured brachial artery diameter and SR pattern using ultrasound and forearm skin temperature using skin thermistors.

Day 1: Maximal incremental cycling test

The incremental maximal cycling test was performed on a cycle ergometer (Lode, Excalibur v1.52, 1991, Groningen, the Netherlands/Ergoline, Ergoselect 200k, Bitz, Germany). After a 2-minute baseline measurement, participants started cycling and workload was increased by 10-25 Watt per minute, depending on the sex, age and height of the healthy participants (Jones *et al.*, 1985) and the estimated physical fitness of the HF patients. Participants were instructed to pedal at a frequency of ≥60 rpm until volitional fatigue. All participants reached volitional fatigue during this test, whilst none of the tests were symptom-limited. During exercise we continuously measured oxygen uptake (breath-by-breath, CPET Cosmed v9.1b, Rome, Italy/LabManager V5.32.0) to determine peak oxygen uptake (VO_{2peak}), which was defined as the average oxygen uptake during the last 30 seconds of the exercise test.

Day 2: Moderate-intensity lower limb cycle exercise

Work rate was matched such that all participants exercised at the same relative intensity. We used heart rate to match work rate between participants, by aiming for a heart rate that corresponded to a certain percentage of the VO_{2peak} (derived from the maximal incremental cycling test). Given the marked differences in fitness between HF and controls, matching at absolute workload would result in extremely low levels of workload for controls (which are not realistic for real-life situation). A 10-minute warm-up at a work rate equivalent to 40% VO_{2peak} was performed, followed by a 30-minute moderate-intensity exercise at a work rate equivalent to 65% VO_{2peak}. To verify intensity during exercise, heart rate was registered

continuously using a heart rate monitor (Polar Electro Oy, Kempele, Finland). Due to practical and technical difficulties, we were not able to continuously measure blood pressure during exercise in our participants. At the end of the warm-up and at 10-minute intervals, the Borg score (6-20 scale) for perceived exertion was obtained (Borg et al., 1987). Participants were allowed to pedal at their preferred rotation frequency, but at least at ≥50 rotations per minute.

Brachial artery shear rate pattern

To measure brachial artery SR pattern the right arm was extended to the side, supported by a memory foam cushion, at an angle of ≈80° from the torso. The right brachial artery was imaged in the distal third of the upper arm by a 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Terason T3000, Aloka, UK) by a well-trained sonographer (NMMB). Ultrasound parameters were optimized to obtain B-mode images from artery lumen and wall. Doppler velocity was measured simultaneously with an insonation angle of <60°. A 2-minute baseline recording was acquired preceding the exercise protocol. During the exercise bout, 1-minute ultrasound recordings were made every 5 minutes. The acquired images were recorded and stored as a digital AVI file for later analysis.

Forearm skin temperature

Previous work has related thermoregulatory changes, or more specifically skin perfusion, to changes in the upstream conduit arteries (Simmons *et al.*, 2011). Unfortunately, technology to assess skin perfusion such as laser-Doppler was not available at the time of testing. In order to acquire information on thermoregulatory changes, we measured forearm skin temperature. Although skin temperature and skin perfusion during exercise seem to follow a similar pattern (Simmons *et al.*, 2011; Demachi *et al.*, 2013), relatively little is known about the relation

between skin temperature and perfusion during exercise and factors influencing this relationship (Taylor *et al.*, 2014). Forearm skin temperature was measured every 30 seconds using iButtons (Thermochron iButton DS1291H, Dallas Maxim). The skin thermistors were attached to the skin using medical tape at the right lower arm, wrist and hand (dorsal side). Forearm skin temperature was calculated as the average skin temperature of these three locations. Baseline values were determined from the average over the 5 minutes preceding exercise. Skin temperature data was analyzed using Matlab (Matlab R2008a, MathWorks, Natick, MA) and for each time-point averaged over the preceding 5 minutes.

Data analysis

Brachial artery diameter and flow velocity images were analyzed using custom-designed edge-detecting and wall-tracking software, which ensures accurate and reproducible analysis (Woodman *et al.*, 2001). This process is described in previous studies (Black *et al.*, 2008; Thijssen *et al.*, 2009a; Thijssen *et al.*, 2009b). In short, the software analysis is based on an icon-based graphical programming language. A pixel-density algorithm automatically identifies the near and far wall of the artery to trace the artery diameter, whilst another algorithm traces the red blood cell velocity signal. Average values of the diameter are calculated, stored and synchronized with blood velocity data to obtain blood flow, SR and oscillatory shear index.

Blood flow was calculated as the product of cross-sectional area of the brachial artery (cm^2) and Doppler mean blood flow velocity (cm/s). SR was defined as 4 X V_m/D, where V_m is Doppler mean blood flow velocity (cm/s) and D is arterial diameter (cm). Retrograde SR is defined as negative SR, in which an increase in retrograde SR entails more negative shear. Oscillatory shear index was determined by |retrograde SR|/(|retrograde SR|+antegrade SR) (Padilla *et al.*, 2010; Simmons *et al.*, 2011). The oscillatory shear index can range from 0 to

0.5, in which 0 indicates unidirectional SR and 0.5 represents maximal shear oscillations (Simmons *et al.*, 2011).

Statistical analysis

Based on pilot work in our laboratory, we calculated that we need 14 participants to detect a difference of 0.13 in oscillatory shear index and estimated SD of this difference of 0.116 (power of 80%, alpha of 0.05) (GPower 3.0.10, Düsselfdorf, Germany). Differences in baseline characteristics between HF patients and controls were compared using independent Student's *t* tests. The sex distribution between HF patients and controls was compared with a Chi-square test. A 2-way repeated measures ANOVA was used to examine whether exercise-induced changes in mean, antegrade and retrograde SR, oscillatory shear index and skin temperature ('time'; within-subject factor) differ between HF patients and healthy controls ('group'; between-subject factor). When a significant main or interaction effect was observed, post-hoc tests with Least Square Difference were used to identify differences between groups (at the various time points) and within groups (when compared to baseline). Due to the large difference in absolute workload between HF patients and controls, we included a subgroup analysis with comparable absolute workload. For this purpose, we included 5 HF patients with the highest and 5 controls with the lowest absolute workload in this explorative, statistically underpowered subgroup analysis. Data are presented as mean ± SD unless stated otherwise. Significance level was set at $P \leq 0.05$.

RESULTS

Subject characteristics

Compared to controls, HF patients demonstrated a higher BMI and lower VO_{2peak}, whilst no significant differences between HF patients and controls were found for age, sex, body weight and blood pressure (systolic and diastolic) (Table 1). Cardiovascular medication use by HF patients is presented in Table 2. Both groups performed exercise at comparable intensity when presented as relative workload (%max), and rate of perceived exertion (Borg score) (Table 3).

Brachial artery SR and blood flow pattern

Brachial artery diameter was not different between groups at baseline and did not change significantly across the exercise bout in both groups (Table 4, 'time*group'-interaction *P*=0.18). Baseline brachial artery SR and blood flow were not different between groups (Figure 1, Table 4). Mean SR (Figure 1A) and blood flow (Table 4) initially decreased in both groups at the onset of exercise (warm-up), followed by a gradual increase when exercise continued (both 'time'-effect: P<0.001). Interestingly, HF patients demonstrated a significantly smaller increase in mean SR (Figure 1A) and blood flow (Table 4) compared to controls (both 'time*group'-interaction: P<0.05). Brachial artery antegrade SR (Figure 1B) and blood flow (Table 4) increased across the lower limb cycle exercise bout in both groups, whilst this increase was significantly lower in HF patients compared to controls (both 'time*group'-interaction: P<0.001). Retrograde SR (Figure 1C) and blood flow (Table 4) increased at the onset of exercise in both groups, and remained increased throughout the exercise period in both groups (both 'time'-effect: P<0.001). To correct for individual differences in antegrade and retrograde SR, we also presented oscillatory shear index. After the onset of exercise, oscillatory shear index increased immediately in both groups (Figure 1D). In controls, oscillatory shear index returned to baseline values after 35 minutes of exercise, whilst oscillatory shear index remained elevated in HF patients across the exercise bout (Figure 1D, 'time*group'-interaction: P=0.029).

Forearm skin temperature

Skin temperature of the arm decreased initially in both groups (Figure 2, 'time'-effect: P<0.001). Lower limb cycle exercise induced a significant increase in skin temperature in controls after 40 minutes, whilst in HF patients skin temperature did not increase above baseline values (Figure 2, 'time*group'-interaction: P=0.002).

Subgroup analysis (comparable absolute workload)

Subject characteristics. In the subgroup analysis, we included 5 HF patients (64±7 yrs, 5 males, peak oxygen uptake 22.5±3.4) and 5 controls (64±7 yrs, 3:2 male:female, peak oxygen uptake 28.4±8.2). Medication use in the HF patient group was: β-blockers (100%), statins (100%), diuretics (60%), angiotensine converting enzyme-inhibitors (60%), aldosterone antagonists (40%), coumarin derivatives (60%), antiplatelet drugs (40%), and angiotensine II antagonists (60%). The exercise bout was performed at comparable absolute workload; 97±13 Watt and 90±22 Watt in HF patients and controls respectively (P=0.59). HF patients and controls performed exercise at comparable intensity when presented as relative heart rate (77±9% *versus* 78±9%, P=0.79) and rate of perceived exertion (14±2 *versus* 14±2, P=0.89).

Brachial artery SR and blood flow. Subgroup analysis at comparable absolute workloads revealed that brachial artery diameter and SR were not different between groups at baseline (all P>0.05, data not presented). A significant main effect of 'time' (all P<0.001) was observed for mean, antegrade and retrograde SR and oscillatory index in the subgroup analysis. A significant 'time*group'-interaction effect was found for mean SR (P=0.030), with post-hoc analysis revealing a smaller exercise-induced increase in mean SR in HF patients compared to controls. Such differences between groups did not reach statistical

significance for antegrade SR, retrograde SR or oscillatory index (Figure 3). Similar to the SR data, a significant main effect for 'time' and 'time*group'-interaction was observed for mean blood flow, but not for antegrade and retrograde blood flow (data not shown). No changes in brachial artery diameter were observed for both groups (data not shown).

Forearm skin temperature. A significant 'time'-effect (P=0.029) was found. A trend for an increase in skin temperature in controls, but not in HF, can be observed ('time*group'-interaction; P=0.09, Figure 4).

DISCUSSION

This study investigated the impact of HF on brachial artery SR and blood flow pattern during lower limb cycle exercise. We have demonstrated that healthy controls as well as HF patients demonstrate a marked increase in oscillatory shear index after the onset of moderate-intensity cycle exercise, which is largely explained by an increase in retrograde SR. Secondly, when exercise continues, oscillatory shear index normalizes in controls, which coincides with a further increase in mean and antegrade SR and increase in forearm skin temperature. In contrast, HF patients demonstrate no normalization of oscillatory shear index, an attenuated increase in mean and antegrade SR and no increase in forearm skin temperature when exercise continues. When analyzing subgroups in which participants performed exercise at comparable *absolute* workloads, although underpowered, the presence of distinct blood flow and shear rate responses between HF patients and controls seems to be confirmed. Therefore, the difference in SR is unlikely to be fully explained by the differences in absolute workload. Taken together, our findings suggest that HF patients show a potentially less favorable SR pattern during exercise than controls.

The primary purpose of our study was to compare the changes in SR during exercise between healthy controls and HF patients. First, we found an attenuated increase in brachial artery mean and antegrade SR during cycle exercise in HF patients compared to controls. A second finding is that, after the characteristic initial increase in retrograde SR and oscillatory shear index during cycle exercise, HF patients demonstrate no change in retrograde SR or oscillatory shear index. The distinct SR responses to exercise in HF patients may relate to the lower absolute workload and/or heart rate in the HF patient group, as a higher workload and heart rate is typically associated with a larger increase in cardiac output (Beck *et al.*, 2006; Fukuda *et al.*, 2012). Due to the large difference in absolute workload between HF patients and controls, we have provided a subgroup analysis in which absolute workload was comparable between groups. This analysis confirmed the presence of an attenuated increase in mean SR during exercise in HF patients compared to controls. Although statistically underpowered, the P-value for between-group differences in retrograde SR approached significance and mean data for antegrade/retrograde SR and oscillatory shear index was comparable to the original analysis. Therefore, difference in the exercise-induced changes in SR and blood flow between groups is unlikely fully explained by the difference in absolute workload.

Various factors may contribute to the distinct SR pattern during exercise between HF and controls. First, SR pattern is directly influenced by peripheral vascular resistance (Baccelli *et al.*, 1985; Thijssen *et al.*, 2014). The inability to attenuate retrograde SR, and hereby oscillatory shear index, in HF patients may relate to an elevated peripheral resistance during exercise. Indeed, an enhanced forearm vascular resistance in HF patients was found previously during cycle exercise (Chiba *et al.*, 2007), supporting this suggestion. One

potential explanation for the enhanced peripheral resistance is an elevated sympathetic nerve system activity in HF patients (Packer, 1988; Triposkiadis *et al.*, 2009) that may remain present during exercise (Chidsey *et al.*, 1962). Unfortunately, due to technical and practical difficulty, we were unable to provide insight into the exercise-induced changes in blood pressure (and therefore peripheral arterial resistance). Alternatively, the distinct SR patterns during cycle exercise may relate to different thermoregulatory changes during exercise as skin cooling is demonstrated to increase the degree of retrograde SR during cycling (Simmons *et al.*, 2011). The absent increase in skin temperature during cycle exercise in HF patients in our study may contribute to the inability to normalize retrograde SR and oscillatory shear index. Lower metabolic heat production may contribute to our observations when comparing all participants (Jay *et al.*, 2011). However, analysis of subgroups with comparable workload suggests that HF patients still demonstrate an absent increase in skin temperature during cycle exercise.

During the initial phase of exercise an increase in antegrade and retrograde SR and oscillatory shear index have been described (Green *et al.*, 2002; Thijssen *et al.*, 2009a). Recently, Simmons *et al.*, found a normalization of retrograde SR and an increase in forearm skin temperature when exercise continues (Simmons *et al.*, 2011). Interestingly, these findings in young subjects contrast with our observations in older humans, as we found that retrograde SR was not normalized during cycle exercise and that forearm skin temperature only demonstrated a late increase in healthy older controls. Although we did not intend to directly compare young and older subjects, these data suggest that advanced age is associated with delayed normalization of brachial artery SR pattern during exercise. Future studies are required to better understand the impact of advanced age on SR pattern during exercise.

Clinical Relevance. Although previous studies investigating the effects of endurance exercise training in HF patients found improvement in brachial artery vascular function after training (Belardinelli *et al.*, 2005; Wisloff *et al.*, 2007), the prolonged exposure to a less favorable shear pattern as observed in our study, may prevent HF patients to optimally benefit from exercise training. Although the differences in shear pattern may be less pronounced when comparing groups who exercised at comparable absolute levels, it should be acknowledged that exercise prescription (especially in rehabilitation settings) is based on relative exercise intensity levels. Therefore, this study adopted an exercise intensity level and duration that is typically applied in rehabilitation settings in HF patients. Importantly, HF patients were on optimal pharmacological treatment, which improves extrapolation of our findings to daily life situations. Whether different types or forms of exercise that are associated with a larger antegrade SR and/or smaller retrograde SR lead to larger improvements in vascular function is currently speculative, and should be subject for future research.

Limitations. A potential limitation is the difference in BMI between HF and controls, since BMI may affect skin temperature responses during exercise. However, with some studies indicating that fat mass does not influence skin temperature responses to exercise (Limbaugh *et al.*, 2013; Adams *et al.*, 2014) and others reporting impaired skin temperature and blood flow responses to exercise (Vroman *et al.*, 1983; Havenith *et al.*, 1995), the effect of fat mass on skin temperature during exercise is unclear. Given the modest differences in BMI between our groups, and the conflicting results from previous work, we believe that difference in BMI between groups unlikely explains our observations. Another potential limitation was the use of β-blockers by HF patients as this induces a lower resting and peak heart rate (Witte *et al.*, 2006). However, since hemodynamic responses to exercise are significantly improved in HF patients by β-blocker therapy (Andersson *et al.*, 1994), withdrawing β-blockers is expected to

392 enlarge the differences in thermoregulatory responses to exercise between HF patients and
393 controls. Moreover, we intended to study thermoregulatory responses during real life
394 situations for HF patients and therefore continued medication. Finally, other limitations of our
395 study are the underpowered subgroup analysis and that we were not able to measure skin
396 perfusion during exercise.
397
398 In conclusion, we found that, when exercise is matched at relative intensity (65% of VO_{2peak}),
399 HF patients demonstrate prolonged exposure to a less favourable brachial artery SR pattern
400 during lower limb cycle exercise. More specifically, HF patients demonstrate an attenuated
401 increase in mean and antegrade SR during exercise, but also a prolonged increase in
402 oscillatory shear index. The latter observation coincides with an absent increase in forearm
403 skin temperature across the exercise bout. These distinct blood flow and SR patterns between
404 groups are unlikely to be fully explained by differences in absolute workload, but possibly
405 relate to between-group differences in vascular regulation. Therefore, our data suggest that,
406 when HF patients perform exercise at a level that is commonly adopted in rehabilitation
407 settings, HF patients are exposed to a less favorable shear pattern compared to controls. The
408 exact consequences for (vascular) adaptation to rehabilitation should be further explored.
409
410

References

Adams JD, Ganio MS, Burchfield JM, Matthews AC, Werner RN, Chokbengboun AJ, Dougherty EK & LaChance AA (2014). Effects of obesity on body temperature in otherwise-healthy females when controlling hydration and heat production during exercise in the heat. *Eur J Appl Physiol*.

Andersson B, Hamm C, Persson S, Wikstrom G, Sinagra G, Hjalmarson A & Waagstein F (1994). Improved exercise hemodynamic status in dilated cardiomyopathy after beta-adrenergic blockade treatment. *J Am Coll Cardiol* **23,** 1397-1404.

Baccelli G, Pignoli P, Corbellini E, Pizzolati PL, Bassini M, Longo T & Zanchetti A (1985). Hemodynamic factors changing blood flow velocity waveform and profile in normal human brachial artery. *Angiology* **36,** 1-8.

Beck KC, Randolph LN, Bailey KR, Wood CM, Snyder EM & Johnson BD (2006). Relationship between cardiac output and oxygen consumption during upright cycle exercise in healthy humans. *J Appl Physiol (1985)* **101,** 1474-1480.

Belardinelli R, Lacalaprice F, Faccenda E, Purcaro A & Perna G (2005). Effects of short-term moderate exercise training on sexual function in male patients with chronic stable heart failure. *Int J Cardiol* **101,** 83-90.

Birk GK, Dawson EA, Atkinson C, Haynes A, Cable NT, Thijssen DH & Green DJ (2012). Brachial artery adaptation to lower limb exercise training: role of shear stress. *J Appl Physiol* **112,** 1653-1658.

Black MA, Cable NT, Thijssen DH & Green DJ (2008). Importance of measuring the time course of flow-mediated dilatation in humans. *Hypertension* **51,** 203-210.

Blackshear WM, Jr., Phillips DJ & Strandness DE, Jr. (1979). Pulsed Doppler assessment of normal human femoral artery velocity patterns. *J Surg Res* **27,** 73-83.

Borg G, Hassmen P & Lagerstrom M (1987). Perceived exertion related to heart rate and blood lactate during arm and leg exercise. *Eur J Appl Physiol Occup Physiol* **56,** 679-685.

Brubaker PH (1997). Exercise intolerance in congestive heart failure: a lesson in exercise physiology. *J Cardiopulm Rehabil* **17,** 217-221.

Chiba Y, Maehara K, Yaoita H, Yoshihisa A, Izumida J & Maruyama Y (2007). Vasoconstrictive response in the vascular beds of the non-exercising forearm during leg exercise in patients with mild chronic heart failure. *Circ J* **71,** 922-928.

Chidsey CA, Harrison DC & Braunwald E (1962). Augmentation of the plasma nor-epinephrine response to exercise in patients with congestive heart failure. *N Engl J Med* **267,** 650-654.

Cui J, Arbab-Zadeh A, Prasad A, Durand S, Levine BD & Crandall CG (2005). Effects of heat stress on thermoregulatory responses in congestive heart failure patients. *Circulation* **112,** 2286-2292.

Demachi K, Yoshida T, Kume M, Tsuji M & Tsuneoka H (2013). The influence of internal and skin temperatures on active cutaneous vasodilation under different levels of exercise and ambient temperatures in humans. *Int J Biometeorol* **57,** 589-596.

Drexler H (1995). Changes in the peripheral circulation in heart failure. *Curr Opin Cardiol* **10,** 268-273.

Drexler H, Hayoz D, Munzel T, Just H, Zelis R & Brunner HR (1993). Endothelial function in congestive heart failure. *Am Heart J* **126,** 761-764.

Fukuda T, Matsumoto A, Kurano M, Takano H, Iida H, Morita T, Yamashita H, Hirata Y, Nagai R & Nakajima T (2012). Cardiac output response to exercise in chronic cardiac failure patients. *Int Heart J* **53,** 293-298.

Green D, Cheetham C, Reed C, Dembo L & O'Driscoll G (2002). Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *J Appl Physiol (1985)* **93,** 361-368.

Green DJ, Maiorana AJ, Siong JH, Burke V, Erickson M, Minson CT, Bilsborough W & O'Driscoll G (2006). Impaired skin blood flow response to environmental heating in chronic heart failure. *Eur Heart J* **27,** 338-343.

Green DJ, O'Driscoll G, Joyner MJ & Cable NT (2008). Exercise and cardiovascular risk reduction: time to update the rationale for exercise? *J Appl Physiol* **105,** 766-768.

Griffin MJ, O'Sullivan JJ, Scott A & Maurer BJ (1993). Core and peripheral temperature response to exercise in patients with impaired left ventricular function. *Br Heart J* **69,** 388-390.

Hambrecht R, Fiehn E, Weigl C, Gielen S, Hamann C, Kaiser R, Yu J, Adams V, Niebauer J & Schuler G (1998). Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation* **98,** 2709-2715.

Havenith G, Luttikholt VG & Vrijkotte TG (1995). The relative influence of body characteristics on humid heat stress response. *Eur J Appl Physiol Occup Physiol* **70,** 270-279.

Jay O, Bain AR, Deren TM, Sacheli M & Cramer MN (2011). Large differences in peak oxygen uptake do not independently alter changes in core temperature and sweating during exercise. *Am J Physiol Regul Integr Comp Physiol* **301,** R832-841.

Jones NL, Makrides L, Hitchcock C, Chypchar T & McCartney N (1985). Normal standards for an incremental progressive cycle ergometer test. *Am Rev Respir Dis* **131,** 700-708.

Laughlin MH, Newcomer SC & Bender SB (2008). Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J Appl Physiol* **104,** 588-600.

Limbaugh JD, Wimer GS, Long LH & Baird WH (2013). Body fatness, body core temperature, and heat loss during moderate-intensity exercise. *Aviat Space Environ Med* **84,** 1153-1158.

Maiorana A, O'Driscoll G, Cheetham C, Collis J, Goodman C, Rankin S, Taylor R & Green D (2000a). Combined aerobic and resistance exercise training improves functional capacity and strength in CHF. *J Appl Physiol* **88,** 1565-1570.

Maiorana A, O'Driscoll G, Dembo L, Cheetham C, Goodman C, Taylor R & Green D (2000b). Effect of aerobic and resistance exercise training on vascular function in heart failure. *Am J Physiol Heart Circ Physiol* **279,** H1999-2005.

Packer M (1988). Neurohormonal interactions and adaptations in congestive heart failure. *Circulation* **77,** 721-730.

Padilla J, Young CN, Simmons GH, Deo SH, Newcomer SC, Sullivan JP, Laughlin MH & Fadel PJ (2010). Increased muscle sympathetic nerve activity acutely alters conduit artery shear rate patterns. *Am J Physiol Heart Circ Physiol* **298,** H1128-1135.

Poelzl G, Frick M, Huegel H, Lackner B, Alber HF, Mair J, Herold M, Schwarzacher S, Pachinger O & Weidinger F (2005). Chronic heart failure is associated with vascular remodeling of the brachial artery. *Eur J Heart Fail* **7,** 43-48.

Shellock FG, Rubin SA, Ellrodt AG, Muchlinski A, Brown H & Swan HJ (1983). Unusual core temperature decrease in exercising heart-failure patients. *J Appl Physiol Respir Environ Exerc Physiol* **54,** 544-550.

Simmons GH, Padilla J, Young CN, Wong BJ, Lang JA, Davis MJ, Laughlin MH & Fadel PJ (2011). Increased brachial artery retrograde shear rate at exercise onset is abolished during prolonged cycling: role of thermoregulatory vasodilation. *J Appl Physiol (1985)* **110,** 389-397.

Takeshita A, Hirooka Y & Imaizumi T (1996). Role of endothelium in control of forearm blood flow in patients with heart failure. *J Card Fail* **2,** S209-215.

Taylor NA, Tipton MJ & Kenny GP (2014). Considerations for the measurement of core, skin and mean body temperatures. *J Therm Biol* **46C,** 72-101.

Thijssen DH, Atkinson CL, Ono K, Sprung VS, Spence AL, Pugh CJ & Green DJ (2014). SYMPATHETIC NERVOUS SYSTEM ACTIVATION, ARTERIAL SHEAR RATE AND FLOW MEDIATED DILATION. *J Appl Physiol (1985)*.

Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT & Green DJ (2009a). Brachial artery blood flow responses to different modalities of lower limb exercise. *Med Sci Sports Exerc* **41,** 1072-1079.

Thijssen DH, Dawson EA, Tinken TM, Cable NT & Green DJ (2009b). Retrograde flow and shear rate acutely impair endothelial function in humans. *Hypertension* **53,** 986-992.

Tinken TM, Thijssen DH, Hopkins N, Black MA, Dawson EA, Minson CT, Newcomer SC, Laughlin MH, Cable NT & Green DJ (2009). Impact of shear rate modulation on vascular function in humans. *Hypertension* **54,** 278-285.

Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT & Green DJ (2010). Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension* **55,** 312-318.

Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G & Butler J (2009). The sympathetic nervous system in heart failure physiology, pathophysiology, and clinical implications. *J Am Coll Cardiol* **54,** 1747-1762.

Vroman NB, Buskirk ER & Hodgson JL (1983). Cardiac output and skin blood flow in lean and obese individuals during exercise in the heat. *J Appl Physiol Respir Environ Exerc Physiol* **55,** 69-74.

Wisloff U, Stoylen A, Loennechen JP, Bruvold M, Rognmo O, Haram PM, Tjonna AE, Helgerud J, Slordahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen O & Skjaerpe T (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* **115,** 3086-3094.

Witte KK, Cleland JG & Clark AL (2006). Chronic heart failure, chronotropic incompetence, and the effects of beta blockade. *Heart* **92,** 481-486.

Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA & Green D (2001). Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol (1985)* **91,** 929-937.

579 Zelis R, Mason DT & Braunwald E (1969). Partition of blood flow to the cutaneous and muscular
580 beds of the forearm at rest and during leg exercise in normal subjects and in patients with heart
581 failure. *Circ Res* **24,** 799-806.

582

583

584

COMPETING INTERESTS

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.M.B., M.T.H., D.H.T., conception and design of research. N.M.B., D.P.L. data acquisition and analysis. N.M.B., M.T.H., D.H.T. interpreted results of research. N.M.B. prepared figures. N.M.B. drafted manuscript. N.M.B., J.P.S., D.P.L., A.P.D., L.B., M.T.H., D.H.T. edited and revised manuscript. N.M.B., J.P.S., D.P.L., A.P.D., L.B., M.T.H., D.H.T. approved final version of manuscript.

FUNDING

Dr. Dick Thijssen is financially supported by the Netherlands Heart Foundation (E Dekker-stipend, 2009T064).

599 **Table 1:** Subject characteristics in HF patients (n=14) and healthy controls (n=14).

Parameter	**Heart failure**	**Controls**	**P-value**
Age (yrs)	65±7	61±5	0.06
Sex (male:female)[1]	13:1	12:2	0.54
Body weight (kg)	91±21	79±16	0.12
Height (cm)	175±5	179±5	0.044
BMI (kg/m^2)	29.4±6.7	24.7±4.6	0.037
NYHA class (I:II:III)	1:10:3		
Systolic blood pressure (mmHg)	130±17	129±15	0.87
Diastolic blood pressure (mmHg)	81±10	85±9	0.29
Resting heart rate (/min)	59±8	60±10	0.76
Peak heart rate (/min)	132±18	166±18	<0.001
Peak oxygen uptake ($mlO_2/kg/min$)	19.9±4.1	38.6±11.4	<0.001
Fasting glucose (mmol/L)[2]	5.47±0.61		
BNP-level (pg/mL)[3]	77±95	9±8	0.010
Current smoker (yes:no)	1:13	1:13	1.00

600 Data is presented as mean ± SD. P-value refers to an unpaired Student's *t*-test for continuous
601 variables.[1]P-value refers to Chi-Square test for sex. [2]Fasting glucose levels were available for
602 10 HF patients. [3]P-value refers to a Mann-Whitney U test for BNP-level. BNP-levels were
603 available for 11 HF patients and 13 control participants. BMI; body mass index. BNP; brain
604 natriuretic peptide.
605

606 **Table 2:** Cardiovascular medication use in HF patients (n=14).

Medication	Number of patients (%)
ACE-inhibitors	9 (64%)
Angiotensin II receptor antagonists	5 (36%)
Aldosterone antagonists	10 (71%)
Diuretics	8 (57%)
β-blockers	13 (93%)
Coumarin derivatives	9 (64%)
Antiplatelet drugs	5 (36%)
Statins	11 (79%)

607 ACE; angiotensine converting enzyme.

608

609 **Table 3:** Characteristics of the cycle exercise bout in HF patients (n=14) and controls (n=14).

Parameter	**HF patients**	**Controls**	**P-value**
Absolute workload (Watt)	73±23	122±29	<0.001
Relative workload (%max)	53±12	50±6	0.43
Average heart frequency (/min)	94±15	129±17	<0.001
Average heart frequency (%max)	72±8	78±7	0.051
Borg score 10min	12±2	12±2	0.54
Borg score 20min	13±2	13±2	0.62
Borg score 30min	14±3	14±2	0.59

610 Data is presented as mean ± SD. %max; percentage of maximally achieved workload/heart
611 frequency.

Table 4: Brachial artery diameter and blood flow at baseline and during exercise in HF patients (n=14) and healthy controls (C) (n=14).

		Time									**2-way ANOVA**		
Parameter	**Group**	*0*	*5*	*10*	*15*	*20*	*25*	*30*	*35*	*40*	*time*	*group*	*time*group*
Diameter (mm)	*HF*	4.4±0.6	4.4±0.6	4.3±0.6	4.3±0.6	4.3±0.7	4.3±0.7	4.3±0.6	4.3±0.6	4.3±0.5	0.249	0.16	0.18
	C	4.0±0.4	4.0±0.5	4.0±0.6	3.9±0.4	4.0±0.5	4.1±0.4	4.1±0.5	4.1±0.5	4.0±0.6			
Mean blood flow (mL/min)	*HF*	52±33	33±36*	41±35	36±34	50±53	55±49	85±68	92±73*	96±75*	<0.001	0.07	0.001
	C	47±34	25±34*	34±38*	50±40	81±61*	118±60*#	140±69*#	146±63*#	177±77*#			
Antegrade blood flow (mL/min)	*HF*	74±32	93±38*	95±30*	121±36*	134±52*	138±49*	164±63*	166±58*	170±67*	<0.001	0.12	<0.001
	C	59±31	95±46*	102±50*	143±61*	168±68*	202±73*#	219±91*	219±72*#	237±86*#			
Retrograde blood flow (mL/min)	*HF*	-22±26	-60±32*	-54±27*	-85±35*	-84±38*	-83±46*	-79±39*	-74±45*	-74±47*	<0.001	0.93	0.32
	C	-12±10	-70±30*	-68±29*	-93±40*	-87±41*	-84±41*	-79±47*	-72±37*	-60±36*			
Heart frequency (beats/min)	*HF*	62±8	75±11*	79±11*	89±12*	93±14*	94±14*	96±16*	97±17*	97±18*	<0.001	<0.001	<0.001
	C	67±11	90±13*#	93±14*#	114±15*#	125±18*#	127±18*#	134±19*#	134±18*#	139±20*#			

Data is presented as mean ± SD. * Post-hoc *t*-test significantly different compared to baseline at P<0.05. # Post-hoc *t*-test significantly different compared to HF patients at P<0.05.

FIGURE LEGENDS

FIGURE 1. A. Brachial artery mean (A), antegrade (B) and retrograde SR (C) and oscillatory shear index (D) at baseline and during exercise in HF patients (n=14) and controls (n=14).

HF patients demonstrate a lower exercise-induced increase in antegrade and mean SR and an increased oscillatory index during exercise. Error bars represent SE. Results from the 2-way repeated measures ANOVA are presented in the figure. *Post-hoc *t*-test significantly different compared to baseline at P<0.05 for individual groups (i.e. significant time*group'-interaction) or both groups combined (i.e. no significant time*group-interaction). #Post-hoc *t*-test significantly different compared to HF patients at P<0.05.

FIGURE 2. Skin temperature of the right forearm at baseline and during exercise in HF patients (n=14) and controls (n=14).

Forearm skin temperature decreased initially in both groups, after which skin temperature increases in controls, whilst forearm skin temperature remains decreased in HF patients. Error bars represent SE. Results from the 2-way repeated measures ANOVA are presented in the figure. *Post-hoc *t*-test significantly different compared to baseline at P<0.05.

FIGURE 3. A. Brachial artery mean (A), antegrade (B) and retrograde SR (C) and oscillatory shear index (D) at baseline and during exercise in a subgroup of HF patients (n=5) and controls (n=5).

HF patients demonstrate a smaller exercise-induced increase in mean SR compared to controls. Differences in exercise-induced antegrade and retrograde SR and

oscillatory index between HF patients and controls did not reach statistical significance. Error bars represent SE. Results from the 2-way repeated measures ANOVA are presented in the figure. *Post-hoc *t*-test significantly different compared to baseline at P<0.05. When no significant time*group'-interaction is found, *relates to both groups combined instead of the seperate groups. #Post-hoc *t*-test significantly different compared to HF patients at P<0.05.

FIGURE 4. Skin temperature of the right forearm at baseline and during exercise in a subgroup of HF patients (n=5) and controls (n=5).

Forearm skin temperature decreased initially in both groups. HF patients show a non-significant lower increase in skin temperature during exercise ('time*group'-interaction effect P=0.09). Error bars represent SE. Results from the 2-way repeated measures ANOVA are presented in the figure. *Post-hoc *t*-test significantly different compared to baseline at P<0.05. There is no significant time*group'-interaction, *therefore relates to both groups combined instead of the separate groups.