# The effects of carotid chemoreceptor inhibition on exercise tolerance in health and chronic heart failure

by

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## Abstract

**Background and Rationale:** Chronic heart failure (CHF) is a condition where heart function is insufficient to meet metabolic demand and is caused by anatomical or physiological abnormalities of the heart. In 2009, one in nine deaths included heart failure as a contributing cause, and the five-year survival rate following a diagnosis of CHF was 51.5% in 2017. CHF is characterized by heightened sympathetic nervous activity, peripheral chemosensitivity (both predictive of mortality in CHF), marked exercise intolerance and an increased ventilatory response to exercise. Amplified carotid chemoreceptor (CC) activity increases sympathetic nervous activity, which may lead to deterioration of cardiac function. Dopamine is known to suppress the CC, and previous work has shown that CC inhibition increases peripheral blood flow both at rest and during exercise in HF animals, suggesting that dopamine administration is a potential therapy to normalize CC activity/sensitivity and improve exercise tolerance in patients with CHF. However, this has not yet been examined in humans with CHF during full-body exercise.

**Purpose and Hypothesis:** The purpose of this study was to determine the effect of CC inhibition on cardiovascular and ventilatory function, and exercise tolerance in health and CHF. It was hypothesized that CC inhibition with low-dose dopamine ($2\mu g \cdot kg^{-1} \cdot min^{-1}$) would result in improvements in exercise tolerance in participants with CHF but not in controls, secondary to reduced ventilation, and improved cardiac output, vascular conductance and muscle oxygenation during whole-body exercise.

**Methods:** Nine clinically stable, optimally medicated patients (treated with $\beta$-blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, aldosterone antagonists and diuretics) with CHF (mean ejection fraction: 44 ± 3.3%) and nine age- and sex-matched controls were recruited. Participants completed 4 testing days: 1) pulmonary function and cardiopulmonary exercise tests; 2) basal chemoreflex testing (hypoxic ventilatory response test, transient hyperoxia, and progressive hypercapnic hyperoxic rebreathe); and 3/4) two time-to-symptom-limitation (TLIM) constant load cycling exercise tests at 75% peak power output with either intravenous saline or low-dose dopamine ($2\mu g \cdot kg^{-1} \cdot min^{-1}$; order randomized). Ventilation was measured using expired gas data and operating lung volume data were determined during exercise by inspiratory capacity maneuvers. Dyspnea and leg discomfort ratings were obtained using a modified Borg scale. Cardiac output was estimated using impedance cardiography, and vascular conductance was calculated as cardiac output/mean arterial pressure. Tissue oxygenation was estimated using near infrared spectroscopy at the vastus lateralis and the $7^{th}$ intercostal space.

**Results:** CHF patients had significantly greater resting CC sensitivity as demonstrated by a higher hypoxic ventilatory response than controls (CHF: 0.74 ± 0.20; Control: 0.28 ± 0.07, p=0.046), while ventilatory responses to transient hyperoxia and $CO_2$ rebreathe tests were not different between groups. There was no change in TLIM in either group with dopamine (CHF: saline 13.5 ± 3.0 vs dopamine 14.0 ± 1.9 min, p=0.81; Control: saline 10.9 ± 1.3 vs dopamine 13.4 ± 1.6 min, p=0.14). Dopamine had no significant effect on exercise cardiac output, ventilation, operating lung volumes, or dyspnea in CHF and controls. Dopamine had no effect on vascular conductance in patients with CHF, however there was a statistically significant increase in conductance at TLIM in controls (saline: 120 ± 8.6 vs dopamine: 139 ± 13 $L \cdot min^{-1} \cdot mmHg^{-1}$, p=0.027).

**Discussion and Significance:** CC inhibition with dopamine does not improve exercise tolerance in chemosensitive CHF patients, suggesting that the enhanced CC sensitivity observed in CHF does not contribute to exercise intolerance. This work highlights the importance of further understanding the mechanisms of exercise intolerance in CHF, and its role in secondary prevention and management as part of the core components of cardiac rehabilitation of patients with CHF.

## Preface

This thesis is an original work by Sophie Élène Collins. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name “Carotid Chemoreception and Exercise in Health and Chronic Heart Failure”, ID No. Pro00000526, January 2011.

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## List of Symbols and Abbreviations

1. CHF: chronic heart failure
2. $CO_2$: carbon dioxide
3. CVP: central venous pressure
4. $DO_2$: oxygen delivery
5. EDV: end diastolic volume
6. EF: ejection fraction
7. ESV: end systolic volume
8. HFPEF: heart failure with preserved ejection fraction
9. HFREF: heart failure with reduced ejection fraction
10. IC: inspiratory capacity
11. LVEF: left ventricular ejection fraction
12. MAP: mean arterial pressure
13. $O_2$: molecular oxygen
14. $P_aO_2$: partial pressure of arterial oxygen
15. $P_AO_2$: partial pressure of alveolar oxygen
16. $P_aCO_2$: partial pressure of arterial carbon dioxide
17. $PO_2$: partial pressure of oxygen
18. $P_ACO_2$: partial pressure of alveolar carbon dioxide
19. $PCO_2$: partial pressure of carbon dioxide
20. $P_{ET}CO_2$: end-tidal partial pressure of carbon dioxide
21. $PO_2$: partial pressure of oxygen
22. Q: cardiac output

23. Q/MAP: vascular conductance

24. $SpO_2$: arterial oxygen saturation

25. SV: stroke volume

26. SVR: systemic vascular resistance

27. TVC: total vascular conductance

28. $\dot{V}CO_2$: carbon dioxide production

29. $\dot{V}_E$: minute ventilation

30. $\dot{V}_E/\dot{V}CO_2$: ventilatory equivalent to carbon dioxide production

31. $\dot{V}O_{2peak}$: peak oxygen consumption

## Glossary of Terms

1. Angiotensin II type 1 receptor: An angiotensin receptor with vasopressor effects that regulates aldosterone secretion.
2. Chronic heart failure: An abnormality of cardiac structure or function where the heart is unable to deliver enough oxygen to meet metabolic demand (McMurray *et al.*, 2012).
3. Chronotrope: A substance that alters the rate of a regularly recurring phenomenon such as the heartbeat (Stedman, 2008).
4. Dopamine: A catecholamine neurotransmitter that when given at low doses, inhibits afferent signaling from the peripheral chemoreceptors in human CHF patients (van de Borne *et al.*, 1998)
5. Ejection fraction (EF): The fraction of blood ejected by the ventricle in relation to its end-diastolic volume (Klabunde, 2011). EF = (SV / EDV) x 100.
6. Hypercapnia: Elevated partial pressure of carbon dioxide in the blood. Arterial partial pressure of carbon dioxide ($P_aCO_2$) > 40mmHg.
7. Hyperoxemia: Elevated partial pressure of oxygen in the blood. Arterial partial pressure of oxygen ($P_aO_2$) > 150mmHg.
8. Hypoxemia: Reduced partial pressure of oxygen in the blood. Arterial partial pressure of oxygen ($P_aO_2$) < 80mmHg.
9. Inspired hypoxia: Insufficient supply of oxygen in the inspired air. Partial pressure of oxygen ($PO_2$) < 150mmHg
10. Tissue hypoxia: Insufficient supply of oxygen to tissues, caused by a low $P_aO_2$ (e.g. pulmonary disease “hypoxic hypoxia”), a reduced ability of blood to carry $O_2$ (e.g. anemia), or a reduction in tissue blood flow (West, 2012).

# CHAPTER I: Introduction

### *1.1 Background*

Chronic heart failure (CHF) is a condition where heart function is insufficient to meet metabolic demand and is caused by anatomical or physiological abnormalities of the heart (McMurray *et al.*, 2012). Due to an increase in prevalence, CHF was confirmed as being a chronic disease epidemic in 2002 (McCullough *et al.*, 2002). In 2011, twenty-three million people were suffering from the disease worldwide, and its prevalence, incidence and mortality rates continue to rise (Bui *et al.*, 2011; Heidenreich *et al.*, 2013). In 2017, the five-year survival after a diagnosis of heart failure was only 51.5% (Taylor *et al.*, 2017).

Independent of its etiology, CHF has been linked to heightened sympathetic nerve activity (SNA) (Floras, 1993; Narkiewicz *et al.*, 1999; Floras, 2009; Andrade *et al.*, 2015). Initially, increased SNA in CHF may be a beneficial adaptation aimed at maintaining cardiac output and blood pressure. However, chronically increased SNA further leads to the deterioration of heart function and is directly related to mortality (Cohn *et al.*, 1984). Increased SNA is provoked by changes in autonomic afferent feedback from desensitized baroreceptors and ergoreceptors (Piepoli *et al.*, 1996) as well as increased chemoreceptor activity and sensitivity (Narkiewicz *et al.*, 1999; Sun *et al.*, 1999*a*; 1999*b*). The carotid chemoreceptors (CC) are located within the carotid body at the bifurcation of the common carotid artery, and are sensitive to circulating stimuli including $O_2$, $CO_2$, inflammation (IL-6, TNF-α) and reactive oxygen species. They play an important role in ventilatory control and sympathetic vasoconstrictor outflow (Guyenet, 2000; Ding *et al.*, 2011; Porzionato *et al.*, 2013). Hypersensitivity of the CC has been shown to independently predict mortality in patients with CHF (Ponikowski *et al.*, 2001; Jankowska *et al.*, 2007; Giannoni *et al.*, 2009). Giannoni and colleagues found that heightened chemosensitivity to both hypoxia (carotid chemoreceptors) and hypercapnia (central chemoreceptors) causes

neurohormonal derangement, ventilatory instability, and ventricular arrhythmias, and concluded that it is therefore a serious adverse prognostic marker in CHF (Giannoni *et al.*, 2009). The authors also found that the four-year survival is only 49% in CHF patients with increased chemosensitivity, compared to 100% in patients with normal chemosensitivity (Giannoni *et al.*, 2009).

An important feature of CHF is severe and marked exercise intolerance (Poole *et al.*, 2012), which is measured objectively as a reduced oxygen uptake ($VO_{2peak}$); also predictive of mortality (Myers *et al.*, 2002; Conraads *et al.*, 2012). This reduction in $VO_{2peak}$ however, cannot be fully explained by impaired cardiac output, since a peripheral blood flow limitation has also been identified (Clark *et al.*, 1996; Piepoli *et al.*, 1999; Poole *et al.*, 2012). Stickland et al. found that basal CC activity is increased during exercise both in health and in CHF animals, even in the absence of changes in circulating CC stimuli. The authors concluded that the CC contribute to the sympathetic restraint of muscle blood flow during exercise in health and in CHF (Stickland *et al.*, 2007). The exaggerated muscle sympathetic nerve activity (MSNA) response to exercise in CHF (Notarius *et al.*, 2001*a*) could contribute to greater sympathetic restraint of exercising muscle blood flow in patients with CHF, and limit blood flow to the working muscles (both skeletal muscles and respiratory muscles), resulting in muscular fatigue and a reduction in functional capacity.

Dopamine infused at low doses has been shown to suppress the CC (Lahiri *et al.*, 1980; Goldberg, 1989; Stickland *et al.*, 2007). Janssen and colleagues found that CC inhibition with low-dose dopamine in healthy humans resulted in a reduction in the ventilatory response ($\dot{V}_E/\dot{V}CO_2$ slope) to exercise (Janssen *et al.*, 2009), however it has been shown not to affect ventilation at rest in healthy controls (van de Borne *et al.*, 1998; Edgell *et al.*, 2015). In patients with CHF, CC inhibition with dopamine has been shown to reduce ventilation (van de Borne *et al.*, 1998), and

improve Q and SV at rest (Edgell *et al.*, 2015). While the CC appears to be activated/sensitized in CHF and play a role in vascular regulation at rest, whether CC inhibition improves cardiovascular function and exercise tolerance in CHF has not been examined.

### *1.2 Purpose*

The primary aim of this study was to evaluate the effects of CC inhibition with low-dose dopamine on exercise tolerance in patients with CHF. The secondary aim of this study was to evaluate the effects of CC inhibition on the cardiovascular and ventilatory responses to exercise in CHF.

### *1.3 Hypothesis*

It was hypothesized that CC inhibition with low-dose dopamine ($2\mu g \cdot kg^{-1} \cdot min^{-1}$) would result in improved exercise tolerance (evaluated through time-to-exhaustion) in participants with CHF secondary to improved cardiovascular function (i.e. stroke volume and conductance (cardiac output/mean arterial pressure) and improved local tissue oxygenation (at the vastus lateralis and the $7^{th}$ intercostal space), while no effect would be observed in controls. CC inhibition was also expected to reduce the drive to breathe during exercise in patients with CHF, and thus reduce ventilation, improve ventilatory efficiency ($\dot{V}_E/\dot{V}CO_2$), and reduce dyspnea.

### *1.4 Delimitations*

CHF is a disease that affects people at a later stage in life, and therefore the female participants were postmenopausal. As a result, the additional confounder of hormonal variance in women (Lloyd-Jones *et al.*, 2010) was avoided without needing to control for menstrual cycle timing.

Limberg and colleagues found that there was individual variability as to the most effective dose of dopamine to inhibit the CC (Limberg *et al.*, 2016). In the current study, we used as standardized dose of dopamine hydrochloride ($2\mu g \cdot kg^{-1} \cdot min^{-1}$) previously used by our group to inhibit the carotid chemoreflex (Stickland *et al.*, 2007; Edgell *et al.*, 2015; Phillips *et al.*, 2018). It is possible that by using a dose-response curve, we may find a more effective individual dose for each patient.

Since the study is a randomized, placebo-controlled crossover design, participants act as their own controls between conditions, as well as be age- and sex-matched with a participant in the other group (CHF or control).

Experimental conditions were randomized. By doing so, we controlled for any learning or time effect, since the trials were completed on a separate day.

### *1.5 Limitations*

By including both men and women in the study, sample heterogeneity is a possibility, since there are known differences between sexes related to electrophysiology, heart failure etiology, coronary heart disease risk factors, pathophysiology, as well as treatments and outcomes (Westerman & Wenger, 2016). To control for this limitation, in addition to matching for age and CV comorbidities, the two groups were matched for sex.

Room temperature was maintained at 21℃ throughout and between tests. Barometric pressure was recorded before each experimental trial. Spirometry flows and gas partial pressures were calibrated before each trial and verified after the trials, to control for temperature and barometric pressure fluctuations.

Since three of the four sessions included exercise and that these sessions involved time-consuming protocols (I.V. catheter insertion, NIRS/ECG/Physioflow® instrumentation, for example) the sessions were spread out over 4 separate days. Each trial was conducted around the same time of day within each subject, however the authors acknowledge the possibility of between day fluctuations in health status and medication titration.

To obtain reliable baseline values for near infrared spectroscopy, the participants were asked to sit in place for a minimum of 5 minutes. The optodes were covered by an opaque material to prevent light interference.

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# CHAPTER II: The effects of carotid chemoreceptor inhibition on exercise tolerance in health and chronic heart failure

### *2.1 Introduction*

Chronic heart failure (CHF) is a condition where heart function is insufficient to meet metabolic demand and is caused by anatomical or physiological abnormalities of the heart (McMurray *et al.*, 2012). CHF is characterized by heightened sympathetic nerve activity (SNA), peripheral chemosensitivity (both predictive of mortality in CHF) (Ponikowski *et al.*, 2001; Jankowska *et al.*, 2007; Giannoni *et al.*, 2009), marked exercise intolerance and an increased ventilatory response to exercise (Chua *et al.*, 1996*a*). Increased SNA in CHF is provoked by changes in autonomic afferent feedback from desensitized baroreceptors and ergoreceptors (Piepoli *et al.*, 1996) as well as increased chemoreceptor sensitization (Narkiewicz *et al.*, 1999). The carotid chemoreceptors (CC) are located within the carotid body at the bifurcation of the common carotid artery, are sensitive to circulating stimuli including $O_2$, $CO_2$, inflammation (IL-6, TNF-α) and reactive oxygen species, and play an important role in ventilatory control and sympathetic vasoconstrictor outflow in CHF (Guyenet, 2000; Ding *et al.*, 2011; Porzionato *et al.*, 2013). Heightened chemosensitivity to both hypoxia (carotid chemoreceptors) and hypercapnia (central chemoreceptors) causes increased SNA, ventilatory instability, and ventricular arrhythmias, and is therefore a very serious adverse prognostic marker in CHF (Giannoni *et al.*, 2009).

Previous work from our group in experimental CHF suggests that CC activity is increased during exercise both in health and CHF, even in the absence of changes in circulating CC stimuli (Stickland *et al.*, 2007). Importantly, it was found that when the CC is inhibited in resting healthy dogs, vasodilation does not occur, however CC inhibition during exercise caused an immediate vasodilatory response. In dogs with pacing-induced CHF, a vasodilatory response was observed both at rest and during exercise with CC inhibition. The vasodilatory response to CC inhibition in

health and CHF was abolished with sympathetic blockade. The authors concluded that the CC play an important role in the sympathetic control of cardiovascular function during exercise in health, and both at rest and during exercise in experimental CHF (Stickland *et al.*, 2007).

More recently, our group found that CC inhibition with low-dose dopamine improved cardiac function (cardiac output and stroke volume) in participants with CHF at rest, but not in risk-matched controls, and that no changes in cardiovascular function or ventilatory response were observed during handgrip exercise in participants with CHF (Edgell *et al.*, 2015). Of note, these patients were optimally medicated receiving $\beta$-blockers, angiotensin-converting-enzyme inhibitors (ACE-I), angiotensin-receptor-blockers (ARB), aldosterone antagonists and diuretics, unlike experimental models of CHF. The role of the CC in optimally medicated patients during full-body exercise is still unclear, and the CC's influence on exercise tolerance, cardiovascular function and the ventilatory response to exercise remains to be fully described. To translate previous work to full-body exercise in patients with CHF, we sought to examine the effects of CC inhibition with low-dose dopamine on cardiovascular and ventilatory regulation and exercise tolerance in patients with CHF. It was hypothesized that CC inhibition would improve exercise tolerance secondary to improvements in ventilation and cardiovascular function.

## *2.2 Methods*

### 2.2.1 Ethical approval and participant description

The study was approved by the University of Alberta Health Research Ethics Board (Pro00000526), and all participants provided written, informed consent. Nine participants with clinically stable CHF, and nine age, sex and risk-matched controls were recruited for the study. Patients with CHF classified as New York Heart Association (NYHA) functional class I – III,

receiving optimal pharmacological treatment (ex: ACE-I/ARB, $\beta$-blockers, aldosterone antagonists and diuretics) with no recent cardiac events within the previous three months were recruited. Participants receiving certain treatments that may affect CC activity/sensitivity, SNA or vascular function such as opioids, peripheral dopamine receptor blockers, certain anxiolytics, and antidepressants, were excluded. Participants with severe renal dysfunction, and severe sleep apnoea (STOP-Bang questionnaire score >3, and apnoea–hypopnoea index > 30 as evaluated by overnight sleep monitoring with ApneaLink Plus, ResMed Ltd, Bella Vista, Australia) were also excluded.

### 2.2.2 Study Design

A double blind, randomized, placebo-controlled crossover design was used to investigate the effects of CC inhibition with dopamine on exercise tolerance, cardiovascular function and ventilation during whole-body exercise. The protocol, completed over a period of 3 weeks, consisted of 4 sessions conducted on separate days: 1) screening session comprised of informed consent, medical history, health-related quality of life questionnaires [EuroQol 5-dimensional 5-level questionnaire (EQ-5D-5L) and the Kansas City Cardiomyopathy Questionnaire (KCCQ)], cardiopulmonary exercise test and pulmonary function test as previously described (Edgell *et al.*, 2015; Stickland *et al.*, 2016); 2) basal chemoreflex assessment; 3) two separate constant work-rate exercise tests to symptom limitation ($T_{LIM}$) at 75% of the maximal work rate (established from prior incremental exercise test) using either intravenous (IV) low-dose dopamine (session A) or placebo saline infusion (session B). The two experimental sessions were randomized with the flip of a coin. Participants were asked to abstain from exercise, alcohol, and caffeine for six hours prior to every visit.

### 2.2.3 Procedures

*Basal chemoreception session*

Basal chemoreception sessions were completed with participants laying on a bed in a semi-supine position while single-lead ECG, brachial blood pressure cuff, and ear-lobe pulse oximeter (N-595; Covidien, Mansfield, MA) were attached and continuously monitored and recorded with a data acquisition system (Powerlab 16/30; ADInstruments, New South Wales, Australia). Data were stored for subsequent analysis using associated software (LabChart 8.0 Pro; ADInstruments). During the tests, participants wore a nose clip and inspired humidified air (HC 150; Fisher and Paykel Healthcare) through a mouthpiece attached to a pneumotachometer (to measure ventilation, respiratory rate, as well as inspiratory and expiratory volumes; 3700 series; Hans Rudolph, Shawnee, KS) and a gas analyzer (to measure inspired and expired partial pressures of $O_2$ and $CO_2$; CD-3A and S-3A; AEI Technologies, Pittsburgh, PA). The mouthpiece and pneumotachometer were connected to a flow-through system to allow the researcher to surreptitiously switch from the hypoxic or hyperoxic gas blender systems during the chemoreflex tests. Participants completed a 10-minute period of quiet, normoxic breathing to ensure a stable baseline prior to initiation of the chemoreflex tests.

The transient hyperoxic ventilatory response test was used to quantify CC activity (Dejours *et al.*, 1958). Following a period of normoxic breathing, participants breathed 100% oxygen ($F_iO_2$: 1.0) for two minutes. After one minute of normoxic breathing, participants repeated hyperoxic breathing for another two minutes. The greatest 15-second average reduction in minute ventilation relative to the one-minute baseline average was used to quantify CC activity. Participants then completed a 10-minute recovery period.

A $CO_2$ rebreathe test ($FiO_2$=0.50, $FiCO_2$=0.07) was completed to estimate central chemosensitivity (Read, 1967). Briefly, a four litre rebreathe bag filled with 50% $O_2$, 7% $CO_2$, and 43% nitrogen was attached to the flow-through system. First, inspired $PO_2$ was raised to ~350mmHg ($F_iO_2$: 0.5) for five minutes, and during end-expiration, the valve was turned over to the rebreathe bag. Participants continued to rebreathe from the bag until an end-tidal partial pressure of $O_2$ ($P_{ET}CO_2$) of 55mmHg was reached or until the participant requested to terminate the test. Central chemosensitivity was evaluated as the slope of the regression line relating the change in ventilation relative to the change in $P_{ET}CO_2$ during rebreathe (Read, 1967).

Following a 10-minute recovery period, the transient hypoxic ventilatory response test was administered to evaluate CC sensitivity (Edelman *et al.*, 1973). During expiration, the researcher turned the gas blender from normoxic gas to 100% nitrogen, where participants inhaled 2-8 breaths, targeting a range of $SpO_2$ concentrations between 75-100%. Following each transient breath(s), participants recovered 2-5 minutes to return to baseline values ($P_{ET}CO_2$ ~ 40mmHg, $P_{ET}O_2$ ~ 100mmHg and $SpO_2$ and heart rate returned to pre-hypoxia baseline) for a minimum of one minute. Each number of transient breaths was repeated a minimum of two times to obtain a range of oxygen saturations (75-100% $SpO_2$). The average of the two largest consecutive breaths yielding the highest ventilation following the hypoxic stimulus was used to calculate the change in ventilation from the one minute baseline immediately preceding the stimulus (Ponikowski *et al.*, 2001). The hypoxic ventilatory response was evaluated as the slope relating the change in ventilation to the change in $SpO_2$ (Chua & Coats, 1995; Chua *et al.*, 1996*a*; Ponikowski *et al.*, 2001).

*Experimental sessions A & B*

Upon arrival, body mass was obtained to calculate infusion rate. Participants were then instrumented with an IV catheter to allow for the infusion of low-dose dopamine hydrochloride (session A, 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Hospira, Lake Forest, IL, USA) or isotonic saline solution (session B) administered through a constant-infusion pump (Alaris, San Diego, CA, USA). Both the study participant and the lead researcher running the trial and interacting with the participant were blinded to the experimental condition (saline or dopamine). Only the nurse, supervising physician and research coordinator were aware of the condition.

Low-dose dopamine (i.e. $2\mu g \cdot kg^{-1} \cdot min^{-1}$) was selected because it has previously been shown to effectively inhibit the carotid chemoreceptors in humans (Lahiri *et al.*, 1980; Stickland *et al.*, 2007) without increases in alpha and beta-adrenergic stimulation. Dopamine does not interact with the central chemoreceptors as it does not cross the blood brain barrier (Zlokovic, 2008), nor is it likely to have peripheral vasodilatory effects through dopamine-1 receptors, as shown in healthy controls (Stickland *et al.*, 2011; Edgell *et al.*, 2015).

Following IV catheter insertion, participants were instrumented with near infrared spectroscopy (NIRS; Oxymon MK III; Artinis Medical Systems, Zetten, The Netherlands). Tissue oxygenation was estimated using NIRS at the belly of the vastus lateralis and the $7^{th}$ intercostal space (refer to appendix for detailed methods). Cardiac output (Q), was estimated non-invasively through impedance cardiography (Physioflow® PF-05, Manatec Biomedical, France) (Bernstein, 1986). Oxygen delivery was estimated using Q, $SpO_2$ and haemoglobin concentration data. Vascular conductance was calculated as cardiac output/mean arterial pressure (MAP).

Once 10 minutes of adequate seated baseline measurements were obtained, infusion of either saline or dopamine was commenced, allowing for a 10-minute infusion period. Participants

were then transitioned to the cycle ergometer (Ergoselect II 1200; Ergoline, Blitz, Germany), where they were instrumented with finger pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA) and 12-lead ECG. Baseline metabolic measurements were obtained for three minutes (Encore229 Vmax; SensorMedics, Yorba Linda, CA, USA) in the upright seated position, followed by a one-minute period of unloaded pedaling, and then a rapid increase in workload to 75% of peak work-rate. The lead researcher (unaware of the experimental condition) provided participants with encouragement and continuous feedback regarding cadence throughout the symptom-limited time-to-exhaustion exercise test at constant load, wherein exercise endurance time was recorded from the onset of constant load to the point of symptom limitation. The test was terminated when participants were no longer able to maintain a cadence at or above 50rpm.

During the last 30 seconds of the first minute of each stage, blood pressure was measured by manual auscultation, after which Borg ratings of breathing and leg discomfort were obtained. Every two minutes, participants completed inspiratory capacity maneuvers (Guenette *et al.*, 2013). ECG, Q, $SpO_2$, NIRS, and metabolic measurements were continuously recorded. To avoid contamination of the expired gas data from the IC maneuvers (Jensen *et al.*, 2008), both ventilatory and cardiovascular measurements were recorded every two minutes, 30 seconds prior to IC maneuver measurements. These data were then linked with the ratings of perceived exertion and IC measurements collected during the final 30 seconds of the respective minute. Haemoglobin concentration ([Hb]) was determined at the beginning of each experimental session (HemoCue 201+; HemoCue AB, Angelholm, Sweden) following IV catheter insertion and immediately after the termination of the TLIM trial, during active recovery. Baseline [Hb] measurements were used to calculate $\dot{D}O_2$ during seated baseline, and the [Hb] measurements obtained during active recovery were used to calculate $\dot{D}O_2$ at TLIM.

### 2.2.4 Statistical analysis

Data are presented as mean ± standard error of measurement (SEM) unless indicated otherwise. For all inferential analyses, the probability of a Type I error was set at 0.05. A three-way, repeated measure analysis of variance (ANOVA) was used to evaluate the effect of: I.V. saline versus I.V. dopamine (factor A) at rest and during exercise (repeated factor) in CHF and controls (fixed factor). A two-way repeated-measures ANOVA was used to evaluate the effect of I.V. saline versus I.V. dopamine on exercise endurance time in CHF and controls. A two-way repeated measures ANOVA was used to evaluate the effect of saline vs. dopamine on key dependant variables at rest and during exercise in each group. If main effects or interaction effects were found, Tukey pairwise comparisons were completed. Unpaired T-tests were used to evaluate cardiopulmonary data, pulmonary function, as well as chemoreception data between CHF and controls. Statistical analyses were completed using Sigmaplot 13.0 (Systat Software, San Jose, CA).

## *2.3 Results*

### 2.3.1 Participants

All participants tolerated study procedures. Patients with CHF were middle aged, with a normal weight, height, and lung function. Mean ejection fraction (EF) at initial CHF diagnosis was 26.2 ± 3.9% (n=7), while EF at study enrolment was 44.0 ± 3.3% (n=9; with a mean improvement of 17 ± 4.7% in EF since initial diagnosis). Seven patients had heart failure with reduced ejection fraction (HFREF), and two had heart failure with preserved ejection fraction (HFPEF). Control

participants were well matched for age, sex, weight, and height. Refer to Tables 1-4 for further descriptive participant characteristics.

### 2.3.2 Health related quality of life

Health related quality of life (KCCQ and EQ-5D-5L) scores can be found in Table 4. Patients with CHF had significantly lower quality of life scores in terms of the EQ-5D-5L index score (CHF: 0.89 ± 0.02 vs controls: 0.94 ± 0.01, p=0.03) but not the EQ-5D-5L visual analog scale (CHF: 81.1 ± 2.6 vs controls: 90.2 ± 2.9, p=0.05) when compared to healthy controls.

### 2.3.3 Lung function and cardiopulmonary exercise test

Cardiopulmonary exercise test and pulmonary function test results are displayed in Tables 1 and 2. CHF patients had significantly worse pulmonary function compared to age- and sex-matched controls. Specifically, percent predicted total lung capacity (TLC), forced expired volume in one second ($FEV_1$), forced vital capacity (FVC) and diffusing capacity ($DL_{CO}$) were reduced in CHF, while no between-group difference in $FEV_1$/FVC ratio was observed.

As compared to controls, patients with CHF had a significantly lower $VO_{2peak}$, as well as peak minute ventilation ($\dot{V}_E$), and heart rate. There was no statistically significant difference in arterial oxygen saturation at baseline between groups, however CHF patients showed higher arterial oxygen saturation at peak exercise compared to controls. Ventilatory efficiency ($\dot{V}_E/\dot{V}CO_2$) was not different between groups at baseline, however $\dot{V}_E/\dot{V}CO_2$ was lower in the CHF group than in the control group at both 60W (highest equivalent work rate) and peak exercise.

### 2.3.4 Central and peripheral chemoreception

Basal chemoreception data can be found in Table 3. There was no statistically significant difference in baseline $\dot{V}_E$ (p=0.87). The change in $\dot{V}_E$ in response to transient hyperoxia was not significantly different between groups (CHF: 3.09 ± 0.85 vs controls: 1.12 ± 0.58 $L \cdot min^{-1}$, p=0.07), but suggests a trend toward greater carotid chemoreceptor activity in CHF. There was no difference in central chemoreflex responses to the progressive hypercapnic rebreathe test between groups (CHF: 1.26 ± 0.23 vs controls: 1.46 ± 0.90 $L \cdot min^{-1} \cdot mmHg^{-1}$, p=0.60). Patients with CHF had significantly higher ventilatory responses to the transient hypoxia test (CHF: 0.74 ± 0.20 vs. Control: 0.28 ± 0.07 $L \cdot min^{-1} \cdot \%^{-1}$, p=0.05). Together, these data suggest that the patients with CHF had greater carotid chemosensitivity than controls, but no differences in central chemosensitivity.

### 2.3.5 The effect of low-dose dopamine on time-to-exhaustion

Experimental trial results are displayed in Table 6. Dopamine did not have an effect on time-to-exhaustion (TTE) in either CHF patients (dopamine: 14.02 ± 1.93 vs saline: 13.55 ± 3.02, p=0.81) or controls (dopamine: 13.05 ± 1.48 vs saline: 11.14 ± 1.17, p=0.14). There was no statistically significant interaction between group (CHF vs control) and condition (saline vs dopamine) for TTE (p=0.521).

### 2.3.6 The effects of low-dose dopamine on the ventilatory response to exercise

The effects of dopamine on physiological and perceptual responses during constant load exercise at 75% $W_{max}$ in CHF patients and healthy controls at four-minute isotime and TLIM are represented in Tables 5 and 6, respectively. The effects of dopamine on $\dot{V}O_2$, $\dot{V}_E$ and $\dot{V}_E/\dot{V}CO_2$ in controls and patients with CHF are displayed in Figure 1. $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were significantly lower in CHF patients in both conditions when compared to controls, due to the reduced metabolic

demand related to lower workload in the CHF group. Interestingly, $\dot{V}_E/\dot{V}CO_2$ was significantly lower in CHF patients than in controls, independent of condition. There were no within group differences in $\dot{V}O_2$ or $\dot{V}CO_2$ at both four-minutes isotime (Table 5) or TLIM in either the CHF or control groups with dopamine (Table 5). Dopamine did not affect $\dot{V}_E$ in either group (Figure 1), however dopamine significantly increased $P_{ET}CO_2$ in CHF patients (p=0.002) and controls (p=0.002) at four-minutes isotime, but not at TLIM. There were no changes in dyspnea in either group between both conditions.

### 2.3.7 The effects of dopamine on the cardiovascular responses to exercise

The effects of dopamine on the cardiovascular responses to exercise can be found in Tables 5 (four-minute isotime data) and 6 (TLIM), and Figures 2, 3 and 4. As expected, cardiac output (Q), heart rate (HR), stroke volume (SV) and mean arterial pressure (MAP), were lower in CHF patients than in controls independent of condition. Additionally, vascular conductance was lower in CHF patients than in controls in both saline and dopamine conditions. There were no significant changes in Q, SV or HR in either group with dopamine at four-minute isotime or TLIM. At TLIM, $DO_2$ was higher with dopamine in the control group (p=0.04), while no change was observed in CHF patients. This improvement in $DO_2$ at TLIM in controls was likely secondary to a trend in increased hemoglobin concentration with dopamine (p=0.08), which was not observed in CHF patients. Vascular conductance was unchanged in CHF with dopamine at TLIM (Figure 2, p=0.07), whereas a significant change in conductance was found in controls at TLIM (saline: 120.2 ± 8.6 vs dopamine: 138.8 ± 13, p=0.03), despite no significant change in MAP (Figure 2). There were no changes in ratings of perceived leg discomfort in either group between both conditions.

Changes in tissue oxygenation during saline and dopamine time-to-exhaustion trials are reported in Figures 3 and 4. In CHF, dopamine did not affect vastus lateralis total-, deoxy-, or oxy-hemoglobin (TotHb, HHb and $O_2$Hb, respectively). In controls, there was no change in vastus lateralis TotHb or $O_2$Hb with dopamine, however, there was a significantly smaller change in HHb with dopamine ($p=0.03$). In both CHF and controls, there was no difference between conditions in any NIRS measurements at the 7$^{th}$ intercostal space.

### *2.4 Discussion*

The current study aimed to evaluate the effects of carotid chemoreceptor inhibition using low-dose dopamine infusion on exercise tolerance and cardiovascular and ventilatory regulation in patients with CHF. Carotid chemoreceptor inhibition had no effect on time-to-exhaustion in either patients with CHF or healthy age- and sex-matched control participants. No changes in MAP or vascular conductance were observed in the CHF group with dopamine, while vascular conductance was improved with dopamine in the control group. Additionally, neither ventilation ($\dot{V}_E/\dot{V}CO_2$ and absolute $\dot{V}_E$) nor perceived breathing discomfort were affected in either group with dopamine. Combined, these data suggest that CC inhibition does not improve exercise endurance time or cardiovascular and ventilatory function in optimally medicated and clinically stable euvolemic CHF patients during whole body cycling exercise.

#### 2.4.1 The effects of dopamine on cardiovascular function during exercise

It has been well documented that patients with CHF have increased CC activity/sensitivity (Chua *et al.*, 1997*b*; Sun *et al.*, 1999*b*; Ponikowski *et al.*, 2001; Stickland *et al.*, 2007; Giannoni *et al.*, 2008; 2009). Previous work has shown that CC inhibition at rest in experimental CHF results

in improvements in vascular conductance (Stickland *et al.*, 2007), while it reduces ventilation and improves cardiovascular function (reduced total peripheral resistance, and increased cardiac and stroke indexes) at rest in CHF patients (Edgell *et al.*, 2015). CC inhibition during exercise in experimental CHF improves vascular conductance (Stickland *et al.*, 2007), while no cardiovascular or ventilatory effects were observed in CHF patients performing hand grip exercise (Edgell *et al.*, 2015). This study furthers previous work by examining the effect of CC inhibition using low-dose dopamine in optimally medicated stable patients with CHF and evaluating its effects on exercise tolerance, as well as cardiovascular function and ventilation during whole-body exercise. Our results suggest that while CC sensitivity is elevated in CHF, CC inhibition did not translate to improved exercise tolerance. Further, consistent with the lack of improvement in exercise tolerance, CC inhibition did not improve cardiovascular function or oxygen delivery during exercise. The lack of improvement in cardiovascular function in CHF in the current study is in contrast with previous work in experimental CHF demonstrating improved cardiovascular function with CC inhibition during exercise (Stickland *et al.*, 2007). These findings suggest that in optimally medicated CHF patients, the CC is not a key mediator in exercise tolerance and cardiovascular control during exercise.

### 2.4.2 The effects of dopamine on ventilation during exercise

It has been well established that patients with CHF have an exaggerated ventilatory response to exercise (Weber *et al.*, 1982; Sullivan *et al.*, 1988; Myers *et al.*, 1992; Koike *et al.*, 1993; Riley *et al.*, 1994; Kobayashi *et al.*, 1996), which can contribute to the sensation of dyspnea (Rubin & Brown, 1984). The CC has been shown to contribute to exercise ventilation (Rebuck *et al.*, 1972; Martin *et al.*, 1978) and enhanced CC sensitivity has been linked to the heightened

ventilatory response to exercise in CHF (Chua *et al.*, 1996*a*). Despite evidence of carotid chemosensitivity, CHF patients in the current study did not exhibit ventilatory inefficiency (i.e. elevated $\dot{V}_E/\dot{V}CO_2$) during exercise. Similarly, CC inhibition with dopamine did not reduce ventilation or dyspnea during exercise in CHF. These results suggest that CC inhibition does not influence ventilation during exercise in stable CHF patients.

### 2.4.3 Central and peripheral chemoreception

Consistent with previous work evaluating CC sensitivity in CHF (Chua *et al.*, 1997*b*; Sun *et al.*, 1999*b*; Ponikowski *et al.*, 2001; Stickland *et al.*, 2007; Giannoni *et al.*, 2008; Giannoni *et al.*, 2009), we found that patients with CHF have increased CC sensitivity compared to controls, as evaluated by the transient hypoxic ventilatory response test. There was a trend (p=0.07) in the ventilatory responses to transient hyperoxia, suggesting increased CC activity in the CHF group. Additionally, there was no statistically significant difference in central chemosensitivity ($CO_2$ rebreathe) between healthy controls and CHF patients in the present study, Our study adds to recent work which found that central chemosensitivity is not increased in patients with chronic systolic HF on optimal medical therapy (Paleczny *et al.*, 2017).

### 2.4.4 Work on CC activity/sensitivity in CHF: past vs present

Historically, work in experimental CHF (Sun *et al.*, 1999; Li *et al.*, 2006; 2007; Stickland *et al.*, 2007; Ding *et al.*, 2008; Marcus *et al.*, 2014) has been on animals that have had pacing-induced (i.e. chronic ischemia) CHF, and these animals typically do not receive CV medications to help manage their disease, nor do they typically have co-morbidities. As a result, there are significant limitations related to the translation of previous findings in experimental CHF to CHF

patients. Additionally, there is variability in humans with CHF in terms of: comorbidities, HF etiologies, and emerging pharmacotherapies being used in CHF patients. Original work demonstrating enhanced CC activity/sensitivity in CHF was completed on patients with lower EF and higher NYHA functional class (Chua *et al.*, 1996*a*; 1996*b*; 1997*b*; Ponikowski *et al.*, 2001) than the patients in the current study. Further, patients in these studies were not treated with *β*-blockers or ARBs but were receiving digoxin. Digoxin is no longer the preferred treatment for CHF patients (Lewis *et al.*, 1989), and has been shown to sensitize the CC (Quest & Gillis, 1971; Janssen:2010hs; McQueen & Ribeiro, 1983; Schobel *et al.*, 1994).

Current pharmacological management of HFREF includes neurohormonal antagonists (ACE-I/ARB, *β*-blocker and aldosterone antagonist) and diuretics (McMurray *et al.*, 2012). At present, no treatment has been shown to improve outcomes in patients with heart failure with preserved ejection fraction (EF≥50%), however, diuretics are recommended to relieve sodium and water retention, and symptoms of edema and dyspnea (McMurray *et al.*, 2012). In the current study, most of the CHF patients received ACE-I/ARBs, aldosterone antagonists, *β*-blockers (in those with HFREF), and diuretics (if needed). Patients were well-managed as demonstrated by clinical stability (as evidenced by symptom stability, with no emergency department visits or hospitalizations within three months prior to study enrolment), and all patients had a relatively good health-related quality of life as compared to previous work in CHF (Heidenreich *et al.*, 2013).

Importantly, the current pharmacological management of these patients may have altered CC activity/sensitivity and the ventilatory response to exercise. In experimental CHF, angiotensin II has been shown to increase CC activity/sensitivity and sympathetic outflow (Allen, 1998; Li *et al.*, 2006). Moreover, angiotensin II receptor type 1 ($AT_1R$) blockade in experimental CHF normalizes the heightened sympathetic response to hypoxic stimulation of the CC, suggesting a

normalization of CC sensitivity with $AT_1R$ blockade (Li *et al.*, 2006). Finally, *β*-blockers do not affect the CC, but have been shown to lower $\dot{V}_E/\dot{V}CO_2$ in CHF (Beloka *et al.*, 2008). As such, despite evidence of carotid chemosensitivity, the CHF pharmacotherapies used by the patients in the present study may explain the absence of further improvements in exercise tolerance, and cardiovascular and ventilatory function with CC inhibition.

### 2.4.5 Limitations

To our knowledge, there is no minimally clinically important difference (MCID) in TTE with constant work-rate exercise in CHF, but the MCID in COPD has been determined to be 90 seconds (Casaburi, 2005). The current study found a 28 second improvement in TTE with dopamine in patients with CHF, which suggests that the current observed effect size is unlikely to be of clinical significance in CHF. A post hoc sample size calculation was completed based on the current mean difference in TTE (0.47 ± 5.6 min), and 1110 CHF patients would be required to detect a significant effect of CC inhibition with dopamine in exercise tolerance ($\alpha$=0.05, $\beta$=0.2, power=0.8). Therefore, we would suggest that the inability to detect a difference in TTE with dopamine is not the result of being statistically underpowered, and that a 28 second change in TTE is unlikely to be physiologically or clinically significant.

Limberg and colleagues found that there was individual variability as to the most effective dose of dopamine to inhibit the CC (Limberg *et al.*, 2016). In the current study, we used a standardized dose of IV dopamine hydrochloride ($2\mu g \cdot kg^{-1} \cdot min^{-1}$) that has been previously used by our group and shown to inhibit the CC without resulting in alpha or beta adrenergic stimulation (Stickland *et al.*, 2007; Edgell *et al.*, 2015; Phillips *et al.*, 2018). It is possible that by using a dose-response curve, we may have found a more effective individual dose of dopamine for each patient.

### *2.5 Conclusion*

In conclusion, this study examined the effect of CC inhibition using low-dose dopamine on exercise tolerance, and cardiovascular and ventilatory function in patients with CHF and healthy controls. CC inhibition did not appear to have a significant effect on exercise tolerance in CHF patients or controls. Additionally, dopamine did not affect cardiovascular or ventilatory function during exercise in CHF patients, but it did improve conductance during exercise in controls. Although patients receiving optimal pharmacological therapy do show evidence of CC sensitivity, the CC does not appear to play a major role in exercise tolerance, or cardiovascular and ventilatory control. The authors suggest that the absence of effects related to CC inhibition may be due to current pharmacotherapy in CHF patients, which may be countering the cardiovascular and ventilatory effects of heightened CC activity/sensitivity traditionally observed in this disease.

Table 1. Participant characteristics

| | Controls | CHF | P-value |
|---|---|---|---|
| Participants | 9 | 9 | |
| Male/Female | 6/3 | 6/3 | |
| Age (years) | 54.5 ± 4.1 | 52.1 ± 4.8 | 0.71 |
| Height (cm) | 169.2 ± 2.9 | 168.8 ± 3.0 | 0.93 |
| Mass (kg) | 74.5 ± 2.9 | 84.5 ± 4.8 | 0.09 |
| BMI ($kg \cdot m^{-2}$) | 26.1 ± 1.0 | 29.5 ± 1.1 | 0.03 |
| NYHA Functional Class (n) | | | |
| I | | 4 | |
| II | | 4 | |
| III | | 1 | |
| Ejection Fraction (%) | | 44.0 ± 3.3 | |
| LV mass ($g \cdot m^{-2}$) | | 100.7 ± 8.1 | |
| Diabetes Mellitus | 0 | 2 | |
| Hypertension (SBP >140) | 1 | 1 | |
| **Medication Use (n)** | | | |
| *β*-blockers | 1 | 7 | |
| ACE-I - ARB | 0 | 9 | |
| Aldosterone antagonists | 0 | 8 | |
| Diuretics | 0 | 4 | |
| Statins | 0 | 3 | |
| **Pulmonary Function** | | | |
| $FEV_1$ (L) | 3.5 ± 0.3 | 3.0 ± 0.3 | 0.24 |
| $FEV_1$ (% pred) | 112.0 ± 4.3 | 89.3 ± 4.7 | 0.003 |
| FVC (L) | 4.6 ± 0.3 | 4.1 ± 0.4 | 0.30 |
| FVC (% pred) | 111.8 ± 3.6 | 96.0 ± 4.8 | 0.02 |
| $FEV_1$/FVC (%) | 76.2 ± 2.1 | 72.8 ± 2.8 | 0.33 |
| $FEV_1$/FVC (% pred) | 99.2 ± 2.3 | 93.2 ± 3.0 | 0.13 |
| TLC (L) | 6.4 ± 0.4 | 5.5 ± 0.4 | 0.12 |
| TLC (% pred) | 102.1 ± 3.2 | 89.2 ± 4.7 | 0.04 |
| RV (L) | 1.7 ± 0.1 | 1.6 ± 0.1 | 0.46 |
| FRC (L) | 3.5 ± 0.2 | 2.7 ± 0.3 | 0.04 |
| IC (% pred) | 121 ± 6.7 | 105 ± 6.9 | 0.12 |
| $DL_{CO}$ (% pred) | 98.0 ± 6.3 | 77.1 ± 4.4 | 0.02 |

Data are presented as n or mean ± SEM. Definition of abbreviations: BMI: body mass index; NYHA: New York Heart Association; LV: left ventricle; ACE-I: angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker; $FEV_1$: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; FRC: functional residual capacity; IC: inspiratory capacity; $DL_{CO}$: diffusing capacity of the lung for carbon monoxide.

Table 2. Cardiopulmonary responses to graded exercise testing

| | Pre-exercise baseline | | | HEWR | | | Peak | | |
|---|---|---|---|---|---|---|---|---|---|
| | Controls | CHF | p-value | Controls | CHF | p-value | Controls | CHF | p-value |
| Work rate (W) | 0 | 0 | 1.0 | 60 | 60 | 1.0 | 211 ± 28 | 139 ± 19 | 0.04 |
| Work rate (% pred) | | | | | | | 135 ± 11 | 83 ± 7.3 | 0.001 |
| $\dot{V}O_2$ (mL·kg$^{-1}$·min$^{-1}$) | 5.8 ± 0.42 | 4.5 ± 0.53 | 0.07 | 15.3 ± 0.8 | 13.0 ± 0.86 | 0.06 | 39.5 ± 4.0 | 26.2 ± 2.7 | 0.01 |
| $\dot{V}O_2$ (L·min$^{-1}$) | 0.433 ± | 0.378 ± | 0.35 | 1.13 ± 0.05 | 1.08 ± 0.06 | 0.51 | 2.94 ± | 2.14 ± 0.25 | 0.02 |
| $\dot{V}CO_2$ (L·min$^{-1}$) | 0.38 ± 0.04 | 0.331 ± | 0.40 | 0.92 ± 0.07 | 1.00 ± 0.05 | 0.37 | 3.23 ± | 2.32 ± 0.26 | 0.06 |
| RQ | 0.86 ± 0.03 | 0.87 ± 0.02 | 0.80 | 0.81 ± 0.04 | 0.93 ± 0.03 | 0.02 | | 1.09 ± 0.02 | 0.85 |
| $\dot{V}_E$ (L·min$^{-1}$) | 14.6 ± 1.3 | 11.2 ± 0.83 | 0.04 | 29.1 ± 1.7 | 29.0 ± 2.2 | 0.96 | 110 ± 13.7 | 69.2 ± 6.7 | 0.02 |
| $P_{ET}CO_2$ (mmHg) | 32.2 ± 0.94 | 35.8 ± 1.2 | 0.03 | 35.9 ± 0.76 | 40.2 ± 0.76 | 0.001 | 31.9 ± | 36.3 ± 1.2 | 0.005 |
| $\dot{V}_E/\dot{V}CO_2$ | 39.1 ± 1.7 | 34.0 ± 2.4 | 0.10 | 32.1 ± 0.8 | 28.8 ± 1.0 | 0.02 | 34.0 ± 0.9 | 30.5 ± 1.2 | 0.03 |
| Nadir $\dot{V}_E/\dot{V}CO_2$ ratio | | | | | | | 27.5 ± 1.0 | 27.8 ± 1.0 | 0.79 |
| $\dot{V}_E/\dot{V}CO_2$ slope | | | | | | | 30.8 ± 1.2 | 28.4 ± 1.0 | 0.08 |
| $f_B$ (breaths·min$^{-1}$) | 17.8 ± 1.5 | 20.0 ± 3.0 | 0.52 | 19.8 ± 1.7 | 21.8 ± 2.1 | 0.50 | 41.7 ± 3.9 | 35.4 ± 3.6 | 0.25 |
| IC (L) | 2.79 ± 0.22 | 2.96 ± 0.20 | 0.56 | 3.16 ± 0.21 | 2.95 ± 0.24 | 0.53 | 3.16 ± | 2.96 ± 0.24 | 0.53 |
| IC % TLC | 44.2 ± 3.4 | 54.0 ± 2.3 | 0.03 | 50.3 ± 2.3 | 53.5 ± 3.1 | 0.42 | 51.1 ± 2.3 | 52.3 ± 3.9 | 0.80 |
| Delta IC | | | | 0.20 ± 0.14 | -0.01 ± 0.10 | 0.25 | 0.33 ± | -0.08 ± 0.18 | 0.09 |
| IRV (L) | 2.02 ± 0.18 | 2.15 ± 0.19 | 0.63 | 1.77 ± 0.22 | 1.54 ± 0.20 | 0.46 | 0.59 ± | 0.79 ± 0.12 | 0.29 |
| IRV % TLC | 31.1 ± 3.5 | 40.9 ± 2.0 | 0.03 | 28.6 ± 3.7 | 28.1 ± 3.3 | 0.91 | 10.1 ± 1.7 | 13.7 ± 1.3 | 0.13 |
| HR (beats·min$^{-1}$) | 78.4 ± 4.5 | 65.2 ± 3.5 | 0.03 | 102.8 ± 7.0 | 88.7 ± 4.0 | 0.10 | 163.4 ± | 110.4 ± | <0.001 |
| $SpO_2$ (%) | 97.3 ± 1.3 | 98.4 ± 0.72 | 0.45 | 96.0 ± 1.8 | 97.3 ± 0.7 | 0.53 | 94.8 ± 1.1 | 95.8 ± 0.91 | 0.05 |
| Dyspnea (Borg) | 0.1 ± 0.1 | 0.3 ± 0.2 | 0.41 | 1.2 ± 0.3 | 2.6 ± 0.4 | 0.03 | 5.8 ± 1.1 | 6.3 ± 0.9 | 0.69 |
| Leg discomfort (Borg) | 0.1 ± 0.1 | 0.1 ± 0.1 | 1.0 | 1.3 ± 0.4 | 3.1 ± 0.6 | 0.03 | 5.7 ± 0.9 | 7.4 ± 1.0 | 0.20 |

Data are presented as mean ± SEM. Definition of abbreviations: HEWR: highest equivalent work rate; $\dot{V}O_2$: oxygen uptake; $\dot{V}CO_2$: carbon dioxide production; RQ: respiratory quotient; $\dot{V}_E$: minute ventilation; $P_{ET}CO_2$: partial pressure of carbon dioxide; $\dot{V}_E/\dot{V}CO_2$: ventilatory efficiency; $f_B$: breathing frequency; IC: inspiratory capacity; IRV: inspiratory reserve volume; HR: heart rate; $SpO_2$: oxygen saturation measured by pulse oximeter.

Table 3. Central and peripheral chemoreceptor reflex responses

| | Control | CHF | P-value |
|---|---|---|---|
| **Transient hyperoxia** | | | |
| Baseline $\dot{V}_E$ ($L \cdot min^{-1}$) | 9.60 ± 1.2 | 9.34 ± 1.0 | 0.87 |
| Nadir (15 s) $\dot{V}_E$ ($L \cdot min^{-1}$) | 8.48 ± 1.4 | 6.25 ± 0.40 | 0.15 |
| Nadir (15 s) change in $\dot{V}_E$ from baseline ($L \cdot min^{-1}$) | 1.12 ± 0.58 | 3.09 ± 0.85 | 0.07 |
| **Hyperoxic hypercapnic rebreathe** | | | |
| $\Delta\dot{V}_E/\Delta P_{ET}CO_2$ slope ($L \cdot min^{-1} \cdot mmHg^{-1}$) | 1.46 ± 0.90 | 1.26 ± 0.23 | 0.60 |
| **Hypoxic ventilatory response** | | | |
| $\Delta\dot{V}_E/\Delta SpO_2$ slope ($L \cdot min^{-1} \cdot \%^{-1}$) | 0.28 ± 0.07 | 0.74 ± 0.20 | 0.045 |

Data are presented as mean ± SEM. Definition of abbreviations: $\dot{V}_E$: minute ventilation; $P_{ET}CO_2$: partial pressure of carbon dioxide; $SpO_2$: oxygen saturation measured by pulse oximeter.

Table 4. Health-related quality of life measures

| | Control | CHF | P-value |
|---|---|---|---|
| **EQ-5D-5L** (n=8/9) | | | |
| Index Score | 0.94 ± 0.01 | 0.89 ± 0.02 | 0.05 |
| Visual Analogue Scale | 90.2 ± 2.9 | 81.1 ± 2.6 | 0.03 |
| **KCCQ** | | | |
| Overall summary score | | 86.1 ± 4.1 | |
| Clinical summary score | | 91.4 ± 3 .9 | |
| Social limitation score | | 84.7 ± 5.0 | |
| Self-efficacy score | | 93.1 ± 3.7 | |
| Quality of life | | 76.9 ± 4.6 | |
| Total Symptom score | | 92.9 ± 2.8 | |
| Physical limitation score | | 89.8 ± 5.1 | |
| Symptom stability | | 61.1 ± 6.1 | |
| Symptom frequency | | 91.4 ± 3.7 | |
| Symptom burden | | 94.4 ± 2.4 | |

Data are presented as mean ± SEM. Definition of abbreviations: EQ-5D-5L: EuroQol 5-dimensional 5-level questionnaire; KCCQ: Kansas City Cardiomyopathy Questionnaire.

Table 5. Effects of dopamine on physiological and perceptual responses during constant load exercise at 75% max work load in patients with chronic heart failure and healthy controls at 4-min isotime

| | Control | | | CHF | | |
|---|---|---|---|---|---|---|
| Condition | Saline | Dopamine | P-value | Saline | Dopamine | P-value |
| Power output (W) | 158 ± 21 | 158 ± 21 | | 107 ± 12 | 107 ± 12 | 0.04 |
| **Metabolic** | | | | | | |
| $\dot{V}O_2$ (L·min$^{-1}$) | 2.51 ± 0.34 | 2.45 ± 0.33 | 0.27 | 1.67 ± 0.15 | 1.71 ± 0.16 | 0.39 |
| $\dot{V}CO_2$ (L·min$^{-1}$) | 2.62 ± 0.36 | 2.59 ± 0.34 | 0.62 | 1.83 ± 0.16 | 1.85 ± 0.17 | 0.67 |
| **Ventilatory/gas exchange** | | | | | | |
| $\dot{V}_E$ (L·min$^{-1}$) | 77.1 ± 9.9 | 71.4 ± 8.2 | 0.08 | 50.1 ± 3.6 | 47.9 ± 3.8 | 0.24 |
| $\dot{V}_E/\dot{V}CO_2$ | 30.0 ± 1.0 | 28.2 ± 0.84 | 0.26 | 27.8 ±0.92 | 26.2 ± 0.83 | 0.19 |
| $f_B$ (breaths·min$^{-1}$) | 28.8 ± 2.4 | 27.1 ± 1.8 | 0.19 | 28.9 ± 1.8 | 26.8 ± 2.3 | 0.17 |
| $V_T$ (L) | 2.66 ± 0.21 | 2.63 ± 0.21 | 0.77 | 1.83 ± 0.20 | 1.91 ± 0.22 | 0.41 |
| EELV, %TLC | 46.4 ± 2.3 | 46.8 ± 2.1 | 0.62 | 46.6 ± 3.0 | 45.6 ± 2.2 | 0.42 |
| IRV, %TLC | 13.1 ± 1.7 | 12.5 ± 2.2 | 0.52 | 20.3 ± 2.2 | 19.7 ± 2.0 | 0.79 |
| $P_{ET}CO_2$ (mmHg) | 36.1 ± 1.2 | 38.6 ± 0.8 | <0.001 | 38.9 ± 1.6 | 41.5 ± 1.3 | 0.002 |
| $SpO_2$ (%) | 98 ± 0.4 | 97 ± 0.5 | 0.09 | 96 ± 1.7 | 96 ± 0.9 | 1.00 |
| **Cardiovascular** | | | | | | |
| Q (L·min$^{-1}$) | 12.9 ± 1.2 | 14.0 ± 1.2 | 0.06 | 8.16 ± 0.8 | 8.49 ± 0.7 | 0.57 |
| SV (mL) | 86.9 ± 6.6 | 94.3 ± 7.6 | 0.16 | 77.6 ± 6.7 | 79.8 ± 6.3 | 0.71 |
| HR (beats·min$^{-1}$) | 147.7 ± 4.8 | 149.2 ± 4.9 | 0.51 | 104.4 ± 2.2 | 107.7 ± 6.7 | 0.51 |
| Q/MAP (L·min$^{-1}$·mmHg$^{-1}$) | 107.8 ± 8.9 | 123 ± 11 | 0.06 | 92.9 ± 9.2 | 101.2 ± 8.1 | 0.28 |
| MAP (mmHg) | 119 ± 3.5 | 115 ± 4.7 | 0.30 | 87.9 ± 3.2 | 84.4 ± 4.0 | 0.16 |
| SBP (mmHg) | 194.1 ± 7.5 | 191.3 ± 7.5 | 0.76 | 130.4 ± 6.0 | 125.8 ± 5.9 | 0.23 |
| DBP (mmHg) | 81.7 ± 2.6 | 76.4 ± 4.6 | 0.09 | 66.7 ± 2.0 | 63.7 ± 3.2 | 0.29 |
| **Subjective** | | | | | | |
| Dyspnea (Borg) | 3.6 ± 0.6 | 3.0 ± 0.7 | 0.08 | 3.2 ± 0.6 | 2.8 ± 0.5 | 0.63 |
| Leg discomfort (Borg) | 4.1 ± 0.6 | 4.0 ± 0.7 | 0.77 | 4.4 ± 0.7 | 4.2 ± 0.5 | 0.75 |

Data are presented as mean ± SEM. Definition of abbreviations: $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; $\dot{V}_E$: minute ventilation; $\dot{V}_E/\dot{V}CO_2$: ventilatory efficiency; $f_B$: breathing frequency; $V_T$: tidal volume; EELV = end-expiratory lung volume; IRV = inspiratory reserve volume; $P_{ET}CO_2$ = end-tidal partial pressure of carbon dioxide; $SpO_2$: oxygen saturation measured by pulse oximeter; Q = cardiac output; SV: stroke volume; HR: heart rate; Q/MAP: conductance; MAP: mean arterial pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure; Hb = hemoglobin concentration.

Table 6. Effects of dopamine on physiological and perceptual responses during constant load exercise at 75% max workload in patients with chronic heart failure and healthy controls at time of symptom limitation

| | Control | | | CHF | | |
|---|---|---|---|---|---|---|
| Condition | Saline | Dopamine | P-value | Saline | Dopamine | P-value |
| Time (mins) | 11.1 ± 1.2 | 13.1 ± 4.5 | 0.14 | 13.6 ± 3.0 | 14.0 ± 1.9 | 0.81 |
| Power output (W) | 158 ± 21 | 158 ± 21 | | 107 ± 12 | 107 ± 12 | 0.04 |
| **Metabolic** | | | | | | |
| $\dot{V}O_2$ ($L \cdot min^{-1}$) | 2.87 ± 0.35 | 2.81 ± 0.37 | 0.25 | 1.95 ± 0.19 | 1.97 ± 0.18 | 0.65 |
| $\dot{V}CO_2$ ($L \cdot min^{-1}$) | 2.81 ± 0.38 | 2.77 ± 0.38 | 0.47 | 2.04 ± 0.18 | 2.04 ± 0.19 | 0.98 |
| **Ventilatory/gas exchange** | | | | | | |
| $\dot{V}_E$ ($L \cdot min^{-1}$) | 99.2 ± 12.5 | 95.7 ± 12.0 | 0.27 | 63.2 ± 5.7 | 60.8 ± 5.0 | 0.22 |
| $\dot{V}_E/\dot{V}CO_2$ | 35.9 ± 1.4 | 35.3 ± 1.0 | 0.67 | 31.1 ± 0.95 | 30.2 ± 0.84 | 0.44 |
| $f_B$ ($breaths \cdot min^{-1}$) | 37.4 ± 2.9 | 39.2 ± 2.6 | 0.16 | 35.7 ± 2.0 | 35.0 ± 3.1 | 0.66 |
| $V_T$ (L) | 2.60 ± 0.15 | 2.41 ± 0.16 | 0.16 | 1.83 ± 0.20 | 1.86 ± 0.22 | 0.77 |
| EELV, %TLC | 45.6 ± 2.2 | 47.1 ± 2.1 | 0.21 | 46.9 ± 3.1 | 45.1 ± 2.7 | 0.13 |
| IRV, %TLC | 13.7 ± 1.3 | 15.1 ± 1.9 | 0.53 | 19.8 ± 2.9 | 21.3 ± 2.2 | 0.51 |
| $P_{ET}CO_2$ (mmHg) | 30.3 ± 1.0 | 30.7 ± 0.9 | 0.55 | 34.6 ± 1.2 | 35.2 ± 1.0 | 0.38 |
| $SpO_2$ (%) | 95 ± 0.7 | 94 ± 0.7 | 0.13 | 96 ± 1.4 | 95 ± 1.1 | 0.17 |
| **Cardiovascular** | | | | | | |
| Q ($L \cdot min^{-1}$) | 14.3 ± 1.0 | 15.6 ± 1.3 | 0.12 | 8.84 ± 0.96 | 9.74 ± 0.87 | 0.13 |
| SV (mL) | 88.1 ± 5.4 | 96.5 ± 8.0 | 0.27 | 76.2 ± 6.7 | 80.9 ± 6.6 | 0.42 |
| HR ($beats \cdot min^{-1}$) | 157.9 ± 5.6 | 162.9 ± 6.2 | 0.47 | 115.0 ± 4.4 | 121.9 ± 7.6 | 0.17 |
| $DO_2$ ($L \cdot min^{-1}$) | 3.04 ± 0.26 | 3.41 ± 0.30 | 0.04 | 1.76 ± 0.20 | 1.97 ± 0.21 | 0.11 |
| Q/MAP ($L \cdot min^{-1} \cdot mmHg^{-1}$) | 120.2 ± 8.6 | 138.8 ± 13 | 0.03 | 94.8 ± 7.5 | 109.1 ± 8.0 | 0.07 |
| MAP (mmHg) | 119.8 ± 3.5 | 113.3 ± 3.4 | 0.16 | 92.2 ± 4.7 | 89.8 ± 4.7 | 0.34 |
| SBP (mmHg) | 192.4 ± 7.6 | 190.4 ± 5.4 | 0.82 | 144.4 ± 8.7 | 142.7 ± 7.9 | 0.64 |
| DBP (mmHg) | 83.4 ± 2.8 | 74.8 ± 3.8 | 0.009 | 66.1 ± 3.6 | 63.3 ± 3.3 | 0.32 |
| Hb ($g \cdot dL^{-1}$) | 16.0 ± 0.52 | 16.6 ± 0.34 | 0.08 | 14.8 ± 0.60 | 15.1 ± 0.55 | 0.52 |
| **Subjective** | | | | | | |
| Dyspnea (Borg) | 7.2 ± 0.6 | 7.2 ± 0.6 | 1.0 | 6.7 ± 0.7 | 7.1 ± 0.6 | 0.34 |
| Leg discomfort (Borg) | 8.3 ± 0.6 | 8.8 ± 0.5 | 0.24 | 8.1 ± 0.6 | 8.8 ± 0.4 | 0.07 |
| Reason for termination | | | | | | |
| Legs | 5 | 6 | | 6 | 7 | |
| Dyspnea | 1 | 0 | | 0 | 0 | |
| Both | 3 | 3 | | 3 | 2 | |

Data are presented as mean ± SEM. Definition of abbreviations: $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; $\dot{V}_E$: minute ventilation; $\dot{V}_E/\dot{V}CO_2$: ventilatory efficiency; $f_B$: breathing frequency; $V_T$: tidal volume; EELV = end-expiratory lung volume; IRV = inspiratory reserve volume; $P_{ET}CO_2$ = end-tidal partial pressure of carbon dioxide; $SpO_2$: oxygen saturation measured by pulse oximeter; Q = cardiac output; SV: stroke volume; HR: heart rate; $DO_2$: oxygen delivery; Q/MAP: conductance; MAP: mean arterial pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure; Hb = hemoglobin concentration.

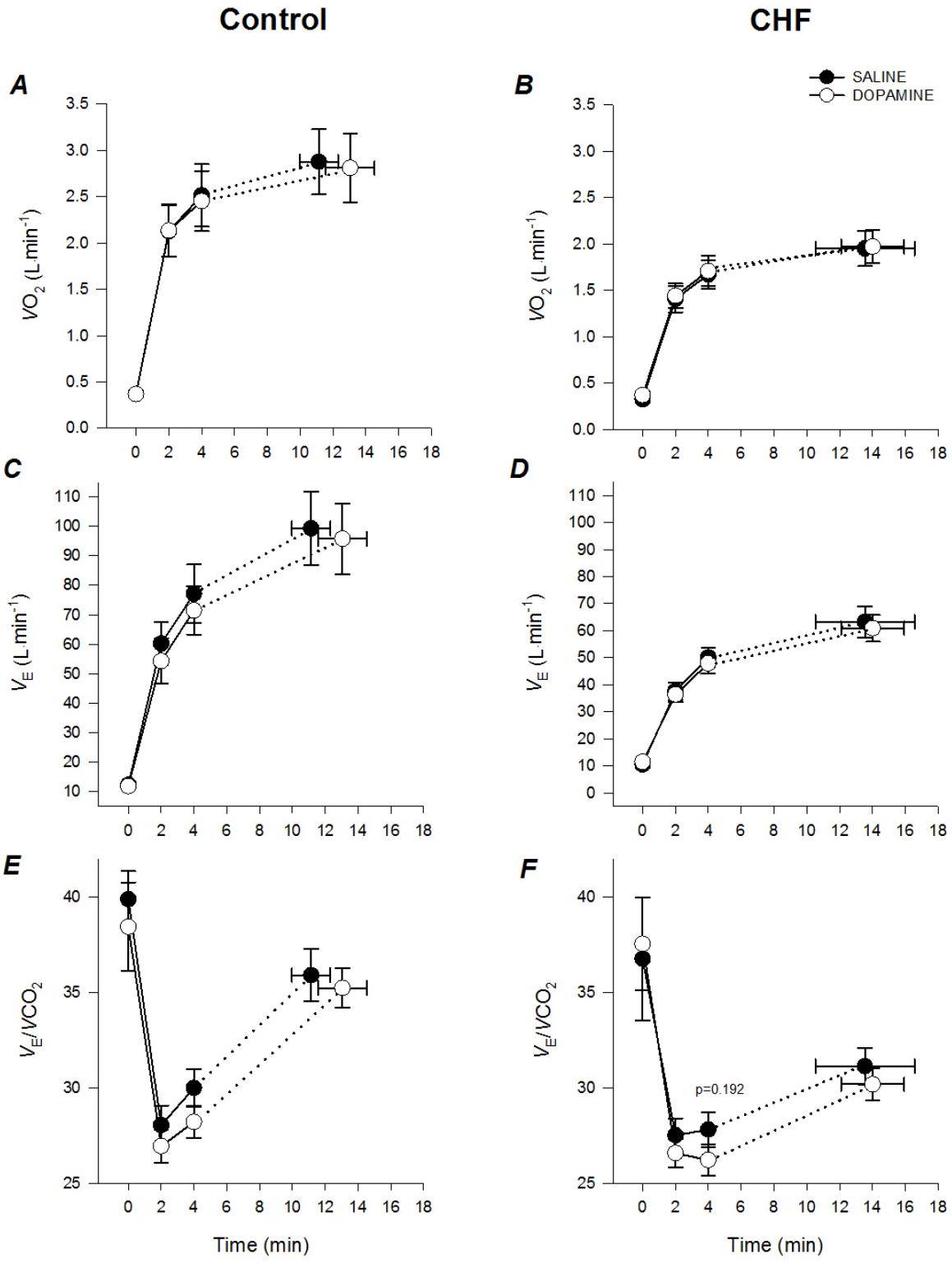


Figure 1: Mean ± SEM oxygen consumption ($\dot{V}O_2$), minute ventilation ($\dot{V}_E$), and ventilatory efficiency ($\dot{V}_E/\dot{V}CO_2$) at rest and during constant-load cycle ergometry in controls (*A,C,E*) and CHF (*B,D,F*). * $p<0.05$ saline vs dopamine within group

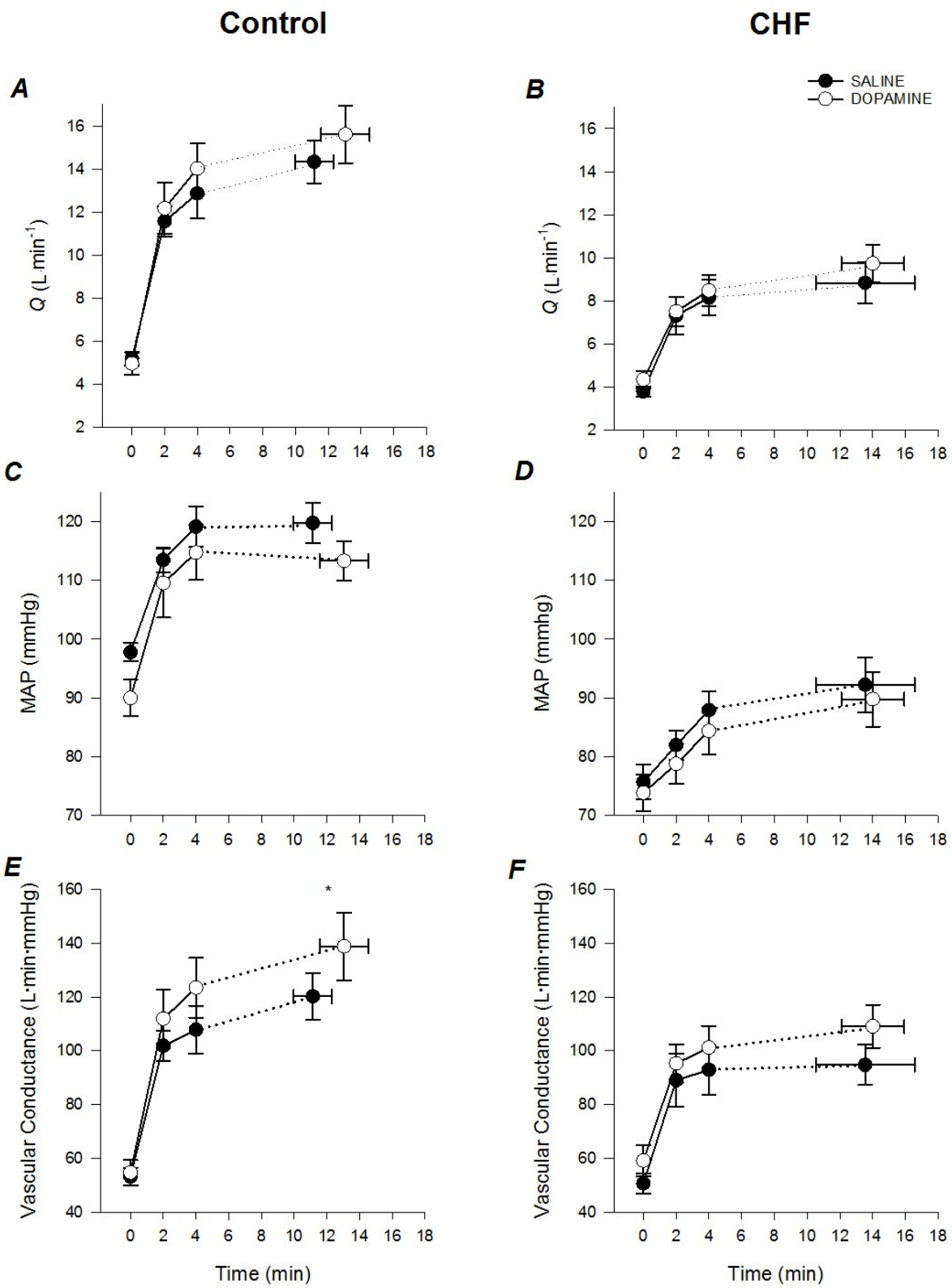


Figure 2: Mean ± SEM cardiac output (*Q*), mean arterial pressure (*MAP*), and vascular conductance at rest and during constant-load cycle ergometry in controls (*A,C,E*) and CHF (*B,D,F*). * p<0.05 saline vs dopamine within group

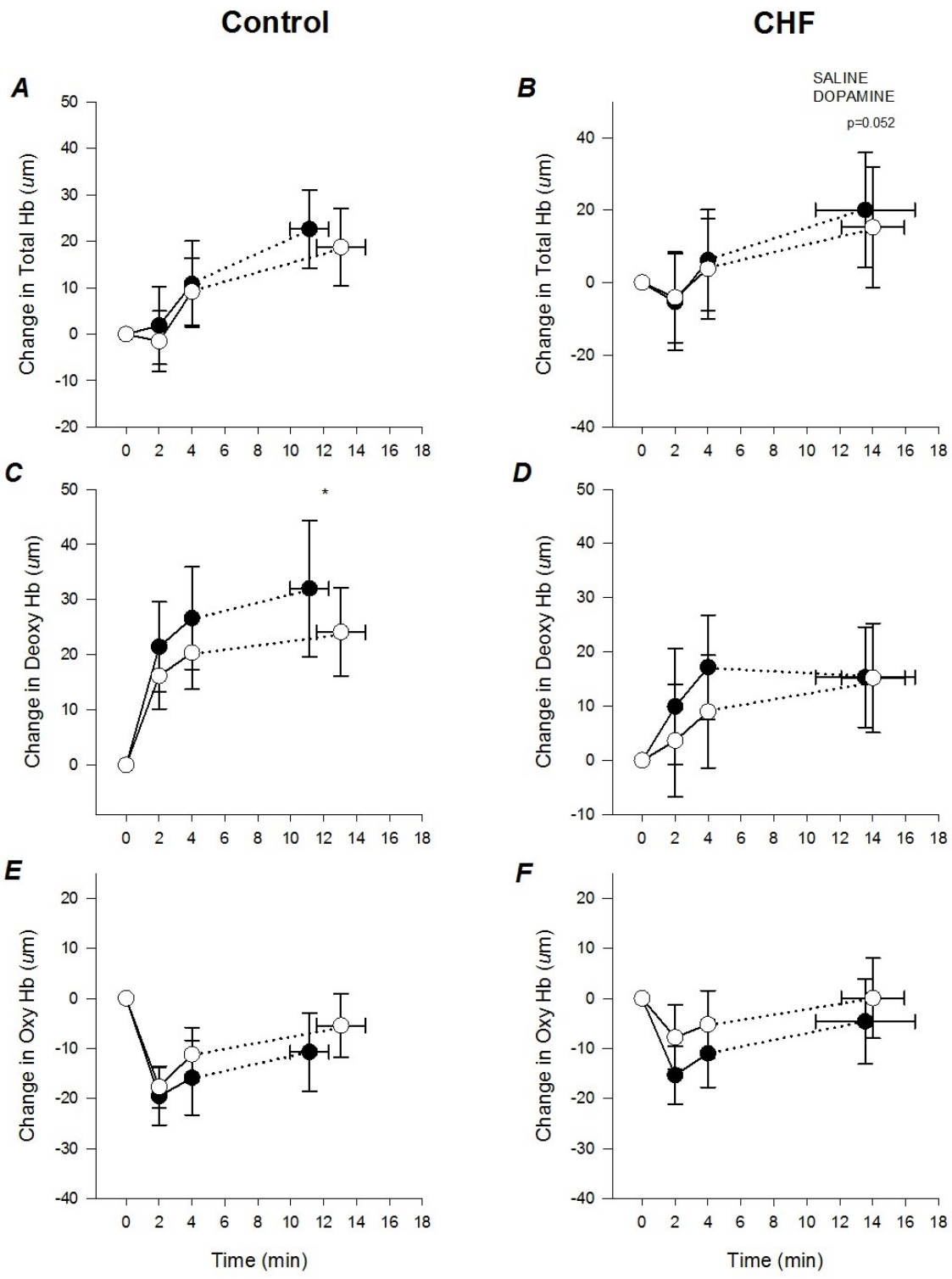


Figure 3: Mean ± SEM changes in total-, deoxy- and oxy-haemoglobin at rest and during constant-load cycle ergometry at the vastus lateralis in controls (*A,C,E*) and CHF (*B,D,F*). * p<0.05 saline vs dopamine within group

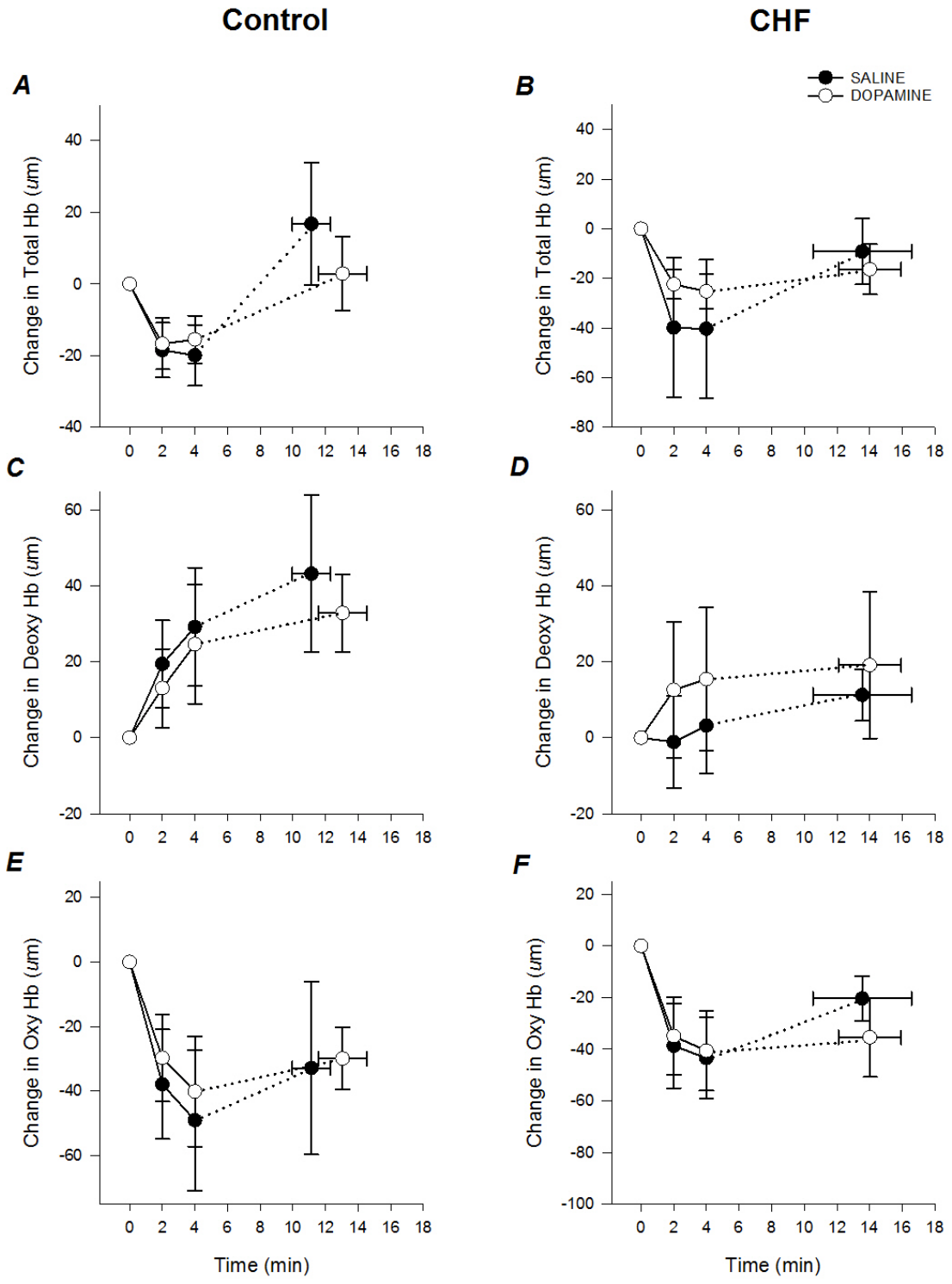


Figure 4: Mean ± SEM changes in total-, deoxy- and oxy-haemoglobin at rest and during constant-load cycle ergometry at the 7th intercostal space in controls (*A,C,E*) and CHF (*B,D,F*). * p<0.05 saline vs dopamine within group

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# CHAPTER III: General Discussion

### *3.1. The Effects of Carotid Chemoreceptor Inhibition on Exercise Tolerance in Chronic Heart Failure*

The purpose of this study was to evaluate the effects of CC inhibition with low-dose dopamine on exercise tolerance and cardiovascular and ventilatory regulation in patients with CHF and healthy controls. We found that dopamine did not have an effect on exercise tolerance or ventilation in either group. Dopamine did improve vascular conductance in controls during exercise while no effect was observed in patients with CHF. These findings are in contrast to previous work showing that CC inhibition in experimental CHF improves vascular conductance both at rest and during exercise (Stickland *et al.*, 2007), and that CC inhibition in resting CHF patients decreases ventilation and improves cardiovascular function (Edgell *et al.*, 2015).

In accordance with previous work, we found that patients with CHF have heightened peripheral chemosensitivity compared to healthy age- and sex-matched controls (Chua *et al.*, 1996*a*; 1996*b*; Ponikowski *et al.*, 1999; 2001; Giannoni *et al.*, 2008; 2009; Niewinski *et al.*, 2013*a*). Early studies on CC activity/sensitivity in CHF patients were completed on patients with lower ejection fraction (EF) and higher NYHA functional class (Chua *et al.*, 1996*a*; 1996*b*; 1997*b*; Ponikowski *et al.*, 2001) than the patients in the current study. The present study included patients that were clinically stable and receiving optimal pharmacological therapy [angiotensin-converting-enzyme inhibitors (ACE-I)/angiotensin receptor blockers (ARB), β-blockers, aldosterone antagonists, and diuretics]. Our results suggest that the CC does not appear to be a key mediator of cardiovascular and ventilatory regulation in patients with CHF NYHA Class I-III, and we suggest the absence of effects from CC inhibition is due to the improved pharmacological management of these patients.

### 3.1.1 The relationship between CC activity/sensitivity and dependent variables

While there were no group effects on cardiovascular or ventilatory variables with CC inhibition, we examined correlations to evaluate the interplay between individual chemoreflex responses and the effects of CC inhibition (see Tables 7-9 in Appendix C). There was no significant correlation between change in time-to-exhaustion with dopamine and baseline CC sensitivity in CHF (r=0.50, p=0.17). CC sensitivity and central chemoreceptor sensitivity has been shown to be related to the ventilatory response to exercise as evaluated by $\dot{V}_E/\dot{V}CO_2$ (Chua *et al.*, 1996*a*). In the current study, we found that the change in $\dot{V}_E/\dot{V}CO_2$ with dopamine during exercise was not correlated with resting CC sensitivity in CHF at four-min isotime (r=0.36, p=0.35) or at time of symptom limitation ($T_{LIM}$) (r=0.52, p=0.14), nor were central and peripheral chemosensitivity correlated in CHF (r=0.09, p=0.82).

We expected that those with the greatest CC sensitivity would have the greatest changes in vascular conductance with dopamine during exercise. In CHF, there was no significant correlation between the change in vascular conductance with dopamine and CC sensitivity (four-minute isotime: r=0.26, p=0.51; $T_{LIM}$: r=0.17, p=0.66), or with the change in time-to-exhaustion (four-minute isotime: r=0.54, p=0.13; $T_{LIM}$: r=0.39, p=0.30). These findings suggest that CC sensitivity is not related to either cardiovascular regulation or exercise tolerance in CHF.

Interestingly, EF at study enrolment was strongly correlated with CC sensitivity in CHF patients (r=-0.91, p=0.0006). However, there was no relationship between CC sensitivity and KCCQ overall score (r=-0.25, p=0.52) or EQ-5D-5L score (r=-0.17, p=0.66) in patients with CHF. Together, these data suggest that while elevated chemosensitivity is not correlated with greater changes in time-to-exhaustion, ventilation or conductance with CC inhibition in CHF or controls, CC sensitivity is indeed related to EF, an objective measure of CHF severity.

### 3.1.2 Breathing responses to incremental cycling exercise tests

An important characteristic of CHF is exertional dyspnea, which worsens with disease progression (Laviolette *et al.*, 2014; Laveneziana *et al.*, 2015). As stated in their review, Dubé and colleagues explain that exertional dyspnea in CHF may be related to "overly increased ventilatory demand and abnormal "restrictive" constraints on tidal volume ($V_T$) expansion with development of critical mechanical limitation of ventilation" (O'Donnell *et al.*, 1999; Laveneziana *et al.*, 2009; Dubé *et al.*, 2016). We found that exertional dyspnea was greater in patients with CHF than in controls at a standardized work load (60W; p=0.03), while there was no significant difference in ventilation between groups (Chapter 2, Table 2). $V_T$ was not different at baseline, or 60W, however, there was a trend toward lower $V_T$ at peak in CHF (p=0.06), despite no difference in respiratory rate between groups. Interestingly, CHF patients had significantly higher $P_{ET}CO_2$ than controls at baseline, 60W and peak, suggesting relative hypoventilation at rest and during exercise. While there was no statistically significant difference in inspiratory capacity (IC) between groups (at baseline, 60W and $VO_{2peak}$), our data suggest a trend toward dynamic hyperinflation in the CHF patients, as IC declined progressively from baseline to peak exercise (ΔIC at peak exercise from baseline; p=0.09) (Chapter 2, Table 2). These data suggest that patients with CHF develop dynamic hyperinflation during exercise, which may contribute to their exercise intolerance and exertional dyspnea (Johnson *et al.*, 2000). We would suggest that this dynamic hyperinflation is independent of CC activity/sensitivity, as CC inhibition did not affect IC in CHF.

### *3.2 Limitations and Considerations*

This study evaluated the effects of CC inhibition with low-dose dopamine in 9 patients with CHF and 9 healthy, age- and sex-matched control participants. Since this study was completed on optimally medicated, stable patients, these results cannot be generalized to all CHF patients, especially those that are not receiving optimal medical therapy, have had recent cardiac events, have more severe CHF, or have co-morbidities that may further affect CC activity/sensitivity. Other limitations include the estimation of oxygen delivery using HemoCue to measure hemoglobin concentration and impedance cardiography to estimate cardiac output, and the changing landscape of CHF pharmacotherapy.

#### 3.2.1 Estimation of oxygen delivery

We aimed to evaluate the effects of CC inhibition on cardiovascular function in patients with CHF compared to controls. Our goal was to follow the oxygen cascade, from inhalation of oxygen through the respiratory system to oxygen delivery ($\dot{D}O_2$) at the working muscles. $\dot{D}O_2$ was estimated non-invasively and is calculated as $\dot{D}O_2 = Q \cdot CaO_2$. Cardiac output (Q) was estimated non-invasively with transthoracic impedance cardiography, while arterial oxygen content ($CaO_2$) was estimated by capillary hemoglobin and arterial oxygen saturation ($SpO_2$). To limit participant discomfort and for practical reasons, hemoglobin was sampled at rest, following intravenous catheter insertion prior to exercise and immediately following the TTE trial during active recovery. Therefore, the study design only allowed us to compare $\dot{D}O_2$ either during resting baseline or at $T_{LIM}$ between both study conditions.

The gold standard of Q measurement is by either the direct Fick method or the thermodilution method using a pulmonary artery catheter (Calbet *et al.*, 2016), however for

practical, patient recruitment, and safety reasons, we opted to use a non-invasive method of estimating Q. Non-invasive impedance cardiography has been validated as an effective and clinically acceptable tool to estimate Q in healthy subjects during incremental exercise (Richard *et al.*, 2001). Further, Physioflow® has been found to overestimate Q in a sample of 10 CHF patients as compared to calculating Q using the direct Fick method or by thermodilution. However, it is suggested that Physioflow® may still be useful to estimate relative changes in Q during exercise (Kemps *et al.*, 2008).

Lastly, peripheral oxygen saturation ($SpO_2$) was used to estimate arterial oxygen saturation, rather than sampling arterial blood gases to limit participant dropout and facilitate participant recruitment. Some of the patients in the current study had [Hb] that would classify them as being anemic. There is concern that anemia may affect the quality of pulse oximetry readings (Lee *et al.*, 1991) as it may increase the signal-to-noise ratio due to lower [Hb]. However, further work has shown that anaemia does not affect the relationship between $SpO_2$ and $SaO_2$ to any clinically important degree (Perkins *et al.*, 2003).

### 3.2.2 Inclusion of patients with heart failure with preserved and reduced ejection fraction

To our knowledge, no study to-date has evaluated CC activity/sensitivity in patients with HFPEF (Andrade *et al.*, 2015), and only one study has been conducted on CC activity/sensitivity and experimental HFPEF (Toledo *et al.*, 2017). Importantly, Toledo and colleagues found that central but not peripheral chemosensitivity is elevated in experimental HFPEF, and that elevated central chemosensitivity aggravates diastolic and autonomic dysfunction (2017). Importantly, patients with HFPEF exhibit neuroendocrine activation and severe exercise intolerance (Kitzman *et al.*, 2002), while few medications have been shown to be of therapeutic benefit (McMurray *et*

*al.*, 2012). Therefore, evaluating the potential role of CC activity/sensitivity in cardiopulmonary regulation and exercise tolerance in this group is of importance.

In our current study, we were not able to compare HFPEF vs HFREF since only 2/9 patients had HFPEF. Consequently, we could not evaluate the chemoreflex responses or the effects of CC inhibition on exercise tolerance in this disease sub-group; an important limitation of the present study. Moreover, since both HFPEF and HFREF patients were analyzed within one CHF group, differential responses between HFPEF and HFREF may have influenced the comparison of the CHF group to our control group.

### 3.2.3 Historical context: CC activity/sensitivity in experimental CHF vs patients with CHF

A strength of previous animal work on CC activity/sensitivity is the direct measurement of multiple downstream effects of CC activation. By infusing low-dose dopamine in the antecubital vein in the present study, we cannot confirm with certainty that the drug is acting specifically on the CC without acting on dopamine receptors outside the carotid body. In previous studies by our group, low-dose dopamine was not shown to cause vasodilation in healthy controls (Stickland *et al.*, 2011; Edgell *et al.*, 2015; Phillips *et al.*, 2018). Vasodilation was, however, shown to occur in diseases related to elevated CC activity/sensitivity such as CHF (Stickland et al. 2007; Edgell et al. 2015) and COPD (Phillips *et al.*, 2018), but not in the present group of CHF patients during whole-body exercise. These results would suggest that intravenous dopamine is an effective intervention to inhibit the CC in humans.

In the current study, it was not possible to use close carotid injections of dopamine to inhibit the CC (Stickland *et al.*, 2007), nor was it possible to obtain direct recordings of carotid sinus nerve activity (Sun *et al.*, 1999*a*), or SNA (Sun *et al.*, 1999*b*) during whole body exercise. While

obtaining these data would have been valuable, we believe that by obtaining Q and blood pressure measurements throughout exercise we would be able to assume that changes in conductance in CHF would have been due to reductions in sympathetic vasoconstrictor outflow (although dopamine did not affect conductance in CHF patients in the current study). Additionally, the goal of this study was not to directly measure the effects of CC inhibition on CC activity and SNA, but to evaluate its downstream effects of CC inhibition on cardiopulmonary function. This clinical research model allowed for the non-invasive evaluation of CC inhibition in clinically stable, well-managed patients with CHF.

### 3.2.4 The current study within the context of rehabilitation medicine

This study may influence the way in which patients with heart failure are managed in the context of rehabilitation medicine by emphasizing the importance of patient assessment, optimization of blood pressure and lipids, as well as nutritional counselling (Balady *et al.*, 2007). The absence of effects of CC inhibition on exercise tolerance suggest that further studies are required to evaluate the mechanisms and causes of exercise intolerance in this disease. In the context of the World Health Organization's International Classification of Functioning, Disability and Health, by better understanding the mechanisms (body function and structure) of exercise intolerance (activity) in CHF, and evaluating potential therapies such as pharmacological interventions, physical activity counseling and exercise training, patients could potentially experience increased participation in meaningful activities. Ultimately, exercise training improves aerobic capacity and normalizes the peripheral chemoreflex (Sun *et al.*, 1999*c*), while participation in meaningful activities would lead to improved patient quality of life (Willenheimer *et al.,* 1998; Negrao & Middlekauff, 2008).

### *3.3 Future Direction*

Previous animal studies have laid the groundwork for important clinical research in CC activity/sensitivity in patients with CHF. Animal models of CHF have allowed for rigorous experimental projects looking at the direct mechanisms and effects of enhanced CC activity/sensitivity in this disease condition. Early projects on CHF patients confirmed the effects of enhanced chemoreflex in CHF. Further, some of these studies were completed before the advent of modern neurohormonal therapies (angiotensin receptor blockers and $\beta$-blockers). With the continually changing landscape of modern medical therapies in cardiology, it is now challenging to translate previous studies to current patients with CHF. Our study furthers previous work showing that the CC is involved in cardiovascular regulation at rest in CHF but not during handgrip exercise (Edgell *et al.*, 2015). Further work is required to understand the mechanisms of elevated CC activity/sensitivity in patients with CHF at rest. Additional studies are also required to investigate the mechanisms of exercise intolerance in HFREF vs HFPEF. We suggest that future studies should follow patients longitudinally from HF diagnosis to establish the effects of pharmacotherapies presently used for the treatment of CHF on CC activity/sensitivity.

### *3.4 Summary*

The effect of CC inhibition with low-dose dopamine on exercise tolerance, and cardiovascular and respiratory regulation was evaluated in clinically stable, optimally medicated patients with CHF and age- and sex-matched controls. It was found that the CC is not a key mediator of cardiovascular function or ventilation during exercise in CHF patients receiving optimal medical therapy, despite evidence of elevated peripheral chemosensitivity in the CHF

group. Further, control participants showed improved vascular conductance with dopamine, but this improvement did not translate to increased exercise tolerance.

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# APPENDICES

# APPENDIX A: LITERATURE REVIEW

Involuntary control of physiological function is coordinated by the autonomic nervous system. At the onset of exercise, changes in sympathetic and parasympathetic activity occur to maintain homeostasis and to ensure $O_2$ delivery to the exercising skeletal muscles. Parasympathetic withdrawal occurs initially (Robinson *et al.*, 1966), increasing cardiac output through augmentations in heart rate. While sympathetic nervous activity increases with exercise, functional sympatholysis occurs, blunting the vascular response to the increased sympathetic nerve activity (SNA) which helps ensure adequate blood flow to exercising muscles. In chronic heart failure (CHF), resting SNA is increased, and patients with CHF show an amplified SNA response to exercise which can reduce blood flow to working muscle and negatively affect exercise tolerance (Notarius *et al.*, 1999). The reason(s) for the increased SNA at rest and during exercise in CHF is not well understood, but evidence suggests that it may be due to increased carotid chemoreceptor activity and sensitivity (Sun *et al.*, 1999*b*; 1999*a*; Ponikowski *et al.*, 2001; Stickland *et al.*, 2007; Giannoni *et al.*, 2008; 2009).

## *A.1 Exercise in Health*

The initial ANS response to exercise is vagal withdrawal (or parasympathetic withdrawal), which causes a rapid increase in heart rate and cardiac output. Vagal withdrawal can be attributed to the initial heart rate response up to a heart rate of 100 beats per minute (Robinson *et al.*, 1966). With the withdrawal of parasympathetic tone, heart rate may reach higher levels in response to increased sympathetic neurotransmitter release. The exercising skeletal muscle increases its metabolic activity and thus has higher $O_2$ demands than does resting muscle. The cardiovascular

system must adapt accordingly to meet the metabolic demands of the exercising muscles, redirecting blood flow towards the skeletal muscle, away from non-exercising tissue (Rowell, 1993), and increasing cardiac output through increases in stroke volume and heart rate to meet metabolic requirements.

### A.1.1 Blood flow regulation during exercise

Maintaining adequate blood pressure is important to maintain blood flow to the active muscles, viscera, and brain. Blood pressure is regulated by cardiac output and vascular conductance. Cardiac output (Q) is proportionally related to mean arterial pressure (MAP), and total vascular conductance (TVC) is inversely related to blood pressure, as displayed by this equation:

$$MAP = Q / TVC$$

Q is the product of heart rate and stroke volume, and TVC is the reciprocal of resistance, therefore:

$$\text{Systemic Vascular Resistance (SVR)} = (MAP - CVP) / Q$$

*CVP: central venous pressure

In the transition from rest to exercise, TVC increases despite concurrent vasoconstriction in non-exercising tissue (Buckwalter & Clifford, 2001). This vasoconstriction, a result of the increase in SNA during exercise, has two roles: the redirection of blood flow toward exercising muscle, and the maintenance of blood pressure (McArdle *et al.*, 2010). The distribution of cardiac output during exercise changes dramatically. In resting, healthy humans, cardiac output is approximately 5 L/min (Laughlin *et al.*, 2012), however, during maximal exercise, cardiac output reaches around 30 L/min, wherein 85-90% of cardiac output is delivered to the exercising skeletal and cardiac muscles (Laughlin *et al.*, 2012). Although delivery of blood is greatly enhanced in exercising

muscle, delivery to the brain, skin, and heart increases only slightly. In addition, flow to the liver and kidneys drops significantly, from 27% and 22% of cardiac output to 2% and 1%, respectively (McArdle *et al.*, 2010).

Skeletal muscle receives the majority of cardiac output during maximal exercise, where a small reduction in skeletal muscle vascular conductance (or increased resistance) can cause a significant rise in mean arterial pressure (Buckwalter & Clifford, 2001). Conversely, in vascular beds receiving a small fraction of cardiac output, changes in conductance will have minimal effects on pressure. Therefore, the fine control of blood vessel diameter in active skeletal muscle plays a crucial role in the maintenance of a stable blood pressure during exercise. Furthermore, sympathetic tone may also limit blood flow, restricting $O_2$ delivery to the exercising skeletal muscle. Consequently, exercise intensity and duration may be severely reduced (Buckwalter & Clifford, 2001) if muscle sympathetic vasoconstriction is overly increased.

### A.1.2 Sympathetic control of blood flow

Seeking to test whether sympathetic changes in blood flow to active skeletal muscles have an effect on muscle oxygen uptake ($V\dot{O}_2$), Joyner and colleagues found that resting forearm blood flow was increased after local anesthetic sympathetic nerve blockade at rest (Joyner *et al.*, 1992). During handgrip exercise (rhythmic maximum voluntary contractions), forearm blood flow was increased during all workloads with sympathetic blockade compared to exercise without blockade (Joyner *et al.*, 1992). For example, at 70-80% of maximum voluntary contraction, forearm blood flow rose from 35.4 to 50.7 $ml \cdot 100ml^{-1} \cdot min^{-1}$ and forearm $V\dot{O}_2$ rose from 45.5 to 54.2 $ml \cdot kg^{-1} \cdot min^{-1}$ during sympathetic blockade (Joyner *et al.*, 1992). The authors concluded that muscle

sympathetic nerves regulate blood flow to exercising muscles during both light and heavy handgrip exercise and that vasoconstriction may restrict $V\dot{O}_2$ of the exercising muscles (Joyner *et al.*, 1992).

*A.1.21 Functional sympatholysis*

Remensnyder and colleagues first described functional sympatholysis (Remensnyder *et al.*, 1962). As oxygen demand increases with exercise intensity, muscle vasculature responsiveness to sympathetic stimulation is attenuated; with resistance decreasing slightly thereafter (Buckwalter *et al.*, 2001). Saltin and Mortensen suggested that functional sympatholysis is the "direct action of one or more exercise-produced compound(s) that block the vasoconstrictive effect of noradrenaline via its receptor on smooth muscles" (Saltin & Mortensen, 2012). Therefore, even though SNA rises in skeletal muscles during exercise, there is still a net increase in blood flow in exercising muscle due to functional sympatholysis.

**A.1.3 Sympathetic nerve activity: reflex responses to exercise**

SNA increases reflexively in healthy humans during dynamic exercise (Victor *et al.*, 1987). Increased SNA occurs with the combined activity of muscle mechanoreceptors, muscle metaboreceptors, arterial baroreceptors and chemoreceptors as well as central command (Rowell & O'Leary, 1990; O'Leary, 1993; Strange *et al.*, 1993; Piepoli *et al.*, 1995; Iellamo *et al.*, 1999; Crisafulli *et al.*, 2003).

The "exercise pressor reflex" was first described by Alam & Smirk (1937). They showed that when exercising a leg with the thigh occluded by inflation of a sphygmomanometer cuff, both systolic and diastolic pressure remained elevated post-exercise cessation. Blood pressure lowered only slightly until the cuff was released and circulation within the thigh returned to baseline levels

(Alam & Smirk, 1937). Since occlusion of the thigh impaired limb blood flow, metabolites built up, stimulating the muscle metaboreflex. McCloskey and Mitchell, and Coote et al. confirmed the importance of afferent neural feedback evoked by skeletal muscles, finding that the reflex cardiovascular and respiratory responses that occur during exercise are initiated inside the skeletal muscle (Coote *et al.*, 1971; McCloskey & Mitchell, 1972).

### *A.1.31 The arterial baroreflex response to exercise*

Baroreceptors are sensory nerve endings that respond to wall stretch due to increased pressure. Arterial baroreceptors are located at the aortic arch and the bifurcation of the common carotid artery within the carotid sinus, and are responsible for the maintenance of a stable arterial pressure both at rest and during exercise; predominantly through adjustments in sympathetic outflow (Bevegård & Shepherd, 1966; Melcher & Donald, 1981; Walgenbach & Donald, 1983; Walgenbach & Shepherd, 1984).

When blood pressure rises, arterial walls stretch and so do the unencapsulated nerve endings of the baroreceptors at the medial adventitial border of the arterial walls (Sheehan *et al.*, 1941). The deformation of the baroreceptors caused by the increased blood pressure leads to an increase in neuronal firing, causing a reflexive increase in parasympathetic activity and a reduction in SNA (Fisher *et al.*, 2011). When blood pressure decreases, the opposite happens; afferent firing slows, increasing SNA and reducing parasympathetic nerve activity (Fisher *et al.*, 2011). In both situations, vascular resistance and cardiac output are adjusted. It has been well established that the arterial baroreflex is continuously reset to regulate blood pressure during exercise (Bevegård & Shepherd, 1966; Potts *et al.*, 1993; Papelier *et al.*, 1994; Fadel *et al.*, 2004; Raven *et al.*, 2006).

During aerobic whole-body exercise, mean arterial blood pressure usually stays around 100 mmHg and does not increase significantly above resting values in young healthy humans (Blomqvist & Saltin, 1983). During heavy exercise in dogs, there is a slight increase in blood pressure where its operating point rises by approximately 15-20 mmHg (Melcher & Donald, 1981). Therefore, the increase in pressure may be less than 20% while cardiac output rises four to eight times its resting value (Joyner & Casey, 2015), attributable to vasodilation. Work in humans (Potts *et al.*, 1993) and dogs (Sheriff *et al.*, 1990) suggests that the carotid baroreflex is highly sensitive to blood pressure during exercise, while also maintaining the capacity to respond to hypotensive stimuli, providing "hypotension protection" during exercise.

### *A.1.32 The skeletal muscle mechanoreflex during exercise*

Mechanoreceptors respond to changes in local mechanical stimuli such as pressure or distortion (Stedman, 2008). Hollander and Bouman found that heart rate increases after muscle contraction caused by electrical stimulation, due to the stimulation of mechanoreceptors located within the affected muscle (Hollander & Bouman, 1975). Findings described by McMahon and McWilliam have supported these earlier findings in the decerebrate (removed cerebrum) cat, suggesting that the muscle group III mechanoreceptors augment heart rate through the withdrawal of vagal tone to the SA node at the onset of exercise (McMahon & McWilliam, 1992). Multiple groups have shown that the buildup of metabolites in skeletal muscles during exercise potentiates the mechanoreflex, increasing SNA and blood pressure (Kaufman *et al.*, 1983; Adreani & Kaufman, 1998; Cui *et al.*, 2008; Murphy *et al.*, 2011).

*A.1.33 The metaboreflex*

Metaboreceptors are afferent nerve endings located in skeletal muscles that elicit responses to metabolites. The metaboreflex induces increases in SNA, regulating blood pressure, cardiac output (by heart rate or stroke volume), and local blood flow distribution (Alam & Smirk, 1937; Klausen *et al.*, 1982; Adams *et al.*, 1992). Increases in sympathetic outflow to the systemic vasculature are both exercise intensity-dependent and time dependent. Increases are observed during moderate to high-intensity exercise, and occur around 1-minute into exercise (Mark *et al.*, 1985; Seals *et al.*, 1988; Victor *et al.*, 1988; Victor & Seals, 1989; Ray *et al.*, 1993; Hansen *et al.*, 1994; Sinoway *et al.*, 1994). As explained by Boushel, Joyner and colleagues found that when blood flow to exercising muscles is insufficient: 1) when blood pressure is increased due to rises in cardiac output and resistance in inactive vascular beds, increased perfusion pressure in working muscles improves blood flow; 2) when cardiac output and vascular conductance do not match in exercising muscle, the metaboreflex responds by inducing vasoconstriction to maintain sufficient blood flow (Joyner, 1991; Boushel, 2010).

*A.1.34 The peripheral chemoreflex*

Chemoreceptors respond to local chemical stimuli through nerve impulses. Peripheral chemoreceptors are located in the carotid bodies at the bifurcation of the common carotid artery, as well as in aortic bodies within the aortic arch. Their nervous impulses relay information to the medulla through the carotid sinus (carotid chemoreflex) and vagus (aortic chemoreflex) nerves. The carotid bodies contain two types of cells; 1) type I glomus cells are stimulated by reductions in the partial pressure of oxygen ($PO_2$), increases in partial pressure of carbon dioxide ($PCO_2$) and decreases in pH; and 2) type II cells comprise 15-20% of the carotid body parenchyma and are

glia-like dormant stem cells that respond to chronic hypoxia by differentiating into type I glomus cells (Pardal *et al.*, 2007).

When active, the CC causes sympathetic vasoconstriction in vascular beds and affects cardiovascular function (de Burgh Daly & Scott, 1962; Rutherford & Vatner, 1978; Murray *et al.*, 1984). Carotid bodies are exceptionally sensitive to hypoxia and respond to small reductions in arterial $PO_2$. Moreover, the reflex response to low $PO_2$ occurs almost immediately after the initial hypoxic stimulus: within seconds (Kou *et al.*, 1991). When a healthy human is breathing at normoxia, nitric oxide (a potent vasodilator) and carbon monoxide inhibit the glomus cells within the carotid chemoreceptor (Prabhakar, 1999). Conversely, hydrogen sulfide has an excitatory effect on the glomus cells, which is demonstrated during transient hypoxia (Peng *et al.*, 2010).

In the presence of oxygen, nitric oxide (produced by endothelial and neuronal nitric oxide synthase (eNOS and nNOS, respectively)) affects carotid body activity through two pathways. Nitric oxide originating from eNOS regulates blood flow to the carotid body (Kline *et al.*, 2000), and since the carotid body is a highly vascularized organ and receives the highest blood flow per gram of tissue (de Burgh Daly *et al.*, 1954; Clarke *et al.*, 1986; Barnett *et al.*, 1988), the effects of endothelium derived nitric oxide are significant. During normoxia, oxygen dependent nitric oxide production by nNOS may mediate efferent inhibition by activating potassium ion channels (Prabhakar, 1999) and inhibiting L-type calcium ion channels in glomus cells (Summers *et al.*, 1999), causing a reduction in sensory activity. In contrast, during hypoxia, nitric oxide production is reduced due to the insufficient supply of oxygen (Prabhakar, 1999), contributing to carotid chemoreceptor excitation (Prabhakar & Semenza, 2012).

Stickland and colleagues evaluated the effects of transient CC inhibition on total and hindlimb conductance, and blood pressure at rest and during exercise in healthy dogs (Stickland

*et al.*, 2007). The authors inhibited the CCs using dopamine (5 to 10 $\mu g \cdot kg^{-1}$) or hyperoxic lactated Ringer's solution through close-carotid injections. Vasodilation was not observed with CC inhibition in resting healthy dogs, however, an instant vasodilatory response occurred with CC inhibition during exercise (increase in conductance and decrease in blood pressure). The vasodilation following CC inhibition during exercise was eliminated with α-adrenergic blockade and was absent in healthy exercising animals following carotid body denervation. These results established an important role for the CCs in cardiovascular control in the healthy animal during exercise. Moreover, the findings showed the occurrence of increased sympathetic vasoconstrictor outflow during exercise secondary to increased CC responsiveness, despite the absence of increased circulating chemoreceptor stimuli within arterial blood, implying an exercise-induced sensitization of the CC (Stickland *et al.*, 2007).

Following the finding that inhibition of the CCs in healthy exercising dogs causes a local vasodilatory response at the level of the exercising skeletal muscle (Stickland *et al.*, 2007), Stickland and colleagues sought to determine whether CC inhibition reduced muscle sympathetic nerve activity (MSNA) in exercising humans (Stickland *et al.*, 2008). The authors found that in healthy humans, the CC contributes to exercise-induced increases in MSNA. They also found that breathing 100% $O_2$ caused an immediate reduction in MSNA during exercise, while no effect was observed with hyperoxia at rest (Stickland *et al.*, 2008). At that point, it was unknown whether the reduction in MSNA with CC inhibition in humans translated to increased blood flow in the exercising skeletal muscle (Stickland *et al.*, 2011).

Stickland and colleagues examined the MSNA response to changes in muscle blood flow at rest and during exercise, during normoxia and hypoxia in healthy humans (Stickland *et al.*, 2011). The authors inhibited the CC at rest, and during leg-extension exercise with transient

hyperoxia and/or low-dose dopamine ($2\mu g \cdot kg^{-1} \cdot min^{-1}$). Additionally, in separate trials, the CCs were inhibited with dopamine at rest and during exercise while the subjects breathed hypoxic gas [target arterial oxygen saturation ($SpO_2$) = 85%]. CC inhibition caused an increase in femoral muscle blood flow and conductance during normoxic exercise. At rest, CC inhibition did not cause a similar increase in peripheral blood flow. An unexpected outcome was that CC inhibition with dopamine did not cause an alteration in cardiovascular function during hypoxia at rest nor during exercise. The rapid vasodilation observed during hyperoxic breathing was blocked with low-dose dopamine, potentially attributable to vasodilation being the direct effect of CC inhibition. The authors concluded that the CC contributes to the sympathetic control of skeletal muscle blood flow during normoxic exercise in health (Stickland *et al.*, 2011).

It has been established that CC stimulation causes an increase in ventilation (Olson *et al.*, 1988; Curran *et al.*, 2000), and provokes increases in sympathetic neural vasoconstrictor outflow to the skeletal muscle, renal and mesenteric vascular beds (Rutherford & Vatner, 1978; Balkowiec *et al.*, 1993; Sun & Reis, 1994; Guyenet, 2000). CC activity/sensitivity is significantly increased with exercise, and when stimulated, its ventilatory response is heightened with exercise (Forster *et al.*, 1974). Additionally, there is an exercise-induced activation/sensitization of the CC in the absence of circulating stimuli in healthy dogs (Stickland *et al.*, 2007). Translating this work to healthy humans, it was found that the CC participates in the sympathetic response to handgrip exercise (Stickland *et al.*, 2008) and that the CC contributes to the sympathetic control of skeletal muscle blood flow during normoxic exercise (Stickland *et al.*, 2011)

## *A.2 Chronic Heart Failure and Exercise*

### A.2.1 The physiology of chronic heart failure

CHF is the inadequacy of the heart as a pump to maintain the circulation of blood, and as a result, delivery of oxygen is insufficient to meet metabolic demand (Dickstein *et al.*, 2008). Although significant improvements have been made regarding treatment for this chronic health condition, the death rate remains high: 51.5% of people diagnosed with CHF will die within 5 years of initial diagnosis (Taylor *et al.*, 2017). As it relates to the International Classification of Functioning, Disability and Health (ICF) (World Health Organization, 2001), the changes in body functions and structures (Churilla *et al.*, 2016) related to heart disease, together with environmental and personal factors such as family and friends, individual attitudes, and social and health services, among others (Cieza *et al.*, 2004), have downstream effects on the performance of activities of daily living (Racca *et al.*, 2010; Dunlay *et al.*, 2015), limiting participation in meaningful activities, and eventually affecting patient quality of life (Racca *et al.*, 2010).

In heart failure with reduced ejection fraction (HFREF), the slope of the end-systolic pressure-volume relationship is decreased and end-systolic volume increases (Klabunde, 2011). As a result, end-diastolic volume increases, and stroke volume and ejection fraction decrease. A diagnosis is made when patients have a left ventricular ejection fraction of <40%. In patients suffering from heart failure with preserved ejection fraction (HFPEF) the slope of the end-diastolic pressure-volume relationship (passive filling curve) increases due to reduced ventricular compliance caused by hypertrophy or hindered myocardial relaxation (Borlaug & Paulus, 2011). Consequently, end-diastolic volume is reduced, and end-diastolic pressure increases. Therefore, stroke volume declines and ejection fraction is unaffected (Klabunde, 2011). Several criteria must be met before a diagnosis of HFPEF is made, including I) clinical signs or symptoms of HF; II)

preserved or normal left ventricular ejection fraction; and III) abnormal left ventricular diastolic function (Vasan & Levy, 2000).

### A.2.2 Increased SNA in chronic heart failure

In 1984, Cohn et al. evaluated hemodynamics, plasma norepinephrine, and plasma renin activity in 106 resting patients with moderate to severe congestive heart failure (Cohn *et al.*, 1984). Of the initial 106 patients, 60 died within 5 years. Among the five significant prognosticators (heart rate, plasma renin activity, plasma norepinephrine, serum sodium, and stroke-work index), plasma norepinephrine, a sympathetic neurotransmitter, alone was identified as being independently related to mortality (Cohn *et al.*, 1984). In untreated heart failure, cardiac norepinephrine spillover is increased as much as 50-fold, which is comparable to levels seen in healthy hearts during maximal exercise (Morris *et al.*, 1997). These findings, among others, indicate that the sympathetic nervous system plays a significant role in the pathophysiology of CHF.

CHF has been associated with higher levels of SNA (Floras, 1993; Narkiewicz *et al.*, 1999; Floras, 2009). Chronic sympatho-excitation in CHF adversely affects excitation-contraction coupling (physiological process of converting electrical stimuli into a mechanical response) (Piacentino *et al.*, 2003) in the myocardium and increases the occurrence of apoptosis (Olivetti *et al.*, 1997) in the myocardium. In addition, chronically elevated SNA increases peripheral vasoconstriction and ventricular afterload, which leads to reductions in cardiac output resulting in tissue hypo-perfusion and ventricular remodeling (Del Rio *et al.*, 2013). Elevated SNA could participate in the development of left ventricular diastolic dysfunction, increasing cardiovascular risk (Grassi *et al.*, 2009). Furthermore, increased SNA activates the renin-angiotensin system leading to congestion and tissue edema as a result of fluid retention. These secondary effects of

chronically increased SNA play key roles in the downward spiral of CHF (LeJemtel & Sonnenblick, 1993).

*A.2.21 Potential mechanisms for increased SNA in chronic heart failure at rest:*

A multitude of afferent and efferent stimuli come into play in the sympathetic activation observed in CHF. As explained in Floras and colleagues review article (Floras, 2009), when a patient develops left ventricular systolic dysfunction, sympathoinhibitory input from primarily ventricular mechanoreceptors declines (Bradley *et al.*, 2003), while efferent sympathetic modulation by the pulmonary stretch receptors and arterial baroreceptors remains intact (Somers *et al.*, 1995). Meanwhile, there is a reduction in the vagal control of heart rate and efferent vagal and sympathetic heart rate responses to arterial baroreflex stimuli (Ebert *et al.*, 1992). Excitatory inputs from an atrial reflex stimulated by increases in cardiac filling pressures add to the increased sympathetic afferent stimuli originating in the CC and skeletal muscles (Malliani & Montano, 2002), and from chemically sensitive ventricular afferent nerve endings, triggered by ischemia (Notarius *et al.*, 2007). Moreover, afferent renal nerves could also provoke sympathoexcitation in patients suffering from renal insufficiency and/or right heart failure (Hausberg *et al.*, 2002). Regarding central excitatory mechanisms, an angiotensin II-AT1 receptor-NADPH-superoxide axis (Gao *et al.*, 2004), and sleep apnea (Somers *et al.*, 1995) can contribute to increased SNA. Facilitation of norepinephrine release (Johansson *et al.*, 1997) and changes in norepinephrine uptake are possible efferent mechanisms. In early systolic dysfunction, there is a selective increase in cardiac norepinephrine release (Rundqvist *et al.*, 1997) as well as a decrease in tonic and reflex vagal heart rate modulation (Binkley *et al.*, 1991), while in advanced heart failure, there is a global increase in SNA (Floras, 2009), blunted vagal and sympathetic heart rate modulation (Horner *et*

*al.*, 1996; Malliani & Montano, 2002), and impairment of the reflex sympathetic regulation of vascular resistance (Zelis *et al.*, 1981) .

*A.2.22 Exercise and sympathetic nerve activity in chronic heart failure*

Sympathetic over-activity contributes to the functional impairment of skeletal muscles in heart failure; since sympathetic-induced vasoconstriction during both rest and exercise restricts muscle blood flow, arteriolar dilation, and capillary recruitment, causing hypo-perfusion, ischemia, increased release of reactive oxygen species, and chronic inflammation (Negrao & Middlekauff, 2008). The effects of chronically increased SNA seen at rest are further exaggerated during exercise in patients with CHF.

In patients with treated heart failure and preserved exercise capacity, MSNA at rest is comparable to that of healthy age- and sex-matched controls (Notarius *et al.*, 2001*a*). Conversely, in patients with HFREF, resting MSNA is heightened and related to the degree of exercise intolerance (Notarius *et al.*, 1999; 2014), as explained earlier. Heightened MSNA is regarded as an independent predictor of premature mortality in this patient group (Cohn *et al.*, 1984; Kaye *et al.*, 1995; Petersson, 2005; Barretto *et al.*, 2009).

*A.2.23 Potential mechanisms for increased SNA in chronic heart failure during exercise:*

Multiple reflexes originating from skeletal muscles can modulate sympathetic activity in CHF during exercise (Floras, 2009). Among these reflexes are: 1) an adenosine stimulated sympathoexcitatory reflex, involving the participation of angiotensin acting via the angiotensin II type 1 receptor as a neural intermediary (Rongen *et al.*, 1998; Notarius *et al.*, 2001*a*); 2) increases in local venous pressure (Chen *et al.*, 1995); 3) muscle mechanoreflex activation during passive

exercise (Middlekauff *et al.*, 2004); and 4) muscle metaboreflex activation during handgrip exercise (Notarius *et al.*, 2001*a*; 2001*b*).

### A.2.3 Evidence of an enhanced carotid chemoreflex in chronic heart failure

Sun and colleagues studied peripheral chemoreflex function in conscious rabbits with pacing-induced heart failure (Sun *et al.*, 1999*b*). They determined that the baseline renal SNA at normoxia was higher in CHF rabbits than in sham rabbits and that the magnitudes of changes in renal SNA and minute volume in response to stimulation of the peripheral chemoreceptors and the slopes of renal SNA-arterial $PO_2$ and minute volume-arterial $PO_2$ curves were larger in CHF than in sham rabbits. By inhibiting the peripheral chemoreceptors through inhalation of 100% oxygen, renal SNA was reduced in CHF but not in sham rabbits. These data indicate that enhanced peripheral chemoreflex activation/sensitization is observed in the rabbit model of pacing-induced CHF and that increased peripheral chemoreflex function contributes to the heightened SNA observed in CHF (Sun *et al.*, 1999*b*).

Ding and colleagues (2011) studied the effect of chronic reductions in blood flow to the carotid body on peripheral chemoreflex function in rabbits. The study design comprised of causing pacing-induced CHF in rabbits, where blood flow in the carotid artery was reduced by approximately 36% after pacing. Compared to sham, carotid artery occlusion caused neural nitric oxide synthase (nNOS) expression and nitric oxide levels to lower, and angiotensin II type 1 receptor protein expression and angiotensin II concentration to increase; similar to CHF rabbit carotid bodies. By using a nitric oxide donor and angiotensin II type 1 receptor antagonist, CC sensitivity was reduced in carotid artery occlusion. Therefore, blood flow reduction to the carotid

body is involved in the augmentation of peripheral chemoreflex sensitivity in CHF (Ding *et al.*, 2011).

### A.2.4 Clinical significance of the carotid chemoreceptor in chronic heart failure

The clinical significance of the CC is highlighted by previous work showing that heightened CC sensitivity/activity is predictive of mortality in patients with CHF (Ponikowski *et al.*, 2001; Jankowska *et al.*, 2007; Giannoni *et al.*, 2009). Ponikowski et al. evaluated CC sensitivity through the hypoxic ventilatory response test (Chua & Coats, 1995; Chua *et al.*, 1996*a*) in 80 patients with HFREF (mean LVEF: 24%) (Ponikowski *et al.*, 2001). The patients exhibited heightened CC sensitivity and reduced baroreflex sensitivity, and out of the initial 80 participants, 37 died during follow-up (median 41 months). The authors established that high peripheral chemosensitivity was quantified as $\geq 0.72 L \cdot min^{-1} \cdot \%SaO_2^{-1}$. Of significance, 3-year survival was 41% in the patients with elevated CC sensitivity, in comparison with 77% in patients with normal CC sensitivity. Importantly, heightened CC sensitivity was identified as being an independent predictor of death (Ponikowski *et al.*, 2001).

Another study investigated the prognostic significance of chemosensitivity to hypercapnia in CHF (Giannoni *et al.*, 2009). One hundred ten HFREF patients were recruited and underwent tests evaluating the hypoxic (Rebuck & Campbell, 1974) and hypercapnic (Read, 1967) (rebreathing technique) ventilatory responses and were followed up for a median period of 29 months. 28% of patients showed enhanced chemosensitivity to hypoxia as well as hypercapnia. Both chemosensitive and non-chemosensitive CHF groups had the same LVEF, however, the chemosensitive patients were more symptomatic, had significantly higher plasma brain natriuretic peptide and norepinephrine, heightened hypercapnic ventilatory response, more Cheyne-Stokes

respiration, and more ventricular arrhythmias. Like Ponikowski et al. (2001) found, four-year survival in chemosensitive patients was reduced to only 49%, compared to 100% in patients with normal chemosensitivity. Consequently, heightened central and peripheral sensitivity to both hypoxia and hypercapnia, provoking neurohormonal derangement, ventilation instability, and ventricular arrhythmias, is a severe adverse prognostic marker in HFREF (Giannoni *et al.*, 2009).

### A.2.5 Evidence of carotid chemoreceptor control of cardiovascular function in chronic heart failure

In their 2007 study, Stickland and colleagues induced CHF in dogs by chronic rapid cardiac pacing. CHF was characterized by impaired cardiac function, heightened chemosensitivity, and greater sympathetic restraint at rest and during exercise (Stickland *et al.*, 2007). These dogs had an HFREF (mean ejection fraction of 18). Unlike healthy dogs, CC inhibition in resting CHF caused vasodilation. A similar vasodilatory response was observed during exercise in CHF and in healthy dogs. Vasodilation following CC inhibition during exercise and in CHF was eliminated with α-adrenergic blockade and was absent in healthy exercising animals following carotid body denervation. Therefore, as well as finding that the CC plays a role in cardiovascular control in health, the results suggest that in an animal model of CHF, cardiovascular control by the CCs occurs both at rest and during exercise (Stickland *et al.*, 2007).

Translating Stickland and colleagues previous work (Stickland *et al.*, 2007; 2008; 2011), Edgell et al. explored the role of the CC in cardiovascular control during both rest and exercise in CHF patients and controls (2015). 11 clinically stable patients with HFREF (EF = 39 ± 5%) and 10 risk-matched controls (EF: 65 ± 2%) performed randomized trials with or without dopamine infusion ($2\mu g \cdot min^{-1} \cdot kg^{-1}$) at rest and during 40% maximal voluntary contraction handgrip (HG)

exercise, and a resting trial of 2 min of inspired 100% oxygen. In resting patients with HFREF, dopamine reduced ventilation, as well as increased cardiac and stroke indexes; likely a result of lower total peripheral resistance index ($TPR_i$). In contrast, dopamine had no effect on these variables in healthy controls. Hyperoxia reduced ventilation in patients with HFREF, but not in controls. These observations imply that the CC is tonically active at rest in HFREF but not in controls with cardiovascular disease risk factors (Edgell *et al.*, 2015). As expected, HG exercise increased heart rate, ventilation, and brachial conductance of the non-exercising arm in both experimental groups (Edgell *et al.*, 2015). Dopamine infusion did not induce changes in mean arterial pressure, heart rate, or ventilatory responses to HG in HFREF nor in controls. Importantly, brachial conductance rose with dopamine in controls. This finding coincides with earlier work in healthy humans and dogs (Stickland *et al.*, 2007; 2011), and is indicative of a reduction in SNA at least partially due to CC inhibition. Contrary to earlier findings in CHF dogs (Stickland *et al.*, 2007), CC inhibition with dopamine did not improve cardiovascular function during exercise in HFREF (Edgell *et al.*, 2015).

The findings of this study were limited by multiple factors, including the authors inability to obtain SNA measurements throughout the entire protocol, the large prevalence of sleep apnea within the cohort which may have an additive effect on CC sensitivity, and cardiovascular function with CHF, and the use of optimal evidence-based pharmacotherapy within the CHF group, which may modulate CC activity/sensitivity. Significant limitations of this study were the authors choice to recruit HFREF patients only, and not to include patients diagnosed with HFPEF, as well as the use of handgrip exercise rather than full-body exercise (Edgell *et al.*, 2015). These limitations highlight the gap in the literature, and the need to further knowledge on the issue of CC activity/sensitivity and its potential implications in CHF and exercise.

### A.2.6 The pulmonary response to exercise in chronic heart failure

Exertional dyspnea is an important symptom of patients with CHF that worsens with disease progression and that severely affects patient quality of life and the ability to function and participate in meaningful activities (Laviolette *et al.*, 2014; Laveneziana *et al.*, 2015). In their review, Dubé and colleagues suggest that symptom limitation is determined by a combination of factors: not only hemodynamics, but also cardio-ventilatory, neurohumoral and peripheral factors (Dubé *et al.*, 2016). It has been shown that as ventilatory demand increases with exercise intensity/duration, restrictive constraints on tidal volume expansion and mechanical limits on ventilation contribute to dyspnea in CHF (O'Donnell *et al.*, 1999; Laveneziana *et al.*, 2009). Additionally, patients with CHF have been reported to have an abnormal ventilatory response to exercise, as shown by a heightened $\dot{V}_E/\dot{V}CO_2$ slope (Clark *et al.*, 1995; 1997; Kleber *et al.*, 2000; Agostoni *et al.*, 2000; 2006; Chua *et al.,* 1996; Laveneziana *et al.*, 2009). In addition to having an exaggerated ventilatory response to exercise at a given workload compared to controls, CHF patients take shallower breaths (tidal volume, $V_T$) but have a higher breathing frequency (Wasserman *et al.*, 1997; O'Donnell *et al.*, 1999; Laveneziana *et al.*, 2009). The CC has been shown to be related to the abnormal ventilatory response to exercise in patients with CHF, and a heightened $\dot{V}_E/\dot{V}CO_2$ slope is an independent prognostic marker in this disease (Chua *et al.*, 1997*a*).

### A.2.7 Potential differences between chronic heart failure etiologies and exercise

As explained by Andrade and colleagues, sympathetic hyperactivity is independent of CHF etiology. A recent study by Niewiński and colleagues (2013) has shown that carotid body denervation may be a clinical treatment option to restore autonomic function and improve outcomes in CHF (Niewinski *et al.*, 2013*b*). In this case study, the patient with HFREF (New York

Heart Association functional class II) experienced reduced CC sensitivity, and an improvement in exercise capacity, sleep disordered breathing and quality of life following denervation. Studies in humans as well as experimental models of CHF, have shown that CC activity and sensitivity, when heightened, contributes to the progression of HFREF. Surprisingly, CC activity and sensitivity has not been studied in HFPEF, neither in experimental models of CHF nor in patients with CHF (Andrade *et al.*, 2015). Kitzman and colleagues found that patients with HFPEF have similar symptoms to those of patients with HFREF, however, their pathophysiological characteristics are not as severe (Kitzman *et al.*, 2002).

### *A.3 Summary*

SNA has been shown to be chronically elevated in patients with CHF (Floras, 1993; Narkiewicz *et al.*, 1999; Floras, 2009). Chronic sympathoexcitation leads to further cardiovascular deterioration in CHF and is a prognosticator of mortality. Indeed, heightened SNA leads to peripheral vasoconstriction, reductions in vascular compliance and increases in ventricular afterload. This reduces cardiac output and causes ventricular remodeling and tissue hypo-perfusion. The reduction in blood flow to exercising muscles may be the cause of the significant exercise intolerance observed in CHF. Evidence suggests that chronically heightened CC activity and sensitivity in CHF contribute to the heightened SNA and sympathetic constraint of exercising muscle blood flow. The effects of CC inhibition on handgrip exercise have been studied in humans with HFREF, however, the effects of CC inhibition on full-body exercise tolerance and tissue oxygenation in HFPEF and HFREF are unknown. Further understanding of the effects of CC inhibition on exercise tolerance will potentially open avenues for the treatment of CHF.

## APPENDIX B: Detailed Methods

### *B.1 Sample size calculation*

Previous work (Stickland *et al.*, 2011) has demonstrated a 21 ± 18% improvement in femoral conductance with low-dose dopamine infusion ($2\mu g \cdot kg^{-1} \cdot min^{-1}$) during leg extension exercise in 9 healthy adults. Assuming that participants with CHF would have similar improvements in conductance during whole-body exercise, an *a priori* sample size calculation predicted that 13 CHF patients would be required to demonstrate a 21% change in conductance ($\alpha = 0.05$; $\beta = 0.2$). An equal number of control subjects were recruited for comparison.

### *B.2 Procedures*

#### B.2.1 Pulmonary function test

The standardized pulmonary function test was completed in conformity with the American Thoracic Society guidelines (2005), wherein standardized spirometry (Buist, 1987; Gardner *et al.*, 1987; Anon, 1987), diffusing capacity, lung volumes, and plethysmography were obtained. The test was completed using the Vmax metabolic system (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA). All breathing maneuvers were repeated a minimum of 3 times, and the subjects were seated in the upright position, with both feet flat on the ground.

#### B.2.2 Cardiopulmonary exercise test

A physician was present during the cardiopulmonary exercise tests. Following a 2-minute resting period (up-right, sitting), participants began cycling exercise using a step protocol, wherein the work rate was gradually increased at every 2-minute stage by either 10 or 20 watt increments.

During the last minute of every stage, blood pressure measurements were obtained, followed by ratings of perceived exertion (modified Borg) (Borg, 1982), and subsequent inspiratory capacity (IC) maneuvers (Guenette *et al.*, 2013).

The exercise tests were deemed maximal if the participants reached a respiratory exchange ratio (RER) ≥ 1.1, a heart rate > 90% predicted max, a patient exhaustion/Borg scale > 9/10, a plateau in $\dot{VO}_2$, or evidence of a ventilatory limitation (breathing reserve <15% and/or significant expiratory flow limitation and/or decrease in IC) (Stickland *et al.*, 2012). It must be noted that some of the CHF patients had cardiovascular limitations to exercise, and therefore did not reach $VO_{2max}$. Upon termination of the exercise test, participants were asked to verbalize the main reason for stopping exercise: such as breathing discomfort, and/or leg discomfort.

### B.2.3 Operating lung volume responses

Operating lung volume changes were estimated from inspiratory capacity measurements (IC) taken during the pulmonary function test and the resting baseline period before the exercise tests and during the last minute of each two-minute stage of exercise. End-expiratory lung volume (EELV) was calculated from the difference in resting total lung capacity (TLC) from IC's, assuming that resting TLC and exercising were the same (O'Donnell *et al.*, 2001). End-expiratory lung volume (EELV) was subsequently estimated by adding tidal volume ($V_T$) and EELV. To properly evaluate operating lung volumes, it is vital that the maneuver is correctly executed and analyzed. Therefore, we practiced the maneuver with the participants before the exercise tests, and recorded ≥4 stable tidal breaths before every IC maneuver (Guenette *et al.*, 2013).

### B.2.4 Near infrared spectroscopy (NIRS)

Skeletal muscle oxygenation was assessed using a commercially-available near-infrared spectroscopy (NIRS) system (Oxymon III, Artinis Medical Systems, BV, The Netherlands). NIRS has been shown to be significantly correlated to the gold standard of non-invasive measurement of skeletal muscle metabolism (phosphorus magnetic resonance spectroscopy) (Sako *et al.*, 2001). The system is comprised of multiple diodes, wherein one fiber optic bundle carrying the NIR light produced by the laser diodes is attached to the surface of the skin nearest to the tissue of interest. A second fiber optic bundle (proximal to the first) returns the transmitted light from the tissue to a photon detector located in the spectrometer. NIRS allows for the determination of oxyhemoglobin ($O_2Hb$) and deoxyhemoglobin (HHb) through measurements of light attenuation at 760 and 864 nm wavelengths, in keeping with the modified Beer-Lambert law. Accordingly, changes in light intensity are related to changes in relative concentrations of hemoglobin (Villringer, 1997). Both the intensities of incident and transmitted light are recorded continuously and used to estimate the changes in $O_2Hb$, HHb, and total hemoglobin (Hbtot) concentration from resting baseline.

One pair of optodes was placed on the belly of the left vastus lateralis to estimate tissue oxygenation, and the other on the left $7^{th}$ intercostal space (Guenette *et al.*, 2008). Skin-fold thickness was measured, then the optodes were secured using double-sided tape, ensuring that the pairs of optodes were separated by approximately 30mm allowing for a depth of penetration of 15mm (Homma *et al.*, 1996).

### B.2.5 Oxygen delivery

Cardiac output (Q), was estimated non-invasively through impedance cardiography (Physioflow® PF-05, Manatec Biomedical, France) (Bernstein, 1986). Estimated $SpO_2$ was

recorded throughout with a finger pulse oximeter. These data were used to compute an estimate of $CaO_2$. Since $CaO_2 = (SaO_2 \times 1.39[Hb]) + (0.003PaO_2)$ and we did not have $SaO_2$ or $PaO_2$ (assuming that $PaO_2$ x 0.003 is negligible), we computed as estimate of $CaO_2$ using the following equation: $CaO_{2est} = SpO_2 \times 1.39[Hb]$. An estimate of $O_2$ delivery ($DO_2$) was then made: $DO_{2est} = Q$ x $CaO_{2est}$, (L/min). Hemoglobin concentration ([Hb]) was measured at the beginning of each experimental session (HemoCue 201+; HemoCue AB, Angelholm, Sweden) and immediately following termination of the TLIM trial, during active recovery.

### B.2.6 Impedance cardiography

In short, impedance is defined as the opposition or resistance to flow (Stedman, 2008). The Physioflow® uses signal morphology impedance cardiography (SM-ICG) to estimate cardiac output (Charloux *et al.*, 2000). Changes in the pulsatile component of thoracic impedance (mainly at the level of the aorta) were measured using a low amplitude/high frequency current directed toward the thorax between two pairs of electrodes (one transmitting electrode and one sensing electrode) placed on the neck and on the back (Strobeck *et al.*, 2000). Additionally, two ECG probes were used to determine heart rate [determined by the R-R interval from the ECG first derivative (dECG/d*t*)]. The device uses the following formula to extrapolate cardiac output: $\dot{Q}c = fc \times SVi \times BSA$, where $\dot{Q}c$ is cardiac output, $fc$ is heart rate, BSA is body surface area, and $SVi$ is stroke volume index (SV/BSA) (Charloux *et al.*, 2000). The change in thoracic impedance during left ventricular ejection was time-corrected to ECG and used to estimate SV.

A total of 6 electrodes (Ag/AgCl, Skintact FS- 50) attached to the Physioflow device were placed on the participant's skin. Importantly, the participant's skin was prepped (i.e., shaved if required, scraped with abrasive tape and wiped with an alcohol swab) prior to electrode placement

to guarantee conduction. Two pairs of electrodes were placed at the left base of the neck above the supraclavicular fossa and on the back at the level of the xiphoid process (on the left lateral to the spine) to estimate the pulsatile component of thoracic impedance. The two ECG electrodes were placed at the V1 and V6 positions (Gordon *et al.*, 2018). Data were continuously recorded in 30 second averages during the time-to-exhaustion trials. Cardiac output increases by 5–6 L/min per unit increase in $VO_2$ (Siebenmann *et al.*, 2015), and in this study, Physioflow® provided estimates of cardiac output that aligned with expected results based on $VO_2$.

## APPENDIX C: Supplemental Tables

Table 7. Correlations of the change in selected parameters at isotime and time of symptom limitation, from dopamine to saline, with the basal hypoxic ventilatory response

| Variable | r-value | p-value |
|---|---|---|
| Δ $\dot{V}_E/\dot{V}CO_2$ at isotime | 0.36 | 0.35 |
| Δ Dyspnea at isotime (Borg) | 0.41 | 0.28 |
| Δ Q/MAP at isotime ($ml \cdot min^{-1} \cdot mmHg^{-1}$) | 0.26 | 0.51 |
| Δ $\dot{V}_E/\dot{V}CO_2$ at $T_{LIM}$ | 0.53 | 0.14 |
| Δ Dyspnea at $T_{LIM}$ (Borg) | 0.42 | 0.26 |
| Δ Q/MAP at $T_{LIM}$ ($ml \cdot min^{-1} \cdot mmHg^{-1}$) | 0.17 | 0.66 |
| Δ Time to exhaustion (min) | 0.50 | 0.17 |

Correlation data are presented as R. Definition of abbreviations: HVR: hypoxic ventilatory response; $\dot{V}_E/\dot{V}CO_2$: ventilatory efficiency; Q/MAP: vascular conductance.

Table 8. Correlations of subject characteristics with the basal hypoxic ventilatory response

| Variable | r-value | p-value |
|---|---|---|
| EF (%) | -0.91 | 0.00056 |
| $\Delta\dot{V}_E$ (Hyperoxia; ml·min$^{-1}$) | -0.20 | 0.60 |
| $\Delta\dot{V}_E/\Delta P_{ET}CO_2$ (Rebreathe; ml·min$^{-1}$·mmHg$^{-1}$) | 0.09 | 0.82 |
| KCCQ (Overall Score) | -0.25 | 0.52 |
| EQ-5D-5L | -0.17 | 0.66 |

Correlation data are presented as R. Definition of abbreviations: HVR: hypoxic ventilatory response; EF: ejection fraction; $\dot{V}_E$: minute ventilation; $P_{ET}CO_2$: end-tidal partial pressure of carbon dioxide; TTE: time-to-exhaustion; Q/MAP: vascular conductance; KCCQ: Kansas City Cardiomyopathy Questionnaire; EQ-5D-5L: EuroQol 5-dimensional 5-level questionnaire.

Table 9. Correlations of the change in selected parameters at isotime and time of symptom limitation with the change in exercise endurance time from saline to dopamine in patients with chronic heart failure

| Variable | r-value | p-value |
|---|---|---|
| Δ $\dot{V}_E/\dot{V}CO_2$ at isotime | 0.03 | 0.93 |
| Δ Dyspnea at isotime (Borg) | -0.20 | 0.61 |
| Δ Q/MAP at isotime ($ml \cdot min^{-1} \cdot mmHg^{-1}$) | 0.54 | 0.13 |
| Δ $\dot{V}_E/\dot{V}CO_2$ at $T_{LIM}$ | 0.46 | 0.21 |
| Δ Dyspnea at $T_{LIM}$ (Borg) | 0.00009 | 1.0 |
| Δ Q/MAP at $T_{LIM}$ ($ml \cdot min^{-1} \cdot mmHg^{-1}$) | 0.39 | 0.30 |

Correlation data are presented as R. Definition of abbreviations: $T_{LIM}$: time of symptom limitation; $\dot{V}_E/\dot{V}CO_2$: ventilatory efficiency; Q/MAP: vascular conductance.

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