The Role of Exercise Central Hemodynamics for the Clinical Classification of Heart Failure Patients

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Abstract

Central hemodynamic evaluation during exercise testing provides critical information for the clinical classification of reduced (HFrEF) or preserved ejection fraction (HFpEF) heart failure (HF) patients. Recent and encouraging observations suggest that non-traditional indices of central hemodynamics can robustly describe cardiac function and pulmonary vascular hemodynamics in healthy individuals and in HF. Therefore, because of emerging evidence in favor of non-traditional indices to describe central hemodynamics in HF, it is unclear what index or indices best describe cardiac function and the heart–lung hemodynamic interaction in HF. Moreover, contributing to the complexity of the pathophysiology underlying the HF syndrome, it is becoming more recognized that neural mechanisms originating within skeletal muscle likely contribute to impaired cardiovascular function and symptoms of these patients. However, it remains unclear what role this neural feedback from skeletal muscle ergoreceptors has in the impaired central hemodynamic response frequently observed during exercise in HF. Therefore, the aims of this dissertation focused on investigating factors to better understand the central hemodynamic response to exercise in HF. In this series of studies we observed that non-traditional measurement of cardiac and pulmonary hemodynamics could describe the central hemodynamic response to exercise in HF. Also, experimental manipulation of neural feedback from skeletal muscle ergoreceptors resulted in observations which suggest pulmonary hemodynamics could be influenced by this mechanism, whereas cardiac function may not be similarly influenced by this pathway in HFrEF. Equally important and relevant to both clinical and research settings, it was

observed that non-invasive measurement of stroke volume at peak exercise could be reliably estimated using echocardiography, acetylene rebreathe, and oxygen pulse in HF.

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3. **EH Van Iterson**, EM Snyder, BD Johnson, TP Olson. Influence of Metaboreflex Stimulation on Circulatory Power and Pulmonary Vascular Capacitance in Heart Failure Patients. Journal of Applied Physiology. 2015 April. Submitted and under review.

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1. **VanIterson, E. H.,** Snyder, E. M., Johnson, B., & Olson, T. (2015). Influence of Metaboreflex Stimulation on Cardiac Power and Stroke Work during Submaximal Constant-Load Exercise in Heart Failure. Journal of the American College of Cardiology, 65(10_S).

2. **VanIterson, E. H.,** Snyder, E. M., Joyner, M. J., Johnson, B. D., & Olson, T. P. (2014). Effect of Locomotor Muscle Afferent Feedback Inhibition on Cardiac Power During Submaximal Exercise in Heart Failure. Circulation, 130(Suppl 2), A16770-A16770.

3. **VanIterson, E. H.,** Snyder, E. M., Joyner, M. J., Johnson, B. D., & Olson, T. P. (2014). Influence of Locomotor Muscle Afferent Feedback Inhibition on Pulmonary Vascular Conductance During Submaximal Exercise in Heart Failure. Circulation, 130(Suppl 2), A16786-A16786.

4. **VanIterson, E. H.,** Snyder, E. M., Johnson, B. D., & Olson, T. P. (2014). Pulmonary Vascular Response to Metaboreflex Stimulation During Submaximal Exercise in Heart Failure. Circulation, 130(Suppl 2), A16602-A16602.

List of Abbreviations

Δ = absolute difference

2-D = two-dimensional

ANOVA = analysis of variance

ACE = angiotensin converting enzyme

ANS = autonomic nervous system

ARB = angiotensin receptor blocker

BP = blood pressure

BMI = body mass index

BSA = body surface area

C_2H_2 = acetylene

$C^{18}O$ = carbon monoxide

$C_{a\text{-}v}O_2$ = arteriovenous difference in oxygen content

CircP = circulatory power

CNS = central nervous system

CO_2 = carbon dioxide

CP = cardiac power

CSA = cross-sectional area

CV = coefficient of variation

DBP = diastolic blood pressure

DL_{CO} = lung diffusing capacity for carbon monoxide

DL_{NO} = lung diffusing capacity for nitric oxide

D_M = alveolar-capillary membrane conductance

ECG = electrocardiogram

ECHO = echocardiography

EDV = end-diastolic volume

EF% = ejection fraction percentage

ESV = end-systolic volume

f_B = breathing frequency

GX_{CAP} = pulmonary vascular capacitance

Hb = hemoglobin

He = helium

HF = heart failure

HFpEF = heart failure with preserved ejection fraction

HR = heart rate

LV = left ventricle

LVOT = left ventricular outflow tract

MAP = mean arterial blood pressure

mm Hg = millimeters of mercury

mPAP = mean pulmonary arterial pressure

N_2O = nitrous oxide

NO = nitric oxide

NTS = nucleus tractus solitarius

NYHA = New York Heart Association

O_2 = oxygen

O_2pulse = oxygen pulse

P_aCO_2 = partial pressure of arterial carbon dioxide

P_AO_2 = partial pressure of alveolar oxygen

P_aO_2 = partial pressure of arterial oxygen

P_b = barometric pressure

PECO = post-exercise circulatory occlusion

$P_{ET}CO_2$ = partial pressure of end-tidal carbon dioxide

$P_{ET}O_2$ = end-tidal partial pressure of oxygen

PH = pulmonary hypertension

PPO = peak exercise power output

PV_{CAP} = pulmonary vascular capacitance

PVR = pulmonary vascular resistance

Q = cardiac output

Q_I = cardiac index

r = correlation coefficient

R^2 = coefficient of determination

RCO = regional circulatory occlusion

RER = respiratory exchange ratio

RPE = rate of perceived exertion

RR = respiratory rate

RV = right ventricle

SBP = systolic blood pressure

SD = standard deviation

SEM = standard error of the mean

SNS = sympathetic nervous system

SPAP = systolic pulmonary arterial pressure

SV = stroke volume

SV_I = stroke volume index

TDI = tissue Doppler imaging

TPR = total peripheral resistance

V_A = alveolar ventilation

V_C = pulmonary capillary blood flow

VCO_2 = carbon dioxide production

V_D = dead space

V_E = minute ventilation

V_E/VCO_2 = ventilatory efficiency

VO_2 = oxygen consumption

V_T = tidal volume

VTI = velocity time integral

Chapter 1

Introduction

Chronic heart failure (HF) is associated with an estimated $34 billion healthcare cost in the United States and accounts for 55,000 deaths per year as a primary cause and contributes to more than 280,000 deaths annually (Heidenreich et al., 2011; Roger et al., 2012). Heart failure contributes to dysfunction of organ systems, which include renal, pulmonary, skeletal muscle, vascular, and nervous systems; and, in parallel, dysfunction of these organs further contributes to the deterioration of health, increasing HF- and non-HF related hospitalizations, and augmenting the risk for premature mortality (Dickstein et al., 2008; Forman et al., 2004; Haldeman, Croft, Giles, & Rashidee, 1999; Komajda et al., 2011; Lee et al., 2003; Paulus et al., 2007; Roger et al., 2012; Vasan et al., 1999).

The etiology of HF is commonly the result of an underlying myocardial and/or cardiovascular disease, of which, coronary artery disease and hypertension together account for an estimated 70–80% of cases (Dickstein et al., 2008; Fox et al., 2001; Paulus et al., 2007; Roger et al., 2012). Despite the current understanding of HF, diagnosis and prognosis of HF are not discernable by a single methodology or approach. Confirmation of HF and therapeutic regimen are reliant on clinical judgment based on a combination of medical and familial history, overt signs and symptoms, and objective tests (Dickstein et al., 2008; Paulus et al., 2007).

Despite the heterogeneity in HF phenotypes, common to the clinical diagnosis of systolic HF, frequently referred to as reduced ejection fraction HF (HFrEF), is left ventricular (LV) dysfunction which is closely associated with an abnormal LV ejection fraction

percentage (EF%) typically <35–40% (Dickstein et al., 2008). In contrast, diastolic HF which is commonly referred to as HF with preserved ejection fraction (HFpEF) demonstrates a clinical phenotype with normal EF% with characteristic impaired LV relaxation, high LV stiffness, and high LV- and- left atrial pressures (Paulus et al., 2007). However, most importantly, HFpEF is now estimated to account for nearly 50% of HF cases while the mortality risk is comparable to HFrEF (Paulus et al., 2007; Vasan et al., 1999).

Despite differentiating phenotypic characteristics between HFrEF and HFpEF, diagnoses are not mutually exclusive. Individuals with HFrEF or HFpEF frequently share similar non-cardiac specific symptoms and signs, which include dyspnea, fatigue, fluid retention, or exertional intolerance (Aronson, Eitan, Dragu, & Burger, 2011; Dickstein et al., 2008; Ghio et al., 2001; McDonagh et al., 1997; Paulus et al., 2007; Roger et al., 2012; Rubin & Brown, 1984). Cardiac-specific signs of HF including measures of atypical structure and function closely relate with hemodynamic and pressure abnormalities in both HFrEF and HFpEF (Dickstein et al., 2008; Grothues et al., 2002; Paulus et al., 2007). In particular, low cardiac output (Q) and stroke volume (SV) at rest are common in both HFrEF and HFpEF despite contrasting EF% between conditions. Therefore, clinical assessment of cardiac as well as non-cardiac specific factors at rest are routinely used to assist in identifying the presence of HF, describing syndrome severity, and predicting prognosis in patients (Dickstein et al., 2008; Olson, Denzer, Sinnett, Wilson, & Johnson, 2013; Paulus et al., 2007).

Scientists now commonly recognize that despite the importance of resting clinical evaluation of HF at rest, patients demonstrate exacerbation of both signs and symptoms

during physical activity (Dickstein et al., 2008; Kao et al., 1997; Mancini et al., 1991; Osada et al., 1998; Sullivan, Higginbotham, & Cobb, 1988; Weber & Janicki, 1985). Moreover, and perhaps most importantly, information attained from objective measurement of cardiac function during clinical exercise testing has been closely associated with robust prognostic power in HF (Cohn et al., 1993; Guazzi, Reina, Tumminello, & Guazzi, 2005; Lang, Agostoni, & Mancini, 2007; Lang, Karlin, Haythe, Lim, & Mancini, 2009; Lavie, Milani, & Mehra, 2004; Mancini et al., 1991; Williams, Jackson, et al., 2005). Thus, because of the importance of clinical exercise testing and assessing cardiac hemodynamics for evaluating syndrome severity and prognosis in HF, this dissertation will address the following gaps in the literature to improve our understanding of the need for increased emphasis of exercise assessment of central hemodynamics for the clinical classification of HF.

Project 1: To study the technical factors which potentially influence the non-invasive measurement of cardiac hemodynamics in HFpEF; and, identify the extent of relationships between measurements of SV during exercise using three non-invasive and readily available techniques. We will use linear regression to study relationships between non-invasive echocardiography (ECHO), acetylene rebreathe, and oxygen pulse (O_2pulse) for the assessment of stroke volume during exercise in HFpEF. In addition to coefficient of determination (R^2), we will assess slopes and intercepts of linear regressions in order to describe relationships and potential biases associated with each of these techniques.

H1: We hypothesized that acetylene rebreathe and O_2pulse would compare favorably to ECHO when measuring SV at rest and at peak exercise in HFpEF.

H2: We hypothesized that gas transfer capacity within lungs would not directly influence SV measured using acetylene rebreathe at peak exercise in HFpEF.

Project 2: To study the influence of metabolite-sensitive group IV neural feedback associated with locomotor muscle on central hemodynamics in HFrEF. We will study in this cross-over experiment both circulatory power (CircP) and pulmonary vascular capacitance (GX_{CAP}) responses to stimulation of the metaboreflex immediately following submaximal locomotor exercise in comparison to a normal recovery condition in HFrEF.

H1: We hypothesized that activation of the skeletal muscle metaboreflex would attenuate both CircP and GX_{CAP} in HFrEF.

Project 3: To study the influence of neural feedback from group III/IV locomotor muscles during submaximal exercise on central hemodynamics in HFrEF. This placebo-controlled cross-over study will assess the influence of inhibiting locomotor muscle group III/IV neural feedback with lower lumbar intrathecal fentanyl injections on CircP and GX_{CAP} in comparison to an exercise condition with placebo inhibition in HFrEF.

H1: We hypothesized that selective μ-opioid blockade of neural feedback from group III/IV afferents using intrathecal fentanyl at the lower-lumbar level during submaximal constant-load exercise would be influential in increasing CircP and GX_{CAP} in HFrEF.

This dissertation begins with Chapter Two and an introduction to the commonly used New York Heart Association (NYHA) classification system in HF. Following, we present an in-depth review of the literature focusing on central hemodynamics, which includes the assessment of traditional measurements of central at rest and during exercise in HF. Next, we will introduce newer non-traditional indices of central hemodynamics and

discuss outcomes of both resting and exercise evaluation in HF. Finally, we will discuss the clinical implications of using central hemodynamic assessment during exercise in HF.

Chapter Three of this dissertation will describe and interpret the central hemodynamic response to exercise in HF using observations from the three dissertation projects.

Chapter Four will discuss in detail the observations, overall interpretations, and clinical implications from the three projects comprising the body of this dissertation.

Chapter Five will provide a brief discussion of the potential limitations which may have influenced the interpretability of the current findings.

Chapter Six will detail future directions for this area of research, as well as providing overall concluding remarks which together lay the framework for advancing the understanding of central hemodynamics in HF.

Chapter 2

Background and literature review

2.1 Introduction

The clinical classification of HF commonly calls into use the NYHA functional classification system which is mainly derived from measures of symptom severity and perception of exercise tolerance (Broek et al., 1992; Dickstein et al., 2008; Scrutenid et al., 1994). Heart failure patients are typically stratified into one of four (I-IV) classes based on a comprehensive examination of both subjective and objective factors administered by the clinician. Class I HF patients are suggested to have the mildest form of HF while demonstrating no symptoms at rest or functional limitations. In contrast, the progressive increase in NYHA class number corresponds with HF patients who demonstrate more frequent and severe occurrences of the signs and symptoms during exertion. Upon categorization into NYHA class IV, HF patients typically demonstrate symptoms at rest in addition to severe functional limitations. However, in addition to being descriptive of syndrome severity, objective factors which are not considered in the NYHA classification of patients have demonstrated the capability to closely associate with prognosis in HF (Broek et al., 1992; Lang et al., 2009; Roul, Germain, & Bareiss, 1998; Roul et al., 1995; Scrutenid et al., 1994).

Individuals who are suspected of having HF, perhaps because of familial history and/or concurrent comorbidities (Table 1), are routinely recommended for comprehensive clinical evaluation, which includes examination of symptoms related to dyspnea and fatigue in addition to objective outcomes. Although valuable in the clinical setting, the

reliability of the NYHA classification system is highly dependent on both the outcomes of clinical testing and judgement of the clinician. Also, because symptoms of HF have been observed to correlate poorly with the magnitude of cardiac dysfunction, it is critical that clinical evaluation of patients includes objective assessment of cardiac function (Stevenson & Perloff, 1989). Overall, however, combined subjective and objective outcomes are closely involved in assessing parameters related to signs or symptoms of HF which frequently mirror cardiovascular system dysfunction (Lipkin, Canepa-Anson, Stephens, & Poole-Wilson, 1986; Lipkin & Poole-Wilson, 1986; Lipkin, Scriven, Crake, & Poole-Wilson, 1986; Spiteri, Cook, & Clarke, 1988).

Table 1. Common causes of heart failure

Etiology
Coronary artery disease*
Hypertensive heart disease/aortic stenosis*
Myocardial infarction
Dilated cardiomyopathy/myocarditis
Hypertrophic cardiomyopathy
Incessant tachycardia
Cor pulmonale
Pericardial effusion/tamponade

*Combined to account for the etiology in ~ 70 – 80% of cases (adapted from Dickstein et al., 2008; Fox et al., 2001; Paulus et al., 2007; Roger et al., 2012)

One of the most common indices to support the diagnosis of HF is abnormal cardiac structure and function that may include a low resting EF%, which is specific to HFrEF. Although, despite a normal EF%, there are several distinct cardiac abnormalities which can be useful in the diagnosis of HFpEF (Table 2).

Table 2. Cardiac structure and function abnormalities common in heart failure

Systolic Heart Failure	Diastolic Heart Failure
Ejection fraction % <35 – 40%	Ejection fraction % >50%
Low cardiac output	Low cardiac output
Eccentric hypertrophy	Concentric hypertrophy
High left ventricular filling pressures	Impaired left ventricular relaxation
Deceleration time <115 – 150 ms	Time constant of relaxation >48 ms
E/E' >15	High left ventricular filling pressure
High ventricular diameters	E/E' >15
End-diastolic >55 – 60 mm	Left ventricular end-diastolic pressure >16 mm Hg
End-systolic >45 mm	Left atrial volume >32 – 40 mL/m^2
Atrial fibrillation	Atrial fibrillation
Left atrial diameter >40 mm	Pulmonary capillary wedge pressure > 12 mm Hg
Global or regional loss of left ventricular function	Global or regional loss of left ventricular function

Structure and function thresholds were determined with echocardiography in most cases. ms, milliseconds; mm, millimeters; mm Hg, millimeters mercury; mL, milliliters; m, meters (adapted from Dickstein et al., 2008; Komajda et al., 2011; Paulus et al., 2007; Roger et al., 2012)

Equally important in the clinical classification of HF are non-cardiac hemodynamic specific signs and symptoms (Table 3). The evaluation of non-cardiac hemodynamic specific signs and symptoms of HF enables the clinician to make judgements based on both subjective and objective measures. For example, dyspnea and fatigue are commonly recognized subjective measures that are well-regarded as valuable measures in the clinical classification of HFpEF and HFrEF (Remes, MlEttinen, Reunanen, & Pyörälä, 1991). In contrast, measurements related to ventilatory function and cardiac structure for example, which can be measured via objective data recording, also demonstrate clinical value in the evaluation of HF (Chua et al., 1997; Cohn et al., 1993; Corra et al., 2002; Francis et al., 2000).

Table 3. Non-cardiac hemodynamic specific symptoms and signs common in heart failure

Symptoms	Signs
Dyspnea at rest or exercise	Fluid retention
Fatigue	Pulmonary edema and/or
Exercise intolerance	Peripheral edema
Peak oxygen consumption <18 mL/kg/min	Raised jugular venous pressure
6-minute walk test <300 m	Cachexia
Nausea	Hypertension (pulmonary/peripheral)
Confusion	Impaired functional sympatholysis
Erectile dysfunction	Left ventricular hypertrophy
Persistent coughing or wheezing	Wall thickness >11 – 12 mm
	Ventilatory inefficiency
	V_E/VCO_2 slope >34
	Tachypnea
	Tachycardia
	Atrial fibrillation
	High natriuretic peptide levels
	BNP >400 pg/mL
	NT-proBNP >2000 pg/mL
	Dysrhythmia on electrocardiogram
	QRS >120 ms

mL, milliliters; kg, kilogram; min, minute; m, meters; mm, millimeters; BNP, brain natriuretic peptide; pg, picograms; NT-proBNP, N-terminal pro b-type natriuretic peptide; ms, milliseconds (adapted from Dickstein et al., 2008; Komajda et al., 2011; Paulus et al., 2007; Roger et al., 2012).

In addition to clinical assessment of HF at rest, it is becoming more common to include an evaluation of many of the indices listed in Tables 2 and 3 during exercise to improve the strength of association between signs, symptoms, and severity with prognosis in HF. The emergence of cardiopulmonary exercise testing in HF has produced an ample body of evidence which suggests that ventilatory function and gas-exchange responses during exercise may be strongly indicative of syndrome severity and prognosis in HF (Chua et al., 1997; Francis et al., 2000; Weber & Janicki, 1985; Weber, Kinasewitz, Janicki, & Fishman, 1982). The value of cardiopulmonary outcomes during exercise testing are important foremost because of their associations with prognosis in HF, but also because cardiopulmonary testing can be performed routinely, non-invasively, and with little risk to the individual (Chua et al., 1997; Cohn et al., 1993; Corra et al., 2002; Francis et al., 2000).

The information attained during cardiopulmonary exercise testing in HF and subsequent interpretation is commonly cross-referenced with the Weber-Janicki Classification system, which assesses functional impairment based on objective physiological responses during incremental treadmill testing in HF (Weber & Janicki, 1985; Weber et al., 1982). The Weber-Janicki Classification system for HF patients relies primarily on measurements of peak oxygen uptake (VO_{2peak}), VO_2 at anaerobic threshold, and peak cardiac index (Q_I) during treadmill exercise testing. As such, stratification of HF severity considers specific thresholds for all three indices with the resultant assessment of syndrome severity ranging from none/mild (class A)- to- very severe (class E) (Weber & Janicki, 1985; Weber et al., 1982).

Despite the valuable information garnered from ventilatory and gas-exchange responses during exercise testing in HF, encouraging observations from notable studies which examined cardiac hemodynamic responses during exercise in HF suggest that exercise assessment of cardiac hemodynamics may be the strongest indicators of syndrome severity and prognosis in these patients (Chua et al., 1997; Francis et al., 2000; Lang et al., 2009; Weber & Janicki, 1985; Weber et al., 1982; Williams, Jackson, et al., 2005; Williams et al., 2001). Several observations suggest that cardiac hemodynamic assessment via non-invasive techniques may provide more robust clinical value compared to more traditional outcomes such as ventilatory responses (Lang et al., 2009; Williams, Jackson, et al., 2005; Williams et al., 2001). Moreover, a growing body of evidence suggests that non-traditional indices of cardiac hemodynamics may add value to cardiac hemodynamic measurements beyond traditional assessment of Q and SV (Lang et al., 2009; Williams, Jackson, et al., 2005; Williams et al., 2001). However, to date, the body

of evidence supporting the use of non-traditional indices of cardiac hemodynamics during exercise for augmenting the strength of the clinical classification of HF is small in number. Although, observations thus far suggest that indices, which include cardiac power (CP), CircP, O_2pulse, and/or GX_{CAP} warrant further examination in the context of exercise testing for augmentation of the clinical classification of HF (Cohen-Solal et al., 2002; Lang et al., 2009; Roul et al., 1995; Taylor, Olson, Chul Ho, Maccarter, & Johnson, 2013; Williams, Jackson, et al., 2005),

2.1.1 Overview of traditional and non-traditional central hemodynamics

The heart is a four chamber pumping organ that has a primary function to continuously match the delivery of nutrients and gases with the transient metabolic demands of the cardiovascular system. As an integral component of the cardiovascular system, the heart receives and then expels blood at high volumes (EF% ~ 50–75%) into the circulation in a rhythmic manner.

Integrated with the heart both in proximity and functional purposes, the pulmonary circulation serves to deliver deoxygenated blood received by the right side of the heart to gas-exchange sites at the alveoli-pulmonary capillary membrane barrier where blood can be oxygenated and then transported to the left side of the heart to be propelled to the systemic circulation. Propulsion of oxygenated blood toward metabolically active tissue (e.g. skeletal and smooth muscle, kidneys, neural) requires a contrasting degree of vascular structure, tone, and distensibility of the peripheral branch of the circulation in comparison to the vasculature comprising the pulmonary circulation since pulmonary vasculature receives the entire Q of the RV and does not need to distribute blood over

long distances or multiple organ systems (Hughes, Glazier, Maloney, & West, 1968; Permutt, Bromberger-Barnea, & Bane, 1962; West, Dollery, & Naimark, 1964).

The pulmonary vasculature has thin walls with high distensibility and develops on average markedly less pressure (~20 millimeters of mercury [mm Hg]; mean pulmonary arterial pressure (mPAP) ~15 mm Hg, systolic/diastolic pulmonary arterial pressure ~ 25/8 mm Hg) compared to that of the thicker walled peripheral vasculature which is purposefully structured for less distensibility and averages nearly five times the pressure of the pulmonary circulation (~100 mm Hg; 120/80 mm Hg systolic/diastolic) (Galie et al., 2009; Simonneau et al., 2009). Because of differences in pulmonary and peripheral vascular structure and function, adjustments in Q can differentially influence marked changes in pulmonary versus peripheral circulatory hemodynamics and pressures.

Hemodynamic homeostasis of the cardiovascular system depends on the continuous maintenance of blood flow and pressure gradients which facilitate circulation of blood toward (veins) and from (arteries) the heart (Pirofsky, 1953; Washburn, 1921). Integral to the process of hemodynamic regulation at the level of the heart are local interactions which include the segmental interrelationships the heart shares with the peripheral and pulmonary vascular systems and can be described using *Ohm's Law*:

$$Flow = \Delta\, pressure/resistance \qquad \text{(equation 1)}$$

Ohm's Law describes blood flow out of the heart as being mediated by the pressure difference between the circulation (pulmonary or systemic) and the atria (left or right) divided by the resistance of the circulation, respectively.

Moreover, to describe the capacity of a specific sector of the circulation to contain blood at a given pressure the following relationship is derived (e.g. RV SV → pulmonary artery) (Guyton, Armstrong, & Chipley, 1956; Guyton, Polizo, & Armstrong, 1954; Mahapatra, Nishimura, Oh, & McGoon, 2006; Mahapatra, Nishimura, Sorajja, Cha, & McGoon, 2006):

$$Vascular\ capacitance = \Delta volume/\Delta pressure \qquad \text{(equation 2)}$$

The relationship between volume and pressure of the circulation is generally positive in healthy individuals, but the magnitude of linearity can be heterogeneous throughout the systemic vasculature. Because of the structural and functional differences of arterial versus venous vasculature, capacitance of the venous circulation is markedly greater compared to the arterial circulation (Guyton et al., 1956). This is an important difference as low capacitance of the arterial circulation assists in maintaining perfusion pressure to the most distal regions of the body while also providing a gradient in which the venous circulation uses to drive blood back to the central circulation.

The direct Fick equation can be used to describe the overall systemic hemodynamic, pressure, and gas-exchange relationships during exercise (Fick, 1870; Hsia, Herazo, Ramanathan, & Johnson, 1995; Johnson et al., 2000; Lipkin & Poole-Wilson, 1985; McMichael & Sharpey-Schafer, 1944; Werko, Berseus, & Lagerlof, 1949):

$$Q = VO_2/C_{a-v}O_2 \qquad \text{(equation 3)}$$

The factors of the direct Fick equation are: VO_2 as oxygen uptake from alveoli of lungs into the circulation per minute, arteriovenous difference in oxygen content ($C_{a\text{-}v}O_2$) as the total uptake of oxygen (O_2) by the peripheral tissues, and Q as the sum and propulsion

strength of blood flow to peripheral tissues or the pulmonary circulation per unit of time (Hsia et al., 1995).

Accordingly, because of the close anatomical and physiological relationships the heart shares with the pulmonary and peripheral vascular systems, and because individuals with HF demonstrate dysfunction of nearly all components of the cardiovascular system, it is critical to understand the implications of these interactions in this population. From a clinical perspective, it is already well-known that cardiac hemodynamic evaluation in HF is important. However, determining cardiac pumping capability as a direct interaction with vascular hemodynamics is central to evaluating syndrome severity and predicting prognosis in HF.

2.1.2 Cardiac hemodynamics

2.1.2.1 Traditional measurements

Cardiac pumping capability has been traditionally assessed using measurements of Q and SV. Cardiac output and SV have proven useful for describing cardiac health because these indices are suggested to be direct estimates of myocardial contractility and rhythmicity separately or together under various conditions of metabolic demand and/or health status (Chomsky et al., 1996; Sullivan, Knight, Higginbotham, & Cobb, 1989a; Wilson, Rayos, Yeoh, & Gothard, 1995). Also important to note, in healthy individuals who are not elite-level athletes, the O_2 cost of breathing is consistent at approximately 3-5% of the total VO_2 and thus it has been suggested that Q is directly proportional metabolic rate (Beck et al., 2006; Bhambhani, Norris, & Bell, 1994; Crisafulli, Piras, et al., 2007; Stringer, Hansen, & Wasserman, 1997; Whipp, Higgenbotham, & Cobb, 1996). Therefore, even though respiratory muscles can demand upwards of 15% of Q during

exercise, because of the close dependence VO_2 has on the cardiac hemodynamic response to exercise, it is suggested that VO_2 may approximate cardiac function in healthy individuals (Bhambhani et al., 1994; Crisafulli, Piras, et al., 2007; Whipp et al., 1996). For example, in using mathematical modeling in healthy individuals, Stringer *et al.* (1997) demonstrated that direct Fick measurements of Q closely related to Q predicted from linear regression between percentage of maximal oxygen consumption (VO_{2MAX}) and measured $C_{a\text{-}v}O_2$ during exercise (coefficient of determination, $R^2 = 0.97$, $p<0.05$) (Stringer et al., 1997).

However, VO_2 is suggested to be highly influenced by non-cardiac factors (e.g. age, muscle deconditioning, motivation, or abnormal pressor reflexes) as well as cardiac hemodynamics during exercise in HF (Becklake, Frank, Dagenais, Ostiguy, & Guzman, 1965; Chomsky et al., 1996; Chua, Clark, Amadi, & Coats, 1996; Ponikowski et al., 2001; Weber et al., 1982; Wilson et al., 1995). As an example, by using a low Q response threshold previously defined as $<0.5 \times VO_2 + 3$ Liters (L)/minute (min) (Higginbotham et al., 1986), it was demonstrated in HFrEF who were classified as having mild (normal Q and ≤20 mm Hg pulmonary wedge pressure during exercise), moderate (normal Q and >20 mm Hg pulmonary wedge pressure during exercise), or severe (low Q and >20 mm Hg pulmonary wedge pressure during exercise) cardiac dysfunction, that VO_{2peak} did not differ between groups nor did it correlate with cardiac hemodynamics during exercise ($p>0.05$) (Wilson et al., 1995). These authors attributed this disconnect to potential differences in non-cardiac factors including skeletal muscle deconditioning and/or motivational differences amongst HF patients (Wilson et al., 1995). Thus, despite observations suggesting a close correlation between VO_2 and Q in HF (Lang, Karlin,

Haythe, Tsao, & Mancini, 2007; Mancini, Katz, Donchez, & Aaronson, 1996; Szlachcic, Masse, Kramer, & Tubau, 1985), caution should be applied when interpreting independent measurements of VO_2 as the surrogate for cardiac pumping capacity in these patients (Chomsky et al., 1996; Griffin, Shah, Ferguson, & Rubin, 1991; Lang et al., 2009; Roul et al., 1995; Wilson et al., 1995).

Stroke Volume

Stroke volume is the volume of blood ejected by the heart per cardiac cycle and is measured as the absolute difference between ventricular filling and ventricular emptying, with the volume entering the pulmonary and systemic circulations typically being identical. The range for SV at rest is 60–80 milliliters (mL) per beat in healthy individuals. Stroke volume can be influenced by lean body mass and therefore indexing to body surface area (BSA, meters^2) is appropriate when body size (e.g. patients or athletes) could influence measurement of cardiac function (Carlsson et al., 2012; Collis et al., 2001; de Simone et al., 1997).

Stroke volume is mainly dependent on preload, contractility, and afterload (Braunwald et al., 1969; Frank, 1895; Gleason & Braunwald, 1962; Patterson & Starling, 1914; Starling, 1918). In brief, preload occurs during diastole and reflects the stretch of the myocardium in response to the total blood volume within ventricles but is highly influenced by numerous factors including venous return, atria, or skeletal muscle pump function for example. In contrast, contractility refers to the capacity of the heart to generate systolic tension which is analogous to the ability of the myocardium to develop force at a given rate of muscle shortening (inverse relationship) and, hence, is related to the number and integrity of actin and myosin relationships within heart muscle at a given preload and

afterload. Lastly, afterload refers to the magnitude of impedance to the ejection of blood from the LV through the aortic valve. Afterload is related to systemic vascular resistance and reflects both the pressure within the ventricle and ventricular wall tension needed during systole to open the aortic valve. Afterload can be influenced by changes in ventricular wall thickness, ventricular dilation, or aortic compliance for example. However, in healthy individuals with normal sized hearts without abnormal wall hypertrophy (concentric), increased preload (i.e. volume → pressure = ↑ myocardial stretch) permits stronger myocardial actin and myosin interactions and thus the potential for higher myocardial systolic wall tension, contractility, and SV.

The overall significance of the integrative relationships between preload, contractility, and afterload can be described by the principles governing the *Frank—Starling Law of the heart* which serve to maintain SV homeostasis (Figure 1) (Frank, 1895; Gleason & Braunwald, 1962; Patterson & Starling, 1914; Schnabel Jr, Sacker, Allen, & Lewis, 1961; Starling, 1918; Villars, Hamlin, Shaw, & Kanusky, 2004).

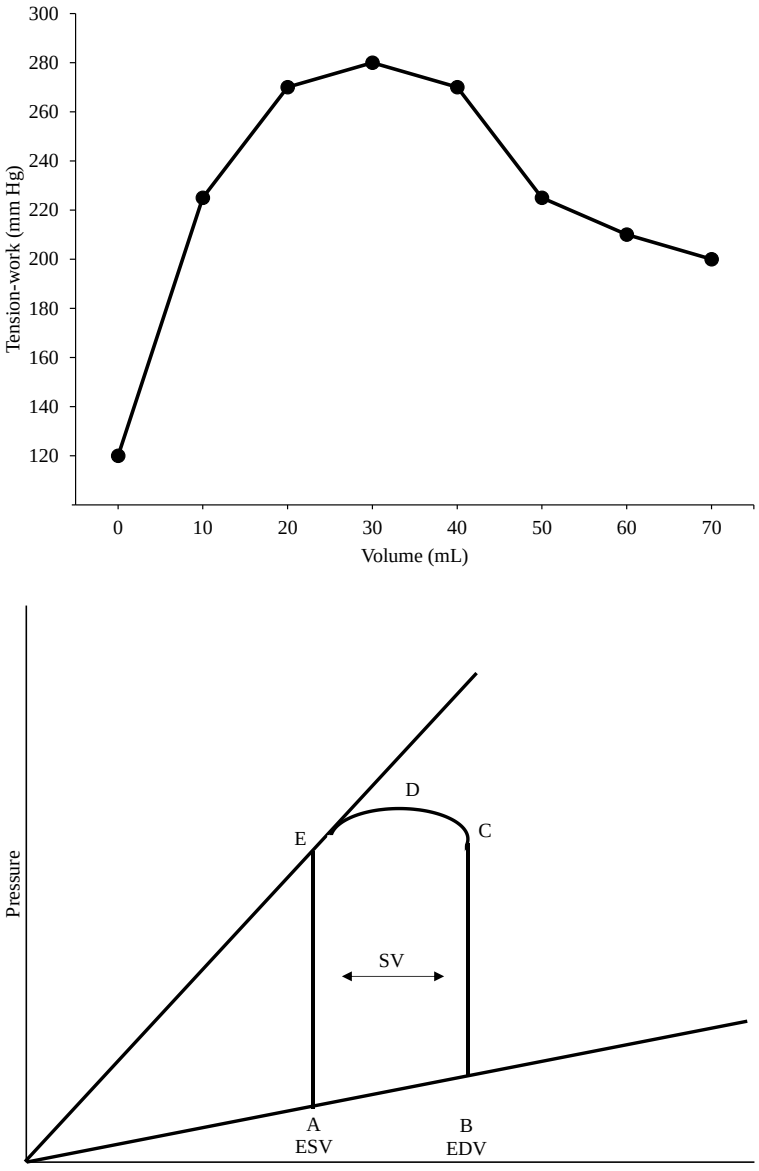

Figure 1: Top: Frank-Starling curve. The myocardial work capacity at any given ventricular end-diastolic pressure. Increases in contractility (e.g. beta agonist) of the myocardium shift this relationship upward and to the left. Decreases in contractility (e.g. heart failure) shift this relationship downward and to the right. Adjustments in these curves suggest that an equal quantity of myocardial work up to a given point can be generated at different conditions of contractility; but, perhaps at the expense of abnormal loading (i.e. end-diastolic pressure)(adapted from Schnabel et al., 1961). Bottom: Ventricular pressure-volume (P-V) relationships for a single cardiac cycle in a healthy individual. Point A represents end-systolic volume (ESV) just before opening of the mitral valve. Point A to B represents early and late filling of diastole. Point B represents end-diastolic volume (EDV). Point B to C represents the isovolumic contraction period. Point C represents opening of the aortic valve. Point C to D represents rapid ejection of blood through the aortic valve. Point D to E represents reduced ejection of blood through aortic valve. Point E represents closure of aortic valve. Point E to A represent the isovolumic relaxation period. Accordingly, stroke volume (SV) is calculated as the absolute difference between EDV and ESV (adapted from Villars et al., 2004).

Briefly, the *Frank—Starling Law of the heart* suggests that due to the myocardium's high sensitivity to venous return with associated pressure (i.e. preload), increased end-diastolic volume (EDV) can lead to high pressure within ventricles and, hence, increased

myocardial stretch that maximizes actin—myosin interactions which can contribute to higher contractility and lead to increased SV in healthy individuals (Frank, 1895; Gleason & Braunwald, 1962; Patterson & Starling, 1914; Starling, 1918). Large increases in Q (e.g. ~ magnitude of 5–7 compared to normal range reported at rest) could not occur without intact normal ventricular relaxation and contractile capabilities concurrent with adequate venous return to stimulate RV and LV filling and loading, particularly during high-intensity exercise when increased HR decreases ventricular filling time (Holmgren, 1956; Horwitz, Atkins, & Leshin, 1972; Poliner et al., 1980; Sullivan, Cobb, & Higginbotham, 1991). For example, Poliner *et al.* (1980) demonstrated that during both upright and supine exercise at low, medium, and peak intensities that EDV progressively increased while end-systolic volume (ESV) was maintained (supine) or trended downward (upright) in healthy individuals, which were accompanied by progressive increases in SV from rest to peak exercise despite significant concurrent increases in HR from rest to peak exercise (Poliner et al., 1980). Thus, although many factors contribute to changes in SV, it could be suggested that the Frank—Starling mechanism in the presence of normal cardiac relaxation and contractility is a primary influence on increases in SV.

Cardiac Output

Cardiac output represents the total volume of blood pumped by the ventricles per unit of time and theoretically accounts for the rate of pumping and the contractile capability of myocardial tissue. Because Q is a product of heart rate (HR) and SV, this measure is subject to the variable influences of each factor, which together can share either a direct (e.g. high-intensity exercise) or an indirect (e.g. low- to moderate-intensity exercise)

relationship with one another (Holmgren, 1956; Horwitz et al., 1972; Poliner et al., 1980; Rodeheffer et al., 1984; Sullivan et al., 1991). Cardiac output should be indexed to BSA when lean body mass would be expected to influence cardiac function (Carlsson et al., 2012; Collis et al., 2001; de Simone et al., 1997; Poliner et al., 1980). The normal range of Q is 4.0–8.0 L/min in healthy individuals, whereas the Q_I range at rest is approximately 2.6–4.2 L/min/m^2.

In a study of HR, SV, and Q at rest and during exercise in healthy individuals, Sullivan *et al.* (1991) demonstrated that Q and Q_I increased in direct relationship with increasing work-load (Sullivan et al., 1991). Moreover, in examining HR and SV responses during exercise in these individuals, it was apparent that the Frank—Starling mechanism was highly influential in contributing to adjustments in cardiac hemodynamics in these individuals. Evidence of this is noted by the progressive increase in HR from rest to peak exercise which paralleled the pattern of increase in Q and Q_I from rest to peak exercise; yet, SV_I mirrored by EDV, remained constant for the duration of exercise following their initial increase from rest to the first stage of exercise (Sullivan et al., 1991). Indeed, these observations suggest that increases in Q_I and maintenance of SV_I during exercise are highly dependent on the Frank—Starling mechanism in healthy individuals, with enhanced relaxation and contractility occurring shortly thereafter the onset of exercise and continuing throughout higher-intensities of exercise.

2.1.2.2 Non-traditional direct measurements

All four chambers of the heart are integral in maintaining cardiac hemodynamic homeostasis, with this interaction being particularly important during excessive loading of the heart (e.g. exercise). The atria serve well to increase the pumping capability of the

ventricles by contributing to the augmentation of the Frank—Starling effect. This occurs because atria accommodate blood from venous return and, therefore, can facilitate preload and pressure development within ventricles. The *Law of Laplace* can be used to describe the influence of concurrent changes in blood volume with pressure and the impact these factors have on the force generating capabilities of the heart (or the myocardial energy required for contraction in response to changes in loading conditions of the heart) (Shipley & Wilson, 1951; Tallarida, Rusy, & Loughnane, 1970):

$$Tension = (pressure \times radius)/2 \qquad \text{(equation 4)}$$

The *Law of Laplace* implicates, although not a perfect cylindrical shape, theoretically, pressure is that within a ventricle, the radius is that of the transverse cross-section of a ventricle, 2 is a numerical constant, and tension being equivalent to the quantity of energy required to generate systolic pressure required to eject blood from a ventricle.

As such, it is important to point out that the right side of the heart is highly sensitive to blood volume changes (i.e. preload dependent volume pump), whereas the left side of the heart although being both preload and afterload sensitive, perhaps may be particularly sensitive to pressure changes (i.e. afterload dependent pressure pump) and is usually larger and thicker in wall structure compared to the right side because of the systemic circulation it needs to supply blood to.

Although the measurement of Q and SV may account for venous return (i.e. volume pump), these indices do not directly assess the influence of the relationship arterial pressure (i.e. pressure pump) shares with cardiac function. Observations in animal models clearly indicate that adjustments in Q are integral in supporting systemic arterial pressure and, hence, perfusion pressure to capillary beds of metabolically active tissue (e.g.

contractile-active skeletal muscle) (Ichinose et al., 2010; Spranger et al., 2013). As a pumping organ, the heart relies on the contributions of the kinetic energy imparted from rhythmic blood flow and concurrent pressure generation within cavities to generate hydraulic power. Hydraulic power can be converted by the heart to mechanical power for propulsion of blood out of the LV with the strength to not only overcome the resistance of the circuit (i.e. systemic vasculature); but, more importantly, to provide continuous perfusion of the cardiovascular system relative to metabolic demands (Lang et al., 2009; Williams, Jackson, et al., 2005; Williams et al., 2001). Indeed, it is crucial for a measure of cardiac function aimed at describing cardiac pumping capability to account for both blood flow (i.e. preload → Q) and pressure (i.e. Q → MAP) generating capacity.

Cardiac Power

The integrative relationship that depicts the hemodynamic and pressure generating capability of the heart pump is CP. The direct components of this index are Q as a product with MAP to estimate hydraulic power (Lang et al., 2009; Tan, 1986; Williams, Jackson, et al., 2005). To further illustrate the utility of CP as a direct indicator of cardiac pumping capability, when Q and total peripheral resistance (TPR) are known the following relationship can be derived:

$$MAP = Q \times TPR \qquad \text{(equation 5)}$$

Thus, if it is assumed that TPR is close to the true resistance to blood flow offered by the peripheral vasculature (i.e. not including the pulmonary vasculature), and that the contribution of central venous pressure to resistance is negligible, it is clear that Q is directly related to MAP. Indeed, MAP represents the pressure generating capacity of Q and, therefore, CP is conceptually a direct measure of cardiac pumping capability:

$$CP = Q \times MAP \quad \text{(equation 6)}$$

Cardiac power can be expressed as the absolute volume of blood per minute as a pressure product (L/min · mm Hg), it can be normalized for power generated in units of watts by multiplying by a correction factor of 2.22×10^{-3} or dividing by 451 (Cooke et al., 1998; Lang, Agostoni, et al., 2007), or it can be indexed to BSA (L/min/m^2 · mm Hg) or body weight (watts/kg).

Early examination of CP in healthy individuals demonstrated resting values of 1.11 ± 0.05 watts (Marshall et al., 2001). More recently, it was observed that CP did not differ at rest in healthy individuals compared to HFpEF (271 ± 53 versus 254 ± 107 L/min/m^2 · mm Hg, respectively; p = 0.624) (Bhella et al., 2011). Although, it was observed by Bromley *et al.* (2006) in healthy individuals that men demonstrated significantly higher peak exercise CP in comparison to women, older men demonstrated significantly lower peak CP in comparison younger men, there appeared to be no age effects in women, and body weight did not appear to have an influence on this measure (Bromley, Hodges, & Brodie, 2006). These observations by Bromley *et al.* (2006) were consistent with another study of healthy men and women which similarly reported a significantly higher CP in men versus women at peak exercise (Bromley et al., 2006; Jakovljevic et al., 2012). Also important, these recent observations suggest that peak CP is directly related to peak Q, SV, and VO_2 in healthy individuals (Jakovljevic et al., 2012). In contrast, but perhaps related to the age (34 ± 9 years) and training status of the participants, Schlader *et al.* (2010) observed the mean CP at peak exercise in well-trained healthy men to be nearly two times higher compared to previous observations in recreationally active men (Schlader, Mundel, Barnes, & Hodges, 2010). Nevertheless, despite being a less studied

cardiac hemodynamic parameter compared to Q and SV in HF, these encouraging observations in healthy individuals provide useful information in interpreting CP in patients (Bromley et al., 2006; Jakovljevic et al., 2012; Schlader et al., 2010).

2.1.2.3 Non-traditional Indirect Measurements

Because of the hemodynamic dependence of VO_2 as well as the physiological principles supporting the direct Fick equation (equation 3) (Fick, 1870; Johnson et al., 2000; McMichael & Sharpey-Schafer, 1944; Stringer et al., 1997; Werko et al., 1949), it has been suggested that VO_2 in parallel with other cardiovascular factors can estimate cardiac pumping capability. The assumption of exact associations between hemodynamics and factors of the cardiovascular system are the framework for the use of indirect cardiovascular based metrics for the estimation of cardiac function.

Oxygen Pulse

Mathematically, if the physiological associations of the direct Fick equation remain constant, the quotient of VO_2 and HR yields the following cardiovascular index, O_2pulse (Crisafulli, Piras, et al., 2007; Kasch, Phillips, Carter, & Boyer, 1973; Saltin, 1964; Whipp et al., 1996):

$$O_2pulse = VO_2/HR \qquad \text{(equation 7)}$$

Oxygen pulse is theoretically an estimate of the product of SV with $C_{a\text{-}v}O_2$ when $C_{a\text{-}v}O_2$ remains invariable, details which are explained in greater detail in studies 1 – 3 of this dissertation. In brief, the units for O_2pulse reported in the literature have been in absolute terms (mL/beat), relative to BSA (mL/beat/m^2), or relative to body weight (mL/beat/kg) (Kasch et al., 1973; Mahler, Parker, & Andresen, 1985; Saltin, 1964; Whipp et al., 1996).

Also, and important to note, our group recently observed that O_2pulse correlated closely with direct Fick measurements of SV during exercise in HFrEF with or without pulmonary hypertension (PH) ($r = 0.91$, $p<0.01$) (Taylor, Olson, et al., 2013). Although, despite these recent observations by our group in addition to evidence which suggests that O_2pulse demonstrates prognostic strength in HF, further testing is needed to confirm whether O_2pulse estimates SV during exercise in HF (Cohen-Solal et al., 1997; Laukkanen, Kurl, Salonen, Lakka, & Rauramaa, 2006; Lavie et al., 2004; Taylor, Olson, et al., 2013).

Circulatory Power

Circulatory power imparts the assumptions which O_2pulse is supported, but may be a better indicator of cardiac pumping capability compared to O_2pulse because CircP also accounts for arterial pressure which is consistent with CP (equation 6). Circulatory power is a gas-exchange based parameter calculated per unit of time:

$$CircP = VO_2 \times MAP \qquad \text{(equation 8)}$$

The principles and theory supporting CircP as an indicator of cardiac pumping capacity similar to CP are explained in detail within studies two and three of this dissertation. Therefore, in brief, the units for CircP have been expressed in absolute terms (mL/min · mm Hg) or adjusted for body weight (mL/kg/min · mm Hg) (Cohen-Solal et al., 2002; VanIterson, Snyder, Joyner, Johnson, & Olson, 2014; Williams, Jackson, et al., 2005; Williams, Tzeng, Barker, & Tan, 2005). Also, in a recent study by Williams *et al.*, it was demonstrated that CircP correlated closely ($r = 0.93$, $p<0.0001$) with CP (via CO_2 rebreathe technique) during exercise testing in 219 NYHA class I-IV HFrEF (Williams, Tzeng, et al., 2005). Our group also recently demonstrated CircP closely relates with

invasive assessment of CP at peak exercise in both HFrEF and HFpEF (r = 0.82 and 0.74, respectively; both $p<0.05$) (*unpublished data from our lab*). Thus, based on these promising, yet limited in number experimental evidence, cardiac pumping capacity during exercise may be estimated using CircP.

2.1.3 Vascular capacitance

Capacitance can be described in general as the magnitude of the total contained blood volume of the vasculature in relation to the transmural pressure imposed on or by the vessels (Guyton et al., 1956; Guyton et al., 1954; Mahapatra, Nishimura, Oh, et al., 2006; Mahapatra, Nishimura, Sorajja, et al., 2006). However, it is important to note that even though this relationship is expressed as a quotient depicting a positive relationship between volume and pressure (equation 2), due to differences in arterial versus venous wall structure as well as the pulsatile nature of blood flow within the arterial circulation, it should not be assumed that these variables are systemically nor constantly inversely related in human or animal systems. As an example, by carefully controlling arterial and venous pressure by acute maximal stimulation of vagus nerves in dogs to control cardiac function, Guyton *et al.* (1956) found that there was a 60 mL increase in arterial blood circulation when the heart was actively pumping versus when circulation was arrested; and, in parallel, there was 60 mL less blood in the venous circulation when the heart was pumping versus when circulation was at a stand-still (Guyton et al., 1956). Moreover, and of further interest, when capacitance of the arterial circulation (thick-walled low distensibility) was compared to capacitance of the venous circulation (thin-walled high distensibility), it was found that the venous circulation has a capacitance 18-30 times greater than capacitance of the arterial circulation. The differences in capacitance

between the two components of the systemic circulation in dogs suggests that blood flow through the arterial circulation could be expected to generate 18-30 times the pressure (i.e. hydraulic power) as that of blood flowing through the venous circulation (Guyton et al., 1956). These findings of Guyton *et al.* (1956) illustrate that peripheral capacitance can influence cardiac hemodynamics; but, perhaps more telling, there appears to be a strong influence of cardiac hemodynamics on peripheral capacitance (Guyton et al., 1956; Guyton et al., 1954). This further supports the hypothesis that in the assessment of cardiac pumping capability it is essential to evaluate the influence that cardiac hemodynamics have on arterial pressure generation associated with blood flow. In contrast, it is also important to assess the influence of attenuated peripheral arterial capacitance on cardiac hemodynamics, particularly because HF patients LV dysfunction and changes in Q which suggest a high degree of afterload dependence.

Pulmonary Vascular Capacitance

The importance of vascular capacitance may be highlighted further when studying the capacitance of pulmonary arterial vasculature and its relationship with cardiac function. Understanding this relationship could be critical because it has previously been reported that low peripheral vascular capacitance was strongly related to survival in HF (Domanski et al., 1999; Mitchell et al., 1997). In addition, it is now well-recognized that individuals with HF are predisposed to in increased risk for pulmonary disease concurrent with HF (i.e. PH) which further increases the mortality risk in patients (Aronson et al., 2011; Butler, Chomsky, & Wilson, 1999; Moraes, Colucci, & Givertz, 2000). Pulmonary hypertension may also be related to the development of interstitial lung fluid accumulation (i.e. pulmonary edema) which is a common symptomatic marker in HF;

although, pulmonary edema in HF is commonly traced back to initial high diastolic filling pressure concurrent with systolic dysfunction and systemic hypertension, which are antecedent events to the onset of "passive and/or mixed" forms of PH in HF (Dickstein et al., 2008; Galie et al., 2009; Lewis, Houssay, Haynes, & Dexter, 1953; Paulus et al., 2007; Pietra et al., 2004; Simonneau et al., 2009).

In early studies of vascular capacitance within the lungs of anesthetized dogs, Sarnoff and Berglund reported several noteworthy findings concerning the relationships between changes in lung blood volume with corresponding pulmonary arterial pressure (Sarnoff & Berglund, 1952). Upon emptying the lungs of blood, these authors observed that when blood was incrementally reinfused into the main pulmonary artery that after reaching a threshold of 15-30 mm Hg, further infusion of blood resulted in a progressive decline in pressure, with the magnitude of decrease being the largest at the highest blood volume. This shift in relationships between blood volume and pressure past 30 mm Hg was suggested to be caused by a relaxation mechanism due to excessive wall stress because it was also shown that when fluid was incrementally withdrawn in the same quantities as injected, pressure decreased in an exaggerated curvilinear pattern (Sarnoff & Berglund, 1952). Moreover, and perhaps relevant to a potential risk for pulmonary edema in HF, these authors noted that fluid losses of 10–20 mL occurred during 10 second pauses between injections and 40–50 mL were lost during 30 second pauses between injections. It is therefore apparent based on these observations that highly variable pulsatile blood flow of persistently elevated volumes from the RV into the pulmonary artery could cause high pulmonary arterial pressure and subsequent interstitial fluid accumulation within lungs of humans. Although, it is also apparent that with intact vessel distensibility and

pressor reflex pathways (e.g. baroreflex), a drop in vascular tone in the presence of high blood volume could occur (i.e. increase in capacitance) (Sarnoff & Berglund, 1952). These novel findings have been subsequently supported by the observations of others using similar animal model techniques (Coleridge & Kidd, 1963).

More recently, the direct relationship between RV hemodynamics and pulmonary arterial hemodynamics has been studied in various cardiopulmonary patients (Friedberg, Feinstein, & Rosenthal, 2007; Mahapatra, Nishimura, Oh, et al., 2006; Mahapatra, Nishimura, Sorajja, et al., 2006; Taylor, Olson, et al., 2013). This is important because traditional assessment of pulmonary vascular resistance (PVR) is not entirely indicative of the direct relationship between pulmonary arterial pressure and RV hemodynamics, it is simply:

$$PVR = \Delta P/Q \qquad \text{(equation 9)}$$

Where ΔP is the pressure difference across the entire pulmonary circulation and Q is flow of blood through the entire pulmonary circulation per unit of time (i.e. ≈Q).

Importantly, PVR primarily reflects pressure within smaller peripheral pulmonary arterioles (diameter, 100–300 μm) which are high resistance vessels that can narrow and lose compliance prior to late stage remodeling of larger arteries of the pulmonary circulation in PH or in individuals with PH secondary to HF (Aronson et al., 2011; Horsfield, 1978; McGoon et al., 2004; McLaughlin et al., 2009; Moraes et al., 2000). Therefore, PVR is not sufficient to describe the integrity nor direct interaction of large pulmonary arterial segments most proximal to the RV. To describe the segmental interaction between the RV and the main pulmonary artery (diameter, 2.72 ± 0.3

centimeters) (Edwards, Bull, & Coulden, 1998), pulmonary vascular capacitance (PV_{CAP}) is useful (equation 2) (Taylor, Olson, et al., 2013).

Focused studies of PV_{CAP} in humans are limited and have mainly centered on individuals with PH likely because of the influence PH has on RV workload and RV SV (Holverda et al., 2006). Although, PV_{CAP} has been successfully assessed using invasive and non-invasive techniques in PH (adults and children), HF, and HF with PH (Friedberg et al., 2007; Mahapatra, Nishimura, Oh, et al., 2006; Mahapatra, Nishimura, Sorajja, et al., 2006; Taylor, Olson, et al., 2013). It was recently observed that PV_{CAP} assessed using cardiac catheterization versus ECHO demonstrated a correlation coefficient $r = 0.74$ in adolescents with PH ($p<0.0001$) (Friedberg et al., 2007).

In one of the first studies of PV_{CAP}, Mahapatra *et al.* (2006) demonstrated that invasive assessment of this index was the strongest indicator of prognosis versus traditional pulmonary function indices in adults with PH (>2.0 mL/mm Hg predicted 100% survival at 4-years) (Mahapatra, Nishimura, Sorajja, et al., 2006). Mahapatra *et al.* (2006) demonstrated PV_{CAP} at rest could range from 0.40 to 3.77 mL/mm Hg in PH (mean, 1.43 ± 0.73 mL/mm Hg), with higher values indicating better prognosis. Subsequent studies in adolescent PH, HF, or HF with PH demonstrated PV_{CAP} values that compared reasonably with measures of PV_{CAP} in adult PH (Friedberg et al., 2007; Mahapatra, Nishimura, Sorajja, et al., 2006; Taylor, Olson, et al., 2013).

The validity of non-invasive assessment of PV_{CAP}, called GX_{CAP}, has recently been demonstrated by our group to closely correlate with direct cath lab measures of PV_{CAP} during exercise in HFrEF ($r = 0.86$, $p<0.01$) and are explained in further detail in studies two and three of this dissertation (Taylor, Olson, et al., 2013). Briefly, to estimate PV_{CAP}

non-invasively using gas-exchange methods, the product of these cardiovascular derived parameters is required (Taylor, Olson, et al., 2013):

$$GX_{CAP} = O_2pulse \times P_{ET}CO_2 \qquad \text{(equation 10)}$$

Summary

The human studies described in this section of central hemodynamics have presented traditional and non-traditional indices proven to be useful in describing the blood flow and pressure generating potential of the heart in HF. Newer indices of central hemodynamics offer several feasible interpretations of the hydraulic power generating capability of the heart. Equally important, many of these indices have been validated against invasive and non-invasive methodology, and the evidence would suggest that non-invasive determination of central hemodynamics can be accomplished with similar accuracy, precision, and repeatability comparable to invasive techniques but without the added risk to the individual or need for additional resources (Friedberg et al., 2007; Johnson et al., 2000; Taylor, Olson, et al., 2013; Whipp et al., 1996; Williams, Tzeng, et al., 2005). Still, despite encouraging evidence which favors newer non-traditional indices (e.g. CP or CircP) for describing central hemodynamics during exercise in HF, the current field remains divided and thus further study is warranted (Cohen-Solal et al., 2002; Lang et al., 2009; Metra et al., 1999).

2.2 Central hemodynamic responses to exercise

2.2.1 Introduction

Irrespective of health condition, the main function of the heart as it pertains to the cardiovascular system is to ensure adequate blood flow (nutrient and O_2 delivery) to

match metabolic demand at rest as well as under all conditions of physiological stress (e.g. cold/heat exposure, gravitational changes, exercise, etc.). Fundamental to this understanding is to point out that the heart does not pump independently. Cardiac pumping capability is influenced by factors of the cardiovascular system (e.g. veins and arteries) which exert their influence on circulation to a great extent during exercise. As such, it is not surprising that independent measurements of cardiac function at rest do not robustly describe cardiac pumping capability in healthy individuals during exercise (Rerych, Scholz, Newman, Sabiston Jr, & Jones, 1978; Stratton, Levy, Cerqueira, Schwartz, & Abrass, 1994). Similarly, in the instance of HF patients, resting measures of cardiac function are not strong indicators for quantifying the overall magnitude of heart pump dysfunction.

2.2.2 Healthy individuals

Overview of cardiac hemodynamics

The total blood volume in humans is approximately 5 L, which, assuming normal cardiac function is intact, this entire volume is pumped through the circulation approximately once per minute at rest. However, as healthy adults transition from rest to dynamic exercise, the rate of VO_2 and Q increase beyond resting ranges, upwards of 20 and 7 times that reported at rest, respectively (Saltin, 1964; Saltin & Astrand, 1967). These specific changes in cardiovascular function which occur during exercise in healthy individuals are particularly evident when examining the early works of Saltin (Saltin, 1964). Saltin observed that both Q and SV changed in near linearity from rest to maximal exercise in healthy individuals performing upright bicycle ergometer exercise (Saltin, 1964). The range of Q and SV for participants was 3.8–6.7 L/min and 54.0–98.0 mL/beat

at rest, respectively. Whereas, the range of Q and SV was 16.0–29.9 L/min and 80.0–166.0 mL/beat at maximal exercise, respectively. Further interesting, the mean Q (18.5 versus 24.1 L/min, significance not reported) and SV (100 versus 134 mL/beat, significance not reported) were lower in women compared to men at maximal exercise which was consistent with differences reported at rest between genders, respectively (Saltin, 1964). Also noted, the linearity between Q and VO_2 throughout exercise became significantly more variable as participants neared maximal exercise (Saltin, 1964), which may have been attributable to body mass (e.g. heart size) and/or more efficient O_2 transport and O_2 utilization related to relationships between hemoglobin (Hb) and arterial blood O_2 content. These observations of Saltin and others subsequently are likely due to Frank—Starling mechanism (Hermansen, Ekblom, & Saltin, 1970; Saltin, 1964; Sullivan et al., 1991); however, additional factors influential to the relationship between Q and VO_2 should be considered (Becklake et al., 1965; de Simone et al., 1997; Freedson, 1981; Ogawa et al., 1992; Salton et al., 2002; Sandstede et al., 2000).

Observations from more recent studies support previous findings in healthy individuals, while providing additional evidence to suggest that there may be a profound influence of diastolic function on the Frank—Starling mechanism for maintenance or even increases in SV at high exercise intensities in highly-trained endurance athletes. Studies of Warburton *et al.* (2002) and Zhou *et al.* (2001) have shown that highly trained cyclists or elite-level runners demonstrate continuous linear increases in SV from rest to maximal exercise (Warburton et al., 2002; Zhou et al., 2001). Further, Warburton *et al.* (2002), using the radionuclide ventriculography method, demonstrated that increases in SV

during exercise were associated with improvements in diastolic function during exercise (Warburton et al., 2002).

In summary, it remains clear that apart from the attenuating influences of aging on cardiac structure and function (Brandfornbrener, Landowne, & Shock, 1955; Gleason & Braunwald, 1962; Hagberg et al., 1985; Sandstede et al., 2000), the cardiac hemodynamic response to exercise via primary influences of the Frank—Starling mechanism could be expected to adequately adjust relative to systemic metabolic demands in healthy individuals.

Overview of pulmonary hemodynamics

Exercise and accompanying increases in Q allows for elevated perfusion of pulmonary capillaries for VO_2 as well as for enhanced release of CO_2. As Q can increase during exercise up to 5–7 times that of resting conditions (Saltin, 1964), the increase in pulmonary arterial hemodynamics and pressure contribute to increases in vascular driving pressure (i.e. difference between pulmonary artery pressure and pulmonary venous pressure) of oxygenated blood to the left atria (Banchero et al., 1966). In contrast, although the pulmonary venous circulation by itself offers little resistance to flow during exercise, increases in left atrial hemodynamics and pressure can cause marked decreases in vascular driving pressure by facilitating increases in pulmonary venous as well as arterial pressures (Eldridge, Braun, Yoneda, & Walby, 2006; Eldridge et al., 1996). However, in the absence of pulmonary vascular disease (e.g. mixed PH), it is suggested that the normal pulmonary arterial hemodynamic response to exercise is suggested to be reflected by a mPAP less than 30 mm Hg in younger (i.e.<50 years age) healthy individuals (~2 × magnitude at rest) in addition to an attenuated rise in pulmonary

capillary wedge pressure (PCWP) and PVR (Eldridge et al., 1996; Galie et al., 2009; Riley, Himmelstein, & et al., 1948; Saltin, 1964; Simonneau et al., 2009).

The overall decrease and/or attenuated rise in PVR during exercise was observed early on by Damato *et al.* (1996) who noted that PVR decreases in parallel with moderate increases in mPAP as exercise intensity increases (i.e. ↑ Q) (Damato, Galante, & Smith, 1966). Damato *et al.* (1966) demonstrated that PVR could drop to nearly 70 dynes · sec/cm^5 · m^2 in the presence of an increase in mPAP upwards of 47 mm Hg (mean 29 ± 8 mm Hg) during the highest level of exercise in young-adult healthy individuals (Damato et al., 1966). However, in the absence of a decrease and/or attenuated rise in PVR during exercise, exaggerated increases in both PVR and mPAP could suggest the presence of abnormal left heart function (i.e. ↑ left atrial pressure related to impaired LV diastolic function), which over-time could contribute to exaggerated resistance of arterioles, poor pulmonary vascular recruitment and dilation, the risk for pulmonary congestion, or PH (Banchero et al., 1966; Fishman, Fritts, & Cournand, 1960; Reeves et al., 1990).

2.2.3 Heart failure

In healthy individuals it has been demonstrated that independent measurements of cardiac function at rest do not robustly describe full cardiac pumping capability (Rerych et al., 1978; Stratton et al., 1994). Similarly, in patients with impaired cardiac function, resting measures of cardiac function are not strong indicators for quantifying the overall magnitude of heart pump dysfunction.

Franciosa *et al.* (1981) observed in HFrEF that resting measurements of cardiac function or structure correlated poorly with total exercise duration (Franciosa, Park, & Barry Levine, 1981). In contrast, Szlachcic *et al.* (1985) observed that cardiac index during

exercise closely related with VO_{2peak} in HFrEF (Szlachcic, Masse, et al., 1985). Also, in HFpEF, Borlaug *et al.* (2006) demonstrated that exercise capacity (i.e. exercise duration and VO_{2peak}) was significantly associated with exercise mediated changes in Q (Borlaug et al., 2006). Indeed, because of these and other observations in HF, it could be suggested that objective measurement of cardiac function during exercise is critical in assessing cardiac pump dysfunction in HF (Borlaug et al., 2006; Borlaug, Nishimura, Sorajja, Lam, & Redfield, 2010; Franciosa et al., 1981; Francis, Goldsmith, & Cohn, 1982; Higginbotham, Morris, Conn, Coleman, & Cobb, 1983; Kronenberg et al., 1998; Szlachcic, Masse, et al., 1985).

Overview cardiac hemodynamics

Much of our understanding of the cardiac hemodynamic response to exercise in HF can be explained by factors which prohibit normal functioning of the Frank—Starling mechanism in patients (e.g. impaired contractility and/or relaxation) (Gleason & Braunwald, 1962; Ross & Braunwald, 1964). In contrast to healthy individuals, there is a large disconnect in the relationships between blood flow and pressure of the central circulation, and secondarily of the peripheral circulation.

Because of gross anatomical (e.g. ischemic/necrotic tissue) and bio-cellular (e.g. desensitized/downregulated β-adrenergic receptors) dysfunctions of the myocardium in HF, the agreement between preload and blood ejected into the systemic circulation cannot always be assumed to be equal (Bristow et al., 1982; Bristow et al., 1986; Dickstein et al., 2008). The disconnect between ventricular filling and emptying as a result of impaired cardiac function and high intracavity filling pressures can be observed at rest, which is then exacerbated further during exercise. This abnormal cardiac

hemodynamic response during physical exertion is frequently associated with the triggering of signs and symptoms that are common in HF.

Lewis *et al.* (1953) in comparing observations from cardiac patients with LV dysfunction to previous findings in healthy individuals suggested that Q_I and SV_I could be similar in HF versus healthy individuals at rest (Dexter et al., 1951; Lewis et al., 1953). In contrast, the similarities between groups at rest did not persist during exercise, demonstrating that differences in Q_I and SV_I were due to a lack of increase in the HF population (Dexter et al., 1951; Lewis et al., 1953). Increases in Q_I during exercise appeared to be largely mediated by adjustments in HR, and changes in SV_I seemed to occur at the expense of abnormally high LV filling pressure in HF. This suggests the presence of abnormal relaxation (i.e. impaired diastolic function) concurrent with an inability to develop adequate wall tension during systole in these patients (Lewis et al., 1953). These findings are supported by the observations of Epstein *et al.* (1967) and Ross *et al.* (1966) who examined cardiac hemodynamic responses at rest and during exercise in healthy individuals and in cardiac patients with LV dysfunction (Epstein, Beiser, Stampfer, Robinson, & Braunwald, 1967; Lewis et al., 1953; Ross, Gault, Mason, Linhard, & Braunwald, 1966). Similarly, these authors also observed that patients did not demonstrate increases in Q_I and SV_I to a similar degree as healthy individuals during exercise despite both groups having comparable Q_I and SV_I at rest (Epstein et al., 1967; Lewis et al., 1953; Ross, Gault, et al., 1966).

Epstein *et al.* (1967) also found that adjustments in Q_I during exercise appeared to be sensitive to adjustments in pulmonary arterial O_2 saturation in patients (Epstein et al., 1967). The relationship between Q_I and pulmonary arterial O_2 saturation was normal until

pulmonary arterial O_2 saturation approached a threshold of approximately 30% (i.e. moderate intensity exercise), whereby any further decrease in saturation (i.e. increasing exercise intensity) did not correspond to adequate adjustments in Q_I (Epstein et al., 1967). The authors found this relationship between Q_I and pulmonary arterial O_2 saturation down to and below 30% useful for identifying LV dysfunction and a potential reason for exercise limitation in cardiac patients.

In contrast, when Epstein *et al.* (1967) compared exercise Q_I responses to exercise intensity (i.e. VO_2) there was no discernable trend in relationships between Q_I and exercise intensity that could be used to indicate at what point, if any, cardiac function was at its true pumping capacity (Epstein et al., 1967). Thus, even at the highest VO_2 measurement it was suggested that the corresponding Q_I should not be assumed to be the absolute limit of cardiac function. As such, with incremental increases in exercise intensity, even with a drop in VO_2, it is not unreasonable to suggest that corresponding increases in $C_{a\text{-}v}O_2$ and Q_I could occur to prolong exercise beyond the point of the highest VO_2 in HF (Epstein et al., 1967; Katz et al., 2000; Sullivan, Knight, Higginbotham, & Cobb, 1989b). It has been observed previously that as VO_2 drops it is possible for parallel increases in O_2 extraction from arterial blood to occur during exercise in both HFrEF and HFpEF (Katz et al., 2000). Moreover, because symptomology (e.g. dyspnea, fatigue, peripheral factors, or discomfort) typically limits exercise capacity in HF prior to reaching a VO_2 limitation, in most instances it would not be accurate to conclude that exercise capacity is commonly limited by VO_2 in HF (Chomsky et al., 1996; Higginbotham et al., 1986; Lipkin, Canepa-Anson, et al., 1986; Piepoli et al., 1999; Wilson et al., 1995). Therefore, Epstein *et al.* (1967) observed that Q, as it related to

pulmonary arterial O_2 saturation was distinctly identifiable at a given threshold of work (i.e. 30% O_2 saturation) whereby upon reaching this threshold, Q increased no further despite the intensity of exercise continuing to increase (Epstein et al., 1967). Therefore, it is reasonable to suggest that the cardiac hemodynamic response relative to pulmonary arterial O_2 saturation during exercise could be effective for objectively describing the limit of cardiac pumping capability in cardiac patients with LV dysfunction.

Included in the study of Ross *et al.* (1966) was the observation that during exercise, the changes in LV end-diastolic pressure in relation to changes in SV_I during exercise were variable and could be considered abnormal in most cardiac patients with LV dysfunction (Ross, Gault, et al., 1966). These observations relate to the functionality of the Frank—Starling mechanism in regulating the blood flow and pressure generating capacity of the heart in patients. In healthy individuals LV end-diastolic pressure could be assumed to be commensurate with LV end-diastolic volume which is directly related with preload and hence SV (Hagberg et al., 1985; Mason, Spann, Zelis, & Amsterdam, 1971; Ross, Morrow, Mason, & Braunwald, 1966; Sandstede et al., 2000). However, the finding of a disconnect between SV_I and LV end-diastolic pressure during exercise in patients foremost suggests that there may be an abnormal disconnect between hemodynamics and appropriate pressure adjustments within the heart. This points to the likelihood that abnormal compliance and relaxation with low contractility could in fact be present in HF and causal for low exercise Q_I and SV_I observed in patients (Braunwald & Ross, 1963). This unique relationship between LV end-diastolic pressure and SV_I that was uncovered during exercise in cardiac patients is significant because many of the patients with LV

dysfunction demonstrated what would be considered normal LV function at rest (Ross, Gault, et al., 1966).

In an extension of these early observations in cardiac patients with LV dysfunction, more recently, investigators have looked to better understand the response of alternative metrics of cardiac function or the hydraulic power generating capacity of the heart (e.g. CP or CircP) during exercise in HF (Cohen-Solal et al., 2002; Lang et al., 2009; Williams, Jackson, et al., 2005). However, in studying CP or CircP for example, it is important to note that these indices do not impart differential mechanisms of action versus Q and SV, rather, it is suggested that they better represent the coordinated influences of venous return, preload, contractility, and afterload (all components which comprise the Frank—Starling mechanism) in describing cardiac hemodynamics (Griffin et al., 1991; Lang et al., 2009; Williams, Jackson, et al., 2005; Williams et al., 2001). Observations from exercise studies which have tested these alternative indices of cardiac hemodynamics have been consistent with studies of Q and SV in HF (Epstein et al., 1967; Lewis et al., 1953; Ross, Gault, et al., 1966), demonstrating that the hydraulic power of the heart in HF does not increase during exercise as would be expected in healthy individuals (Cohen-Solal et al., 2002; Griffin et al., 1991; Lang et al., 2009; Williams, Jackson, et al., 2005; Williams et al., 2001).

Heart failure patients with reduced ejection fraction have been observed to demonstrate CP values upwards of 6.79 watts at peak exercise (lowest = 1.2 watts); although, the average peak exercise CP in this sample of HFrEF patients was markedly lower at 2.96 ± 1.2 watts (resting mean = 0.99 ± 0.29 watts) (Williams et al., 2001). This response of CP to peak exercise testing is consistent with the hypothesis of impaired pump function in

HF, given these patients also demonstrated low Q and VO_2 at peak exercise (12.0 ± 3.4 L/min and 23.1 ± 9.23 mL/kg/min, respectively). Further, Lang *et al.* (2009) recently observed that peak exercise CP appears to track with severity of HF, in that NYHA class I and II patients have nearly 1–1.5 units higher CP compared to patients in NYHA class III and IV (Lang et al., 2009). The differences in peak exercise CP observed between these studies are likely attributable to the larger number of NYHA class II patients in the former study versus a larger number of NYHA class III and IV patients in the latter study (Lang et al., 2009; Williams et al., 2001). Nevertheless, despite differences in CP between studies, the CP response at peak exercise in both studies still suggests impairment of cardiac hydraulic power generating capacity in HFrEF.

The interpretation of impaired cardiac pumping capacity from observations of peak exercise CP in HFrEF are further supported by observations of CP at peak exercise in healthy individuals which have been observed to reach upwards of 7.94 watts with an average of 4.5 ± 1.2 watts (Bromley et al., 2006). Also, in healthy men and women (>45 years old), average CP at peak exercise has been demonstrated to be 4.85 ± 0.79 watts and 3.69 ± 0.68 watts, respectively (Bromley et al., 2006). Thus, the impaired cardiac hemodynamic rise during exercise in HF likely contributes to symptoms since blood flow demands to metabolically active tissue (e.g. respiratory and skeletal muscles) are augmented during exercise (Olson, Joyner, Dietz, et al., 2010).

Accompanying CP observations in HFrEF are newer evidence which extend this area of study to HFpEF. Bhella *et al.* (2011) recently demonstrated that despite no differences in CP at rest between HFpEF and healthy individuals (254 ± 107 versus 271 ± 53 L/min/m^2 · mm Hg, p = 0.624), at peak exercise there was a significant difference between groups

(893 ± 174 versus 1136 ± 240 L/min/m^2 · mm Hg), respectively (Bhella et al., 2011). Indeed, these observations of Bhella *et al.* (2011) provide encouraging evidence which suggests that the hydraulic power generating capabilities of the heart likely related to mechanisms governing the Frank—Starling mechanism (i.e. diastolic dysfunction, poor relaxation, and high intracavity filling pressure) are impaired in HFpEF, perhaps in a manner which supports the likelihood that diastolic dysfunction is more influential in HFrEF than previously thought (Bhella et al., 2011).

Studies of CP during exercise in HF have been followed-up by examinations of CircP during exercise in HF (Cohen-Solal et al., 2002; VanIterson et al., 2014; Williams, Jackson, et al., 2005). Because CircP is suggested to be the surrogate of CP it could be assumed that like CP, CircP would also demonstrate an attenuated rise during exercise in HF. However, using CircP during exercise in HF may have additional benefits because this surrogate uses VO_2 in place of Q in its calculation. Although, as mentioned previously, VO_2 may be highly influenced by non-cardiac factors (e.g. obesity, motivation, skeletal muscle deconditioning) in HF, and consequently it cannot be assumed that VO_2 can always estimate Q in these individuals (Becklake et al., 1965; Chomsky et al., 1996; Chua et al., 1996; Ponikowski et al., 2001; Weber et al., 1982; Wilson et al., 1995).

Nevertheless, observations in HF have indicated that the behavior of CircP during exercise is closely associated with that of CP (Cohen-Solal et al., 2002; Williams, Jackson, et al., 2005; Williams, Tzeng, et al., 2005). Circulatory power assessed at peak exercise is consistently higher in healthy individuals compared to the sickest HF patients (e.g. 3573 ± 1273 versus 2567 ± 984 mL/kg/min · mm Hg, $p<0.001$) (Cohen-Solal et al.,

2002). To help support these previous findings, our group has demonstrated that CircP is indeed lower in HFrEF compared to healthy individuals during submaximal exercise (2721 ± 618 versus 1413 ± 257 mL/kg/min · mm Hg, respectively; $p<0.05$) (VanIterson et al., 2014). Thus, the strength of CircP as a cardiac hemodynamic surrogate in HF is further strengthened when also considering the closeness of relationships observed between CircP and CP during peak exercise testing in HF (HFrEF, $r = 0.93$, $p<0.0001$ (Williams, Tzeng, et al., 2005), and HFpEF, $R^2 = 0.68$, $p<0.05$ (*unpublished research from our lab*)).

Consistent with the observations of CircP during exercise in HF, the rise of O_2pulse has been observed to be attenuated in HF (Cohen-Solal et al., 1997; Cohen-Solal et al., 2002). This could be expected since this parameter is derived from assumptions which suggest that VO_2 changes in parallel with cardiac hemodynamics during exercise (Crisafulli, Piras, et al., 2007; Whipp et al., 1996). In a comparison study of O_2pulse at peak exercise, Cohen-Solal *et al.* (1997) demonstrated that peak O_2pulse was markedly lower in HF compared to healthy individuals (Cohen-Solal et al., 1997). Cohen-Solal *et al.* (1997) reported mean O_2pulse values of 8.8 ± 3.0 mL/beat in NYHA class II-IV HF (Cohen-Solal et al., 1997), whereas Bhambhani *et al.* (1994) previously demonstrated mean maximal O_2pulse values in trained and untrained healthy individuals to be 23.8 ± 5.1 mL/beat and 20.8 ± 3.9 mL/beat, respectively (Bhambhani et al., 1994). Thus, although O_2pulse is considered an indirect measurement of cardiac pumping capability, observations of O_2pulse at peak exercise in HF are consistent with traditional (i.e. SV) assessment of cardiac hemodynamics in these patients. Low O_2pulse suggests that the ability to generate blood flow from the heart is impaired.

In summary, observations of traditional and non-traditional cardiac hemodynamic responses to exercise in HF indicate that the heart is incapable of generating the proper blood flow and perfusion pressure to accommodate the increased metabolic demands of exercise. It is likely that a significant contribution to impaired pump function in HF is attributable to an unknown degree of both diastolic and systolic dysfunction (Kitzman, Higginbotham, Cobb, Sheikh, & Sullivan, 1991; Ross & Braunwald, 1964). Impaired diastolic function disrupts the ascending arm of the Frank—Starling curve by inhibiting the ability to generate end-diastolic pressure at an appropriate volume of blood (i.e. causing excessive loading and myocardial stretch) and therefore causing a rightward shift in the curve while also causing large end-diastolic and systolic volumes (Ross & Braunwald, 1964). Whereas, systolic dysfunction also in the presence of diastolic impairment could further shift the Frank—Starling curve rightward and downward due to the additional loss of contractility and wall tension development during systole (Ross & Braunwald, 1964). Still, even with impaired systolic and/or diastolic function, it remains possible for HF to generate blood flow with the Frank—Starling mechanism during exercise. Although, in comparison to healthy individuals, in HF this is believed to occur on the descending arm (i.e. less myocardial distension) of the Frank—Starling curve in addition to the fact that any increase in Q and SV from resting levels comes at the expense of abnormal intracavity blood volume and pressure throughout diastole and systole (Ross & Braunwald, 1964).

Overview of pulmonary hemodynamics

Briefly mentioned earlier, it is now well-recognized that the pulmonary hemodynamic response to exercise in HF is not consistent with responses demonstrated in healthy

individuals (Butler et al., 1999; Epstein et al., 1967). In general, there appears to be a severe increase in mPAP (>30 mm Hg) and attenuated decreases in PVR with increasing exercise workload in HF. Epstein *et al.* (1967) demonstrated that mPAP was 53 ± 25 mm Hg in HF at maximal exercise, which was over two times the values demonstrated in healthy individuals (29 ± 5 mm Hg, $p<0.05$) (Epstein et al., 1967). These findings were extended by Butler *et al.* (1999) who observed that elevated levels of mPAP (28 ± 11 versus 40 ± 13 mm Hg), PCWP (18 ± 9 versus 23 ± 10 mm Hg), and PVR (2.45 ± 1.57 versus 2.41 ± 1.52 wood units) at rest were exacerbated during exercise in HFrEF (Butler et al., 1999). Additionally, it was observed that severity of pulmonary hemodynamic dysfunction appeared to directly relate with the degree of cardiac hemodynamic impairment in HF. Individuals with HF who had resting PVR greater than 3.5 wood units demonstrated significantly worse peak exercise mPAP, PCWP, Q, and VO_2 compared to patients with a PVR less than 1.5 wood units (Butler et al., 1999). Together, these observations of abnormal pulmonary hemodynamic responses during exercise suggest that dysfunctional regulation of pulmonary pressures should be considered a serious concern in HF; and, that the magnitude of cardiac impairment during exercise may be related to the severity of pulmonary hemodynamic impairment in these patients (Butler et al., 1999; Epstein et al., 1967; Franciosa, Baker, & Seth, 1985).

Pulmonary hemodynamic responses during exercise in HF have been reported by Franciosa *et al.* (1985) to average 50.4 ± 12.9 mm Hg while reaching nearly 60 mm Hg in certain individuals (Franciosa et al., 1985). Also, consistent with the sharp rise in mPAP observed from rest to maximal exercise was the finding that PVR failed to drop significantly at maximal exercise. Somewhat surprising, however, despite abnormal

mPAP at maximal exercise, these parameters did not appear to correlated with exercise capacity (i.e. VO_{2MAX}) in HF. In contrast, Q_I, which is attenuated in HF appears to be a rather strong correlated of VO_{2MAX} (Franciosa et al., 1985). However, it is possible that the abnormal pulmonary hemodynamic responses to maximal exercise was secondary to the primary influences of impaired cardiac function; and, perhaps related to combined diastolic and systolic dysfunction in these patients (Franciosa et al., 1985). An elevated PCWP at maximal exercise is suggestive of elevated left atrial pressure (related to poor LV relaxation) which likely leads to back pressure toward the pulmonary venous circulation and contributes to the pulmonary hemodynamic responses that are observed in HF (Franciosa et al., 1985).

In support of the potential role of diastolic dysfunction in contributing to abnormal pulmonary hemodynamics during exercise in HF, more recent evidence suggests that mPAP and systolic pulmonary arterial pressure (SPAP) at peak exercise are nearly two-fold higher in HFpEF compared to healthy individuals (Borlaug, Nishimura, et al., 2010). Heart failure with preserved ejection fraction patients are known to have impaired LV relaxation at rest which is exacerbated during exercise. Moreover, in comparing cardiac with pulmonary hemodynamic responses between HFpEF and controls at rest and peak exercise, the evidence strongly suggests that exercise exacerbates abnormal cardiac and pulmonary hemodynamic function in HFpEF (Borlaug, Nishimura, et al., 2010). Thus, HFpEF patients demonstrate abnormal pulmonary hemodynamic responses to exercise which, despite differences in HF classification, appear to be similar to HFrEF (Borlaug, Nishimura, et al., 2010; Butler et al., 1999; Franciosa et al., 1985).

In summary, there is more than a negligible interaction between left sided HF, abnormal pulmonary hemodynamics, and impaired right heart function during exercise in patients with chronic HF. In addressing the latter consequence of chronic LV diastolic dysfunction, with or without concurrent systolic dysfunction, it is crucial to understand pulmonary hemodynamic responses to exercise as direct interactions with cardiac function given SV is received by the pulmonary circulation and this appears to occur under exaggerated pulmonary pressures during exercise in HF (Borlaug, Nishimura, et al., 2010; Butler et al., 1999; Epstein et al., 1967; Franciosa et al., 1985).

2.3 Peripheral neural factors influencing central hemodynamics during exercise

2.3.1 Introduction

Input to the brain originating from peripherally located sensory neurons can facilitate adjustments in cardiovascular function in all individuals. This afferent signal transduction comes from pathways of several types of receptors which includes contraction and/or metabolically activated skeletal muscle ergoreceptors—mechanoreceptors and metaboreceptors (Batman et al., 1994; Hayes, Kindig, & Kaufman, 2005; Herr, Imadojemu, Kunselman, & Sinoway, 1999; Kaufman, Longhurst, Rybicki, Wallach, & Mitchell, 1983; Kniffki, Mense, & Schmidt, 1978; MacLean, Imadojemu, & Sinoway, 2000; McCloskey & Mitchell, 1972; Piepoli, Clark, & Coats, 1995; Piepoli, Dimopoulos, Concu, & Crisafulli, 2008; Rotto & Kaufman, 1988). More recently it has been suggested that the strength of activity originating from these reflexes and others (e.g. baroreceptors and chemoreceptors) changes depending on health status (e.g. HF versus healthy individuals) as well as resting versus exercising conditions (Crisafulli et al., 2011; Crisafulli et al., 2006; Crisafulli, Salis, et al., 2007; Dibner-Dunlap & Thames, 1992;

Hammond et al., 2001; Hansen, Thomas, Jacobsen, & Victor, 1994; Johnson, Joyner, & Olson, 2014; Leshnower, Potts, Garry, & Mitchell, 2001; Mitchell & Schmidt, 1983; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1995; Piepoli et al., 1996; Piepoli et al., 1999; Scott et al., 2000; Scott et al., 2004). Studies examining the function of the ergoreflex suggests that changes in efferent neural drive during exercise can be influenced by neural feedback originating within skeletal muscle (Amann et al., 2010; Asmussen & Nielsen, 1964; Coote, Hilton, & Perez-Gonzalez, 1971; Daley, Khan, Hogeman, & Sinoway, 2003; Freund, Rowell, Murphy, Hobbs, & Butler, 1979; Mark, Victor, Nerhed, & Wallin, 1985; McCloskey & Mitchell, 1972; Mitchell, Kaufman, & Iwamoto, 1983; Sinoway & Prophet, 1990; Vorluni & Volianitis, 2008). Studies examining the influence of afferent feedback from skeletal muscle suggests this feedback may lead to beneficial adjustments of the autonomic nervous system (ANS) by enhancing the exercise responses of the central circulation and cardiac tissue (i.e. ↑ sympathetic drive, ↓ parasympathetic drive, and ↑ Q) and vasculature of the peripheral circulation in an attempt to regulate homeostasis of the circulation (Crisafulli et al., 2011; Crisafulli et al., 2006; Fisher et al., 2010; Floras, 1993; McDaniel et al., 2010; Spranger et al., 2013; Tsuchimochi, Matsukawa, Komine, & Murata, 2002; White, Lykidis, & Balanos, 2013).

In HF, however, evidence suggests this peripheral neural feedback is dysfunctional and contributes to exercise intolerance and exertional symptoms characteristic of the syndrome (Piepoli, 1998; Piepoli et al., 1999). Dysfunction of this neural feedback in HF is closely related to impaired ANS function, skeletal muscle myopathy (e.g. cachexia), and cardiovascular dysfunction (Coats, Clark, Piepoli, Volterrani, & Poole-Wilson, 1994; Minotti et al., 1991; Piepoli et al., 1996; Piepoli et al., 2006; Piepoli et al., 1999;

Szlachcic, Massie, Kramer, Topic, & Tubau, 1985; Wilson, Martin, & Ferraro, 1984). Moreover, it has been suggested that the ergoreceptor pathway is tonically dysfunctional in HF and a main contributor to chronic sympathoexcitation and symptomology observed at rest and during exercise in these patients (Crisafulli, Salis, et al., 2007; Floras, 1993; Hammond et al., 2001; Johnson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999; Scott et al., 2004; Smith, Mitchell, Naseem, & Garry, 2005; Smith, Williams, Mitchell, Mammen, & Garry, 2005). Nevertheless, despite convincing observations with regard to the negative influence of the ergoreceptor pathway on cardiovascular function (i.e. ventilation and peripheral hemodynamics) in HF, it remains unclear what influence this pathway has on cardiac and pulmonary vascular hemodynamic function associated with exercise in HF.

2.3.2 Anatomy and physiology of the ergoreceptor pathway

The ergoreceptor pathway is a reflex arc linking the peripheral nervous system to the central nervous system. Ergoreceptors are unencapsulated afferent nerve endings found within the interstitium and around the vasculature and connective tissue and skeletal muscle. Upon stimulation, the neural feedback from skeletal muscle ergoreceptors synapse at cells located within the laminae of the dorsal horn of the spinal cord at which point signal transduction converges and is relayed toward the brainstem via second-order spinal neurons for organization, integration, and eventual modulation at higher-brain centers (Ciriello & Calaresu, 1977; Coote et al., 1971; Iwamoto, Parnavelas, Kaufman, Botterman, & Mitchell, 1984; Iwamoto, Kaufmann, Botterman, & Mitchell, 1982; Mark et al., 1985; McCloskey & Mitchell, 1972; Mitchell & Schmidt, 2011; Piepoli et al., 1995; Randle Adair, Hamilton, Scappaticci, Helke, & Gillis, 1977; Sato, 1973; Strange et

al., 1993; Victor, Seals, & Mark, 1987). The brainstem houses neurons responsible for processing and integrating afferent input to regulate respiration, heart rhythm, and blood pressure; but, more specifically, contained within this medullary region are the ventrolateral medulla and dorsal caudal nucleus tractus solitarius (NTS) which are critical sites for sensory processing of somatic and cardiorespiratory neural feedback related to ergoreceptors. The ventrolateral medulla and caudal NTS are the terminals for afferent neural traffic from ergoreceptors, however, after convergence and organization, the resultant integrated neural signaling is relayed to higher brain centers (e.g. central command—hypothalamus, motor cortex, and cerebellum) for further modulation at which point appropriate efferent neural signals are sent to facilitate cardiovascular pressor responses (Ciriello & Calaresu, 1977; Iwamoto et al., 1984; Iwamoto et al., 1982; Iwamoto et al., 1985; Mark et al., 1985; McCloskey & Mitchell, 1972; Potts, Fuchs, Li, Leshnower, & Mitchell, 1999; Randle Adair et al., 1977; Sato, 1973).

Ergoreceptors—Metaboreceptors and Mechanoreceptors

Two specific types of sensory nerve fibers make up the umbrella term ‘ergoreceptors’. These polymodal muscle afferents have been identified as having high sensitivity to mechanical and/or metabolite specific stimuli related to skeletal muscle contraction. More specifically, group III myelinated C-fibers are considered mechanoreceptors and demonstrate a primary sensitivity to non-noxious mechanical deformation and pressure changes of skeletal muscle fibers with secondary responsiveness to metabolic byproducts of muscle contraction (Hanna, Hayes, & Kaufman, 2002; Hanna & Kaufman, 2004; Li & Sinoway, 2002; Middlekauff et al., 2004; Middlekauff et al., 2001; Smith, Mammen, Mitchell, & Garry, 2003; Smith, Mitchell, et al., 2005). In contrast, group IV

unmyelinated A-δ fibers are termed metaboreceptors and are primarily sensitive to ischemic metabolites, metabolic byproducts, and acidosis as a function of muscle contraction (e.g. H^+, pH, capsaicin, bradykinin, prostaglandins, K^+, lactic acid, or arachidonic acid) (Clark, Piepoli, & Coats, 1995; Hanna et al., 2002; Kaufman, Iwamoto, Longhurst, & Mitchell, 1982; Kaufman et al., 1983; Kaufman & Rybicki, 1987; Kaufman, Rybicki, Waldrop, & Ordway, 1984; Li & Sinoway, 2002; McCord, Tsuchimochi, & Kaufman, 2009; Piepoli et al., 1995; Rotto, Stebbins, & Kaufman, 1989; Rybicki, Kaufman, Kenyon, & Mitchell, 1984; Scott, Wensel, et al., 2003; Stebbins, Maruoka, & Longhurst, 1986; Victor, Bertocci, Pryor, & Nunnally, 1988). Although it may be commonly recognized that metaboreceptors and mechanoreceptors function in two distinct mechanistic pathways, the associated reflex pathways of these afferents are not mutually exclusive in that both types of ergoreceptors can lead to adjustments in ANS activity and sympathoexcitation in humans (exercise pressor reflex, EPR). The stimulation of mechanoreceptors (mechanoreflex) or metaboreceptors (metaboreflex) have been closely associated with ventilatory, cardiac, and peripheral hemodynamic adjustments which occur in healthy individuals during and immediately following exercise (Amann et al., 2010; Coote et al., 1971; Fisher et al., 2010; Mark et al., 1985; McCloskey & Mitchell, 1972; Spranger et al., 2013).

The earliest evidence demonstrating muscle contraction could evoke a pressor reflex was by Alam and Smirk (1937). These authors demonstrated that direct increases in blood pressure could be elicited using circulatory occlusion of the leg (via sphygmomanometer cuffs around the upper thigh) during seated dynamic calf exercise. This study also showed that increases in blood pressure which occurred during exercise were augmented

at the cessation of exercise by maintaining suprasystolic cuff pressure for an additional 3–4 min post-exercise (Alam & Smirk, 1937). Because of the exaggerated blood pressure response demonstrated individual using this technique it was speculated that the heightened response during post-exercise with occlusion was caused by retention of the byproducts from muscle contraction (e.g. H^+, lactic acid, K^+) within the muscle which facilitated a pressor reflex (i.e. ↑ sympathetic drive) via afferent signal transduction (Alam & Smirk, 1937).

2.3.2.1 Metaboreflex

Metaboreceptors are stimulated by the accumulation of metabolites as opposed to the act of contracting muscle. Therefore, it is suggested that rapidity of firing of group IV afferents is consistent with time needed for the accumulation of byproducts of skeletal muscle metabolism, however, these afferents may also fire in direct proportion to magnitude of metabolite buildup (Kaufman et al., 1983; Kaufman, Rybicki, et al., 1984; Kaufman, Waldrop, Rybicki, Ordway, & Mitchell, 1984).

The laboratory of Coote *et al.* (1971) was one of the first to directly confirm the pressor response of the metaboreflex by using isolated electrical stimulation of lumbar and sacral ventral roots to simulate hind-limb exercise in cat preparations (Coote et al., 1971). Observations from this novel study suggested that increases in arterial blood pressure, HR, and ventilation were reflex responses which originated within contracting muscle. Also demonstrated was that when vascular occlusion to the contracting limb was induced to attenuate arterial blood flow, the rise in arterial blood pressure was augmented compared to contractions during non-ischemia, suggesting that the origin of the metaboreceptor pressor response was chemical in nature and caused by the accumulation

of metabolites from skeletal muscle metabolism (Coote et al., 1971; Coote & Perez-Gonzalez, 1970; Mark et al., 1985; Piepoli et al., 1995). Moreover, to confirm the muscular origins of the metaboreflex response, these authors further demonstrated that increases in arterial blood pressure were abolished when muscular contraction was inhibited by cholinergic blockade using gallamine triethiodide, as well as when sensory input to the spinal cord was eliminated via transection of dorsal roots of lumbar and sacral regions (Coote et al., 1971).

McCloskey and Mitchell subsequently demonstrated, in a similar feline model, that anodal blockade of the L_7–S_1 dorsal roots did not influence cardiovascular responses to muscle contraction, whereas applying a topical anesthetic to dorsal roots of this same region did not inhibit group I and II sensory nerves but did attenuate cardiovascular responses to muscle contraction (McCloskey & Mitchell, 1972). Thus, it was suggested that skeletal muscle group IV and to a lesser extent group III nerve afferents stimulated by exercise, ischemic exercise (arterial and venous occlusion), and post-exercise ischemia (PEI) could unequivocally evoke chemically mediated pressor reflex response increases in arterial blood pressure, HR, and ventilation (Coote et al., 1971; McCloskey & Mitchell, 1972). Further, in a more recent study in humans using adrenergic or cholinergic blockade, Fisher *et al.* (2010) observed that the blood pressure response to isometric handgrip exercise (i.e. 25.0% and 40.0% of maximum voluntary contraction, MVC) or PEI was dependent on metaboreflex feedback (Fisher et al., 2010). Additionally, these authors found that during PEI, the engagement of the metaboreflex resulted in an augmented HR response which paralleled blood pressure changes (Fisher et al., 2010). Thus, these newer data in healthy individuals suggest that the intensity of the

metaboreflex response may be workload dependent and that this may influence cardiac sympathetic drive while contributing to adjustments in central hemodynamics (i.e. ↑ Q and SV) in addition to its known impact on peripheral hemodynamics. There is substantive evidence to suggest that the metaboreflex initiated during exercise in healthy individuals contributes to ANS activity directed toward central and peripheral vascular to benefit cardiovascular function.

2.3.2.2 Mechanoreflex

Mechanoreceptors are abundant within and around skeletal muscle of humans and animals. Yet, despite the similarity in anatomical origin of these receptors, the polymodal mechanoreceptor is unique because of its rapid and preferential sensitization by non-noxious mechanical (e.g. stretch/contraction) and to a lesser extent chemical stimuli (Adreani & Kaufman, 1998; Batman et al., 1994; Hayes et al., 2005; Herr et al., 1999; Kaufman, Waldrop, et al., 1984; McDaniel et al., 2010). Observations from studies in both healthy individuals and animals suggest that the mechanoreflex is particularly active during periods of passive and/or low- to- moderate intensity rhythmic exercise (Batman et al., 1994; Gladwell & Coote, 2002; Hayes et al., 2005; Herr et al., 1999; McDaniel et al., 2010). Upon sensitization, the mechanoreflex has the capacity to influence adjustments in ANS activity (↑ sympathetic drive) in order to facilitate changes in cardiovascular function which can result in beneficial adjustments to HR, blood pressure, and ventilation in order meet the metabolic demands of the cardiovascular system during exercise in healthy individuals (Gladwell & Coote, 2002; McCloskey & Mitchell, 1972; McDaniel et al., 2010; Mitchell et al., 1983; Vorluni & Volianitis, 2008; White et al., 2013).

Initial findings by McCloskey and Mitchell and later of Adreani and Kaufman and Tsuchimochi *et al.* (2002) indicate that mechanoreceptors in addition to metaboreceptors within cat skeletal muscle during PEI and ischemic and non-ischemic static and/or dynamic exercise can facilitate reflex responses of cardiac, cardiovascular, and ventilatory systems (Adreani & Kaufman, 1998; McCloskey & Mitchell, 1972; Tsuchimochi et al., 2002). Moreover, to extend the observations of McCloskey and Mitchell (1972) (McCloskey & Mitchell, 1972), in a similar feline model in which ventral root stimulation was used to facilitate static hind-limb exercise, Kaufman *et al.* (1983) quantified the relative activity and pattern of responses of group III and IV nerve afferents required to elicit cardiovascular responses (Kaufman et al., 1983). Kaufman *et al.* (1983) demonstrated that both groups of nerve afferents contributed to reflex responses, but also that there was contrasting and preferential activity of group III and IV nerve afferents specific for mechanical and chemical stimuli, respectively, elicited from contracting skeletal muscle (Kaufman et al., 1983). In more recent studies of healthy individuals during exercise, there is evidence which suggests that sensitized skeletal muscle mechanoreceptors contribute to augmented sympathetic drive during exercise that can contribute to normal cardiac, vascular, and ventilatory function which is observed during exercise (Batman et al., 1994; Gladwell & Coote, 2002; Herr et al., 1999; McDaniel et al., 2010; Vorluni & Volianitis, 2008, 2010; White et al., 2013).

Herr *et al.* (1999) measured muscle sympathetic nerve activity (MSNA) of non-exercising legs of eight healthy men during 12-repetitions (each lasting 20.0 seconds of duration) of static left-leg quadriceps exercise at 25.0% of relative MVC (Herr et al., 1999). These authors suggest that mechanoreflex mediated increases in MSNA occurred

within 4-6 seconds of the onset of low-intensity intermittent quadriceps contractions. Also observed, beginning with repetition number three, it was apparent that significant increases in MSNA occurred as a function of exercise repetition number and, hence, the mechanoreflex is potentially related to exercise intensity or work performed. Stimulation of the mechanoreflex was also observed to be closely related to adjustments in cardiovascular outcomes, with both HR and MAP increasing with each successive exercise repetition starting from baseline (Herr et al., 1999). In contrast to the findings of Herr *et al.* (1999), metaboreceptor-mediated elevation of MSNA has been demonstrated to take upwards of 30–60 seconds (Mark et al., 1985).

To extend the observations of Herr *et al.* (1999) (Herr et al., 1999), White *et al.* (2013) found that stretch activation of skeletal muscle mechanoreceptors could influence significant increases in SPAP, peripheral blood pressures, Q, SV, and HR in healthy individuals during ischemic plantar-flexor exercise (White et al., 2013). Moreover, SPAP during PEI (i.e. metaboreflex activation alone) was not associated with changes in Q during this condition (White et al., 2013). Whereas, when PEI combined with passive-stretch was present (i.e. chemical and mechanical stimuli present), it was demonstrated that significant increases in Q (via ↑ in HR and SV) occurred in the absence of significant changes in SPAP (White et al., 2013). Thus, importantly, these observations suggest that the mechanoreflex has the capacity to influence metaboreflex-mediated pulmonary vasodilation and/or attenuation of metaboreflex-mediated increases in PVR. This potentially could occur via mechanoreflex-mediated increases in cardiac sympathetic drive and parasympathetic-vagal withdrawal, as would be suggested by the increases in HR and SV during PEI + passive-stretch (White et al., 2013). As such, the observations

of this study in healthy individuals as well as those of others in animals and humans clearly indicate that the mechanoreflex is excitable with deformation of skeletal muscle (i.e. passive, static, and/or dynamic exercise) which likely contributes to increases in MSNA that are closely associated with cardiac, vascular, and ventilatory reflex responses (Adreani & Kaufman, 1998; Batman et al., 1994; Hayes et al., 2005; Herr et al., 1999; Kaufman, Waldrop, et al., 1984; Lykidis, White, & Balanos, 2008; Mark et al., 1985; McCloskey & Mitchell, 1972; White et al., 2013). It also remains possible that stimulation of the mechanoreflex may occur prior to that of the metaboreflex and therefore an earlier contributor to adjustments in MSNA than the metaboreflex. Lastly, the strength of the mechanoreflex pressor response may be augmented in accordance with exercise intensity or skeletal muscle work output as well as in the presence of additional chemical stimuli (Adreani & Kaufman, 1998; Gladwell & Coote, 2002; Hayes et al., 2005; Herr et al., 1999; Kaufman et al., 1983; Leshnower et al., 2001; McDaniel et al., 2010; Tsuchimochi et al., 2002; White et al., 2013).

2.3.2.3 Exercise pressor reflex and central hemodynamics

Cardiac hemodynamics

In contrast to the evidence supporting the association between the EPR and ventilatory and peripheral vascular control during exercise, our understanding of the magnitude of association between the EPR and the adjustments which occur to central hemodynamics during or immediately following exercise in healthy individuals is less clear. In this light, a few studies have demonstrated evidence to suggest an association between the EPR and cardiac hemodynamics during exercise in an attempt to regulate circulation in healthy individuals.

In a recent study on the influence of the metaboreflex on Q in the canine model during treadmill running, Ichinose *et al.* (2010) observed that when the metaboreflex was activated by an acute reduction in hindlimb blood flow there was a significant increase in Q (↑ HR and SV) which was sustained during the course of ischemia (Ichinose et al., 2010). Moreover, when Q was not permitted to increase beyond the level that observed during non-metaboreflex activated exercise, the metaboreflex mediated a significant increase in peripheral vascular resistance and decreased vascular conductance in non-ischemic limbs (Ichinose et al., 2010). This reduction in vascular conductance was significant compared to the condition where Q was freely able to adjust. Thus, these contrasting responses of hemodynamics in response to metaboreflex activation during exercise in canines suggest that metaboreflex-mediated adjustments in Q occur in an attempt to restore blood flow to hypo-perfused metabolically active tissue (Ichinose et al., 2010). Whereas, when cardiac reserve is impaired (e.g. HF), metaboreflex stimulation leads to increases in peripheral vascular resistance. These findings suggest that stimulation of the metaboreflex can increase cardiac contractility (i.e. SV) in non-failing hearts, and that Q is an important mediator of arterial pressure which reaffirms the role of the heart as a hydraulic power generator.

In a similar experimental set-up to study the association between the metaboreflex and Q responses in canines, observations by Spranger *et al.* (2013) support the relationship between Q and the metaboreflex demonstrated by Ichinose *et al.* (2010) (Ichinose et al., 2010; Spranger et al., 2013). However, to extend these findings and attempt to isolate the metaboreflex to a greater extent versus the potential influences of the mechanoreflex, Spranger *et al.* (2013) also studied the Q response during post-exercise recovery with

metaboreflex activation (Spranger et al., 2013). These authors found significantly higher Q (via HR and lesser SV) and MAP during post-exercise recovery with metaboreflex activation compared to recovery without metaboreflex stimulation (Spranger et al., 2013). This was accompanied by no significant differences in vascular conductance between conditions which could be expected since Q was freely able to adjust and no central command was present as these canines were in a state of static rest. Also, although not significant, SV was still higher during recovery with metaboreflex activation versus normal recovery (Spranger et al., 2013). These observations during and immediately following exercise suggest that there is a strong influence of the metaboreflex on contributing to increased HR, SV, and Q in a condition where cardiac function is not impaired. It is likely that adjustments in factors (e.g. MAP and vascular conductance) influencing perfusion pressure to metabolically active tissue are closely associated with the ability of cardiac hemodynamics to adjust at rest and during exercise healthy individuals.

To extend these observations to healthy individuals, Crisafulli *et al.* (2011) recently observed that Q responses to metaboreflex activation during exercise and post-exercise were consistent with the observations of Ichinose *et al.* (2010) and Spranger *et al.* (2013) in healthy canines (Crisafulli et al., 2011; Fisher et al., 2010; Spranger et al., 2013). It was shown that increased Q during hand-grip exercise with an metaboreflex stimulation (via upper arm suprasystolic cuffing) was significantly higher versus hand-grip exercise without metaboreflex stimulation. Alternatively, during post-exercise recovery with suprasystolic cuffing, Q was significantly higher versus rest, whereas Q during normal recovery was no different from rest (Crisafulli et al., 2011). Additionally, the SV

response during post-exercise recovery with metaboreflex augmentation was significantly higher versus rest as well as that of normal recovery. These differences in SV between recovery conditions contrasted the HR response which recovered to resting values with and without metaboreflex stimulation. These observations suggest that metaboreflex stimulation increases in HR and Q in healthy individuals (Crisafulli et al., 2011). Whereas, because of observations during post-exercise recovery with sustained augmentation of the metaboreflex, it is possible that increases in Q via elevated SV are significantly influenced by group IV metabolite-sensitive neural feedback. Accordingly, although it is difficult to interpret during exercise whether metabo- or mechano-reflexes are the primary mediators of this reflex, it would appear as a result of post-exercise responses of SV that the metaboreflex can contribute to increased cardiac contractility (i.e. SV) in healthy individuals.

Pulmonary hemodynamics

Contrasting what would appear to be a favorable influence of the EPR on cardiac hemodynamics in healthy individuals, recent findings from studies suggest that pulmonary hemodynamics may be impaired by the EPR in healthy individuals. It was observed that sustained isometric exercise of handgrip muscles followed immediately by upper arm regional circulatory occlusion (RCO) during recovery was associated with elevated SPAP versus a resting condition immediately followed by RCO (control) (Lykidis et al., 2008). This elevation in SPAP during exercise and recovery with RCO was significantly higher versus resting values of SPAP, while also demonstrating that SPAP did not return to baseline until the second minute of recovery following release of the upper arm cuff. These adjustments in SPAP appeared to relate with changes in Q and

SV which also may have been associated with adjustments in peripheral blood pressure (i.e. MAP, SBP, and DBP). Cardiac output and peripheral blood pressure were significantly higher compared to rest during exercise and recovery with RCO, whereas SV was higher in these same comparisons albeit not significantly (Lykidis et al., 2008). It was also apparent that exercise and recovery with RCO was associated with higher HR, SV, and Q versus the control condition. Thus, the metaboreflex may play a role in regulating the pulmonary vasculature as well in healthy individuals. Alternatively, it is possible that because of the higher distensibility of pulmonary vasculature compared to that of the peripheral arterial vasculature, metaboreflex-mediated increases in SPAP are protective to the pulmonary vasculature to assist in increasing pulmonary capillary perfusion while also preventing over distension of vessels during periods of increasing pulmonary blood flow (Lykidis et al., 2008).

This same group subsequently studied the combined influence of metabo- and mechano-reflex activation on pulmonary hemodynamics in healthy individuals (White et al., 2013). In a unique study design using active plantarflexion exercise or passive dorsiflexion stretch of calf muscles, it was shown that ischemic exercise results in significantly higher SPAP compared to rest and that this significance persisted during ischemic recovery and then during ischemic dorsiflexion of muscles. The changes in SPAP also appeared to be markedly higher versus the control condition of no exercise followed by ischemic rest and ischemic dorsiflexion. Similar to previous observations with hand-grip exercise (Lykidis et al., 2008), changes in SPAP appeared coincide with changes in HR, SV, and Q during plantarflexor exercise and ischemic recovery (White et al., 2013). However, in extending earlier observations, it was observed during ischemic dorsiflexion in the

exercise condition that HR, SV, and Q were significantly higher compared to rest, whereas this did not occur in the control condition. Moreover, changes in SV and Q during ischemic recovery and ischemic stretch following exercise appeared to mirror adjustments in blood pressure (i.e. MAP and DBP). These findings corroborate previous observations to suggest that active mechanoreflex stimulation with augmentation of the metaboreflex can contribute to increases in SPAP which parallel Q, HR, and peripheral blood pressure in healthy individuals (Lykidis et al., 2008; White et al., 2013). These observations of the central and peripheral hemodynamic response to metabo- and mechano-reflex activation shed light on the integrated role each of these pathways has on circulatory regulation during exercise in healthy individuals.

In summary, the metaboreflex contributes to elevations in SPAP in healthy individuals (Lykidis et al., 2008; White et al., 2013); however, the highest level of SPAP achieved in these individuals would not typically be considered abnormal in comparison to the level reached at rest or during exercise in the disease conditions such as HF or PH (Aronson et al., 2011; Galie et al., 2009; Simonneau et al., 2009). Therefore, although unclear, it is unlikely that the elevation in SPAP in response to metaboreflex activation is indeed a harmful response in healthy individuals. Rather, this stiffening of pulmonary arteries would appear to be a normal mechanism to protect the pulmonary vasculature in response to increased Q and SV, which could evoke excessive distention of pulmonary vessels due to augmented vascular loading related to augmented pulmonary blood flow.

2.3.3 Exercise pressor reflex and the "muscle hypothesis" in heart failure

Despite the capacity of the EPR to beneficially influence cardiac, cardiovascular, and ventilatory function during exercise in healthy individuals (Amann et al., 2010; Crisafulli

et al., 2011; Fisher et al., 2010; Mark et al., 1985; McDaniel et al., 2010; White et al., 2013), mounting evidence suggests that EPR pathways are dysfunctional in HF and that these pathways are associated with tonic neurohumoral hyperexcitation, ergoreceptor hyper- or hypo-sensitization, and impaired cardiovascular function; which, suggests that peripheral neural mechanisms may be closely associated with symptoms and prognosis in HF (Crisafulli, Salis, et al., 2007; Dibner-Dunlap & Thames, 1992; Johnson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999; Ponikowski et al., 2001; Scott et al., 2000; Smith, Williams, et al., 2005). However, it remains unclear whether cardiac hemodynamics are influenced by afferent feedback in HF, whereas observations in healthy individuals clearly indicate that cardiac hemodynamics are favorably influenced by group III/IV neural feedback associated with exercise (Crisafulli, Salis, et al., 2007; Dibner-Dunlap & Thames, 1992; Johnson et al., 2014; Jondeau et al., 1992; Olson, Joyner, & Johnson, 2010; Piepoli et al., 2008; Ponikowski et al., 2001; Scott, Davies, Coats, & Piepoli, 2002; Smith, Williams, et al., 2005).

Exercise intolerance, dyspnea, and fatigue are hallmark symptoms of HF have traditionally been attributed to dysfunction of central hemodynamics and/or pulmonary congestion. More recently, an emerging body of evidence suggests that peripheral factors may play a larger role than once believed (Coats et al., 1994; Johnson et al., 2014; Jondeau et al., 1992; Middlekauff et al., 2004; Minotti et al., 1991; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999; Smith, Mitchell, et al., 2005; Szlachcic, Massie, et al., 1985). This alternative explanation for symptoms of HF suggests that exercise intolerance, dyspnea, and fatigue are likely attributable to impaired mechanisms in the periphery and explainable by the “muscle hypothesis” (Coats et al., 1994; Middlekauff et

al., 2004; Minotti et al., 1991; Olson, Joyner, & Johnson, 2010; Piepoli et al., 2006; Piepoli et al., 1999; Smith, Mitchell, et al., 2005). The muscle hypothesis suggests that increased neural feedback from locomotor muscle is most striking during times of physical exertion likely because increased contractile activity of poorly functioning skeletal muscle causing exaggerated neural feedback from already dysregulated ergoreceptors in these patients (Clark, Poole-Wilson, & Coats, 1996; Li et al., 2004; O'Leary et al., 2004; Shoemaker, Kunselman, Silber, & Sinoway, 1998; Smith, Mitchell, et al., 2005; Smith, Williams, et al., 2005).

2.3.3.1 Metaboreflex in heart failure

In contrast to the observations in healthy animals and humans, the effects of the metaboreflex in HF appear paradoxical. Hammond *et al.* (2000) observed in the pacing-induced HF canine model that during exercise with graded aortic ischemia, skeletal muscle metaboreflex pathways are not only sensitized by these conditions, but the cardiovascular responses are markedly different compared healthy individuals (Hammond et al., 2000). Hammond *et al*. (2000) demonstrated that the metaboreflex contributes to exaggerated increases in sympathetic drive to cause peripheral vasoconstriction (Hammond et al., 2000). Further, the Q response to stimulation of the metaboreflex was negligible in the HF condition. These observations and those of others help to support the hypothesis that in the presence of HF, the metaboreflex is active but dysfunctional and associated with abnormal pressor reflex responses of the central and peripheral circulation during exercise in comparison to non-HF (Hammond et al., 2000; Hammond et al., 2001; O'Leary et al., 2004).

In the rat model of HF, complimentary studies of Li *et al.* (2004) and Smith *et al.* (2005) demonstrated that the EPR was dysfunctional (Li et al., 2004; Smith, Williams, et al., 2005). However, because capsaicin administered to the neonate rat is known to destroy unmyelinated (i.e. metaboreceptors) but not myelinated (i.e. mechanoreceptors) neural afferents in rats (Jancso, Kiraly, & Jancso-Gabor, 1977; Nagy & van der Kooy, 1983), by treating neonatal rats with capsaicin, Smith *et al.* (2005) observed an enhanced cardiovascular (i.e. HR and MAP) pressor response to muscle contraction which was comparable to pressor response of rats with HF (Smith, Williams, et al., 2005). Thus, because mechanoreceptors and hearts of capsaicin treated neonatal rats were determined to be fully functional, in addition to the similarities in cardiovascular responses to muscle contraction in HF rats, the observations of Smith *et al.* (2005) suggested that the abnormal HR and MAP responses to muscle contraction were likely due to the removal of metaboreceptors (Smith, Williams, et al., 2005). These observations suggest that part of the link between abnormal cardiovascular responses in HF may be related to blunted sensitivity of metaboreceptors due to desensitization and/or downregulation of these nerve afferents.

Similar to the interpretations of Smith *et al.* (2005) (Smith, Williams, et al., 2005), although using a different methodology, Li *et al.* (2004) observed that the sensitivity of metaboreceptors could be blunted in HF (Li et al., 2004). Previous observations suggest that when capsaicin is injected into the intra-arterial circulation of contracting hindlimbs that this can be used to selectively stimulate metaboreceptors and evoke a pressor response of the cardiovascular system in rats (Guo, Vulchanova, Wang, Li, & Elde, 1999; Michael & Priestley, 1999). Therefore, following the injection of capsaicin into the

arterial circulation of hindlimbs in healthy rats and rats with HF, in the absence of muscle contraction, it was observed that blood pressure was significantly increased in healthy rats versus rats with HF. Thus, similar to the conclusions of Smith *et al.* (2005) (Smith, Williams, et al., 2005), it could be possible based on the blood pressure response to intra-arterial capsaicin injection that the sensitivity of metaboreceptors are blunted in HF and related to abnormal cardiovascular responses that are commonly observed in HF.

To extend the findings of animal models to human HF our group recently examined the influence of the metaboreflex on ventilatory responses during post-exercise recovery in NYHA class I and II HF compared to healthy individuals (Olson, Joyner, & Johnson, 2010). We observed that a four-minute bout of moderate intensity (60.0% VO_{2peak}) steady-state cycling-exercise results in an attenuated return of ventilation to resting levels during metaboreflex stimulation (via bilateral supra-systolic blood pressure cuffing around upper thighs) This finding suggest that the metaboreflex pathway is stimulated by exercise and also that this reflex is abnormally augmented in HF in comparison to healthy individuals.

Subsequently, we have demonstrated that the exercise blood pressure response is also associated with heightened metaboreflex activity (Keller, Johnson, Joyner, & Olson, 2014). It was observed that significantly elevated MAP and SBP during exercise persisted during post-exercise recovery with RCO-mediated metaboreflex stimulation in HF. In contrast, MAP and SBP did not continue to increase during recovery in healthy individuals. Overall, the contribution of these respiratory and peripheral blood pressure observations in response to metaboreflex stimulation help to support the body of evidence which suggests that the mechanisms regulating the influence of metabolite-sensitive

neural feedback on cardiovascular and ventilatory function are dysfunctional in HF (Keller et al., 2014; O'Leary et al., 2004; Olson, Joyner, Dietz, et al., 2010; Shoemaker et al., 1998).

2.3.3.2 Mechanoreflex in heart failure

In addition to the pathophysiologic changes noted in the metaboreflex a outlined above, studies have suggested that the mechanoreflex is also abnormal in HF (Middlekauff et al., 2001; Smith et al., 2003). In revisiting the observations in rats with HF from the group of Li *et al.* (2004), in addition to evidence suggesting decreased responsiveness of the metaboreflex in HF, these authors further suggest that the augmented afferent feedback in HF could be due to hypersensitivity of mechanoreceptors (Li et al., 2004).

In describing the potential hypersensitivity of the mechanoreflex in HF, it is important to note that adenosine triphosphate (ATP) accumulation within the muscle interstitium has been previously demonstrated to occur during muscle contraction in decerebrate cats (Li, King, & Sinoway, 2003); and, that the mechanoreflex has been observed to be augmented in the presence of ATP as well as intra-arterial injections of α,β-methylene ATP via purinergic (P2X) receptors which can be found on group III afferent fibers (Hanna et al., 2002; Hanna & Kaufman, 2003; Li et al., 2003; Li & Sinoway, 2002). Previous observations suggests a significant pressor response (i.e. MAP and HR) when α,β-methylene ATP is injected into the intra-arterial circulation of hindlimbs in rats with HF prior to manual stretch in comparison to rats with HF that received saline and rats without HF receiving α,β-methylene ATP or saline (Li et al., 2004). These data suggest that HF may be associated with an augmented sensitivity of mechanoreceptors to mechanical stretch as well as to by-products of metabolism (e.g. ATP).

To complement the observations of Li *et al.* (2004) and to further understand the contribution of mechanoreceptors to the abnormal EPR in HF (Li et al., 2004), Smith *et al.* (2005) studied rat hindlimbs using various techniques to isolate the mechanoreflex pathway (Smith, Mitchell, et al., 2005). By selectively activating or blocking group III neural afferents using electrical stimulation of lumbar ventral roots to simulate static muscle contractions, gadolinium injectate (blocks mechanoreceptors), passive stretching, and/or treatment with capsaicin. These authors have found that mechanoreceptor activity is augmented in the presence. In response to both passive stretch and static contraction of hindlimbs prior to and following gadolinium administration, relative and absolute measures of HR and MAP were significantly higher in both rats with HF and neonatal rats treated with capsaicin compared to rats without HF further suggesting that mechanoreflex-mediated EPR pathways are dysfunctional in HF (Smith, Mitchell, et al., 2005). Moreover, although passive and static contraction during gadolinium exposure contributed to increases in the pressor response that were significantly less than pre-gadolinium contractions in all rats, the magnitude of increase remained significantly higher in both rats with HF and neonatal capsaicin rats compared to rats without HF (Smith, Mitchell, et al., 2005). In contrast, this pressor response was not seen when saline was used in place of gadolinium during contractions in all rats. These observations and those of others provide evidence that the sensitivity of mechanoreceptors may be heightened in HF which likely contributes to the augmented pressor reflex during exercise in HF (Li et al., 2004; Middlekauff et al., 2004; Smith, Mitchell, et al., 2005; Wang, Li, Gao, Zucker, & Wang, 2010).

Consistent with the observations in animal models of HF, testing of the mechanoreceptor in humans with HF supports that the mechanoreflex is abnormally augmented in HF (Li et al., 2004; Middlekauff et al., 2004; Middlekauff et al., 2001; Smith, Mitchell, et al., 2005; Smith, Williams, et al., 2005). Middlekauff *et al.* (2004) in a mechanistic study was one of the first to directly confirm the abnormal activity of mechanoreceptors in individuals with HF (Middlekauff et al., 2004). Middlekauff *et al.* (2004) demonstrated that passive wrist exercise coupled with microneurography could be used to isolate, excite, and quantify the activity of mechanoreceptors, which indicated accentuated MSNA in NYHA class II–III HF but not in healthy individuals. These authors also observed that low-level (20.0% of relative MVC) rhythmic (30 contractions/min for three-minutes) handgrip exercise could be used to isolate and excite mechanoreceptors in HF and healthy individuals (Middlekauff et al., 2004). Although both groups demonstrated mechanoreceptor-mediated elevations in MSNA attributable to low-level rhythmic exercise; perhaps more importantly, increased MSNA was significantly exaggerated and occurred earlier in HF compared to healthy individuals. These data suggest a heightened sensitivity and level of activity of mechanoreceptors in individuals with HF and confirm prior observations in animal models of HF (Li et al., 2004; Middlekauff et al., 2004; Smith et al., 2003; Smith, Mitchell, et al., 2005; Smith, Williams, et al., 2005; Wang et al., 2010).

2.3.3.3 Exercise pressor reflex and central hemodynamics in heart failure

Cardiac hemodynamics

Our current understanding of the influence of the afferent feedback on cardiac hemodynamics in HF is unclear (Amann et al., 2014; Piepoli et al., 1999). Observations

from several studies in animals with or without HF suggest that the response of cardiac hemodynamics in HF is not similar to what could be expected in the non-HF state (Ansorge et al., 2005; Hammond et al., 2000; O'Leary et al., 2004; Sala-Mercado et al., 2007). Briefly, as discussed previously, when Q and SV are permitted to freely adjust in proportion to metabolic demand arterial pressure and vascular conductance adjusted in parallel. In contrast, when adjustments in Q and SV are restricted during periods of increased hemodynamic demand changes in arterial pressure and vascular conductance occurred in response to changes in vascular tone as opposed to the influence from cardiac hemodynamics (Crisafulli et al., 2011; Ichinose et al., 2010; Spranger et al., 2013).

Hammond *et al.* (2000) have demonstrated in a cross-over experiment that the metaboreflex did not elicit adjustments in Q and SV in dogs with HF comparable to healthy canines (Hammond et al., 2000). Both Q and SV were lower in HF versus non-HF at rest and during exercise in both the absence and presence of metaboreflex stimulation. Although, with metaboreflex activation during exercise, Q and SV responses were diminished but in the presence of normal changes in HR and right atrial pressure (RAP) in HF compared to no HF (Hammond et al., 2000). Cardiac output and SV changes during exercise in HF with metaboreflex activation did not rise above exercise values without metaboreflex activation, but HR and RAP did. In contrast, Q, SV, and HR during exercise with metaboreflex activation in the absence of HF increased significantly versus exercise without metaboreflex activation. Also important, it appeared that adjustments in arterial pressure and systemic vascular conductance occurred in a manner which reflected adjustments or lack of adjustments in Q and SV in both the non-HF and HF condition, respectively (Hammond et al., 2000). These changes in arterial pressure

and systemic vascular conductance in HF were mainly due to peripheral vascular constriction, whereas changes in the non-HF condition were largely due to increases in cardiac hemodynamics. It could be suggested that in the absence of HF, the metaboreflex increases HR and cardiac contractility which result in increases in Q and SV that contribute to changes in arterial pressure and vascular (Hammond et al., 2000). In contrast, in HF, metaboreflex influenced increases in HR are not accompanied by increased cardiac contractility (potentially due to ischemic tissue and/or β-receptor abnormalities) and therefore Q and SV cannot increase, which attenuates the ability of Q to contribute to arterial pressure and vascular conductance. This results in exaggerated peripheral vascular constriction in attempts to maintain driving pressure for venous return (Sheriff, Augustyniak, & O'Leary, 1998). The observations and interpretations of Hammond *et al.* (2000) are supported by others (Ansorge et al., 2005; O'Leary et al., 2004; Sala-Mercado et al., 2007; Sala-Mercado et al., 2006; Sheriff et al., 1998).

In addition to confirming the impaired cardiac hemodynamic response to metaboreflex activation in dogs with HF, more recent studies have provided further evidence to suggest that there are critical relationships between changes in HR, cardiac contractility, and venous return during metaboreflex activation. In healthy dogs, it would appear that metaboreflex activation can contribute to increases HR and maintenance of central venous pressure which mirror increases in myocardial contractility (similar to an inotropic agent) and augmentation of the Frank—Starling mechanism (O'Leary et al., 2004; Sala-Mercado et al., 2007; Sala-Mercado et al., 2006; Sheriff et al., 1998). In HF, because of factors related to abnormal β-receptor function and/or necrotic myocardial tissue for example, the ability to increase cardiac contractility either by the metaboreflex

and/or Frank—Starling mechanism may be severely limited and, therefore, despite maintained central venous pressure via metaboreflex activation, dysfunctional myocardial tissue likely prohibits normal increases in Q and SV (Ansorge et al., 2005; O'Leary et al., 2004; Sala-Mercado et al., 2007). Furthermore, because metaboreflex activation contributes to increases in both HR and arterial resistance (i.e. afterload), these factors in parallel with blunted cardiac contractility likely exacerbate the attenuated rise of Q and SV in HF.

In contrast to the numerous observations in animal models of HF regarding relationships between the EPR and cardiac hemodynamics, there is a considerable gap in the understanding of the EPR and cardiac hemodynamics in human HF. Nevertheless, and perhaps unexpectedly, it was recently observed by Amann *et al.* (2014) that by blocking group III/IV neural feedback during exercise in HFrEF that Q and SV failed to reach the level of Q and SV attained during exercise without inhibition of group III/IV neural feedback (Amann et al., 2014). Further, it appeared that HR during exercise was negligibly influenced by group III/IV neural feedback blockade in HF; and, despite observing blood pressure and vascular conductance responses to inhibition of group III/IV neural afferents similar to our recent findings (Olson, Joyner, Eisenach, Curry, & Johnson, 2014), these responses were not consistent with adjustments in both Q and SV (Amann et al., 2014). During exercise with inhibited group III/IV afferents arterial pressure was significantly lower and vascular conductance was significantly higher versus exercise without group III/IV afferent neural blockade in HF; which, was surprising, given Q and SV were also depressed therefore making it unclear what mechanism was driving central venous pressure with exercise in patients (Amann et al.,

2014). Moreover, it might also be expected that blockade of group III/IV neural afferents from exercising muscle would not be sufficient to lower the sympathoexcitation of cardiac tissue to a point where contractility but not HR would be influenced. This should be considered since it has been observed in patients with HF that significant increases in HR occur in parallel with metaboreflex stimulation (Olson, Joyner, & Johnson, 2010). Also, despite reported differences in catecholamines between inhibited exercise and non-inhibited exercise, these differences alone do not indicate uptake and/or sensitization of cardiac β-adrenergic receptors (Amann et al., 2014). It is clear that these recent observations present controversial evidence regarding relationships between afferent feedback and cardiac hemodynamic responses during exercise in HF. Further study is warranted to better understand the afferent reflex pathway and cardiac hemodynamics in HF.

Exercise pressor reflex and pulmonary hemodynamics in heart failure

The influence of metabo- and/or mechano-receptors on pulmonary hemodynamics remains undefined. It is hypothesized, that because pulmonary vasculature shares anatomical and physiological similarities with the peripheral vasculature that the pulmonary hemodynamic response to afferent feedback in HF may be similar to that of peripheral hemodynamics (Harrison, Pirages, Robison, & Wintroub, 1969). Presented earlier was evidence which suggests adjustments in pulmonary hemodynamics were closely related to both metabo- and mechano-receptor activation in healthy individuals (Lykidis et al., 2008; White et al., 2013). With the lack of increase in Q and SV in HF, it may be possible that afferent feedback mediated adjustments in pulmonary

hemodynamics might be aimed at increasing overall pulmonary vascular permeability in effort to improve ventilation-perfusion matching.

Afferent feedback mediated adjustments in pulmonary hemodynamics in HF may also be aimed at preventing or attenuating backflow caused by high pressures in the left atria (regurgitation into pulmonary vein) in the instance of diastolic dysfunction. Although, this response by the pulmonary vasculature over the long-term could result in "mixed" PH which may lead to pulmonary congestion and impaired ventilatory function which are also known to be common in HF (Butler et al., 1999; Dickstein et al., 2008; Moraes et al., 2000; Paulus et al., 2007). Thus, it is possible that excessive pulmonary vascular pressures (i.e. arterial, venous, or total pressure) in HF are related to an augmented afferent feedback system and therefore a contributing cause in the development of PH and/or pulmonary congestion.

Summary

In summary, although there is ample evidence in animal models which suggest that there are close relationships between the dysfunctional EPR and abnormal central hemodynamics in HF (Ichinose et al., 2010; Lykidis et al., 2008; O'Leary et al., 2004; Spranger et al., 2013; White et al., 2013), there are obvious gaps in our current understanding of the impact of locomotor muscle neural feedback on central hemodynamics in human HF. It is critical to improve this understanding as these neural afferents have been closely linked with dysfunctional ventilation and peripheral hemodynamic responses at rest and during exercise in HF; which, more importantly, have been associated with symptoms and prognosis in this population (Middlekauff et al.,

2004; Olson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli, 1998; Piepoli et al., 1996; Piepoli et al., 1999).

2.4 Clinical implications of central hemodynamic measurement during exercise

2.4.1 Introduction

The importance of central hemodynamic assessment during exercise for the clinical evaluation of HF cannot be understated. Recent and encouraging observations suggest that non-traditional indices, which include CP, CircP, or PV_{CAP} assessed during exercise provide robust clinical value for evaluating syndrome severity and survival risk in HF (Bhella et al., 2011; Cohen-Solal et al., 2002; Lang et al., 2009; Mahapatra, Nishimura, Oh, et al., 2006; Taylor, Olson, et al., 2013; Williams, Jackson, et al., 2005; Williams et al., 2001; Williams, Tzeng, et al., 2005). Studies in HF examining the clinical value of traditional versus non-traditional cardiac hemodynamic parameters indicate that evaluating hydraulic power generation (e.g. CP) is more valuable than simply estimating blood flow alone (e.g. Q or SV) (Lang et al., 2009; Williams, Jackson, et al., 2005). Moreover, compared to common ventilatory parameters assessed during exercise (i.e. VO_2), it has recently been suggested that metrics such as CP may be more valuable in the clinical classification of HF (Cohn & Rector, 1988; Lang et al., 2009; Williams, Jackson, et al., 2005). Thus, although central hemodynamic evaluation using non-traditional indices during exercise is still an emerging area of study and not yet commonly agreed upon, the observations to date are encouraging and suggest central hemodynamic evaluation during exercise is critical at any stage of the evaluation of the HF patient.

2.4.2 Traditional measurements

Assessment of Q and SV, and perhaps even RV and LV EF% provide valuable information regarding HF severity. Evaluation of these parameters during exercise offers an even greater level of information for the clinical evaluation of HF (Borlaug et al., 2006; Franciosa et al., 1985; Franciosa, Leddy, Wilen, & Schwartz, 1984; Franciosa et al., 1981; Francis et al., 1982; Szlachcic, Massie, et al., 1985). As such, aside from the most important consequence of HF which is an increased risk for early mortality, it is important to point out that functional impairment (e.g. exercise intolerance) closely associated with HF greatly affects these patients which is also associated with prognosis (Cleland, Dargie, & Ford, 1987; Cohn & Rector, 1988; Jessup Likoff, Chandler, & Kay, 1987; Pilote, Silberberg, Lisbona, & Sniderman, 1989; Szlachcic, Massie, et al., 1985). Therefore, a better understanding of how cardiac and pulmonary hemodynamic factors during exercise are related to exercise intolerance in HF is crucial.

Early observations from Francis *et al*. (1982) suggest that there is little direct association between resting LV function and exercise capacity in HF (Francis et al., 1982). In support of this, Francis *et al*. (1982) demonstrated that resting Q_I, SV_I, and LV EF% correlated poorly with VO_{2peak} in HFrEF (Francis et al., 1982). Benge *et al.* (1980) similarly demonstrated in HFrEF, that despite low resting LV EF%, it could be possible for patients to have preserved exercise capacity (i.e. duration) (Benge, Litchfield, & Marcus, 1980). More recently, Piepoli *et al.* (1999) observed that resting LV EF% related poorly with VO_{2peak} measured during arm or leg exercise in HFrEF (Piepoli et al., 1999). Together, these observations indicate that although resting assessment of cardiac hemodynamics can be useful for assisting in identifying the presence of impaired cardiac function, these measurements at rest do not accurately describe cardiac functional reserve

or functional capacity. Hence, resting assessment of cardiac hemodynamics cannot evoke the full magnitude of pumping impairment in HF. This is likely due to a multitude of factors which are highlighted in this dissertation, which include lack of competition for blood flow at rest versus during exercise, depressed contractile capabilities related to ischemic/necrotic cardiac tissue, or influence of receptor dysfunction (e.g. β-cardiac adrenergic or ergoreceptors) (Bristow et al., 1986; Bristow et al., 1990; Kannengiesser, Opie, & van der Werff, 1979; Olson, Joyner, Dietz, et al., 2010; Piepoli et al., 1999).

In contrast, Szlachcic *et al.* (1985) observed that exercise capacity (i.e. VO_{2peak}) closely correlated with exercise measures of Q_I, but correlated poorly with resting measures of Q_I, LV or RV EF%, PVR, and mPAP (Szlachcic, Massie, et al., 1985). More importantly, Szlachcic *et al.* (1985) further demonstrated at one-year that patients with a VO_{2peak} <10 mL/kg/min had a 77% higher mortality versus patients with a VO_{2peak} >10 mL/kg/min, suggesting that there could be important clinical implications for the relationship between exercise central hemodynamics and exercise capacity in HF (Szlachcic, Massie, et al., 1985). Similarly, Franciosa *et al.* (1984) observed in the first of two separate investigations, that measurements of Q_I, SV, and PCWP during exercise significantly and closely correlated with exercise capacity (i.e. workload) in HFrEF; and, that maximal Q_I correlated closely with VO_{2MAX} (Franciosa et al., 1984). Further, Franciosa *et al.* (1985) observed that Q_I assessed during exercise significantly correlated with exercise capacity (i.e. VO_{2MAX}) as did total PVR in HFrEF; whereas, relationships were less between resting measures of Q_I and PVR with exercise capacity (Franciosa et al., 1985). Kitzman *et al.* (1991) similarly observed that exercise capacity (i.e. workload performed) significantly and closely correlated with changes in SV_I from rest to peak exercise in

HFrEF (Kitzman et al., 1991). Also, more recently, it was observed that change in Q from rest to peak exercise in HFpEF correlated closely with both exercise duration and VO_{2peak} (Borlaug et al., 2006).

Despite observations which suggest that there are important direct associations between specific changes in central hemodynamics during exercise and exercise tolerance and prognosis, contrasting observations indicate that neither rest nor exercise measurements of central hemodynamics are indicative of exercise tolerance or risk stratification in HF (Higginbotham et al., 1983; Meiler, Ashton, Moeschberger, Unverferth, & Leier, 1987; Willens et al., 1987). However, it may be argued that traditional measurements of central hemodynamics (e.g. Q, SV, mPAP) potentially lack diagnostic strength because these parameters do not quantify pressure and blood flow relationships, and they do not fully describe the integrated anatomy and physiology that pulmonary hemodynamics share with cardiac hemodynamics. Thus, it is suggested that non-traditional parameters such as CP, CircP, or PV_{CAP} can better describe the hydraulic pump as well as the interaction the heart shares with the pulmonary vasculature and, hence, these newer indices may be more useful in the clinical classification of HF (Bhella et al., 2011; Cohen-Solal et al., 2002; Lang et al., 2009; Mahapatra, Nishimura, Oh, et al., 2006; Mahapatra, Nishimura, Sorajja, et al., 2006; Taylor, Olson, et al., 2013; Williams, Jackson, et al., 2005; Williams et al., 2001; Williams, Tzeng, et al., 2005).

2.4.3 Non-traditional measurements

Assessment of the blood flow and pressure generating capability of the heart describes hydraulic power and total pumping capacity. As previously described, parameters such as CP or CircP integrate both factors of flow and pressure to quantify the hydraulic power of

the heart. Consistent with Q and SV, these non-traditional metrics of cardiac hemodynamics are lower at rest and during exercise in HF compared to healthy individuals (Bhella et al., 2011; Bromley et al., 2006; Cohen-Solal et al., 2002; Gelberg et al., 1979; Hecht et al., 1982; Jakovljevic et al., 2012; Lang et al., 2009; Schlader et al., 2010; Williams, Jackson, et al., 2005; Williams et al., 2001). However, more importantly, non-traditional indices of the pumping capability of the heart have been observed to predict prognosis in HF to a greater magnitude than traditional measurements of cardiac and ventilatory function (Lang et al., 2009; Williams, Jackson, et al., 2005).

Although the extent of testing is limited, because PV_{CAP} considers the direct interaction of cardiac hemodynamics with pulmonary hemodynamics, it has been suggested that this index measured during exercise may be a better indicator of the pulmonary hemodynamic response to exercise versus traditional parameters in HF (Mahapatra, Nishimura, Oh, et al., 2006; Mahapatra, Nishimura, Sorajja, et al., 2006; Taylor, Olson, et al., 2013). Moreover, PV_{CAP} has shown prognostic strength in patients with primary PH (Mahapatra, Nishimura, Oh, et al., 2006; Mahapatra, Nishimura, Sorajja, et al., 2006). Pulmonary vascular capacitance is important because individuals with HF are at an increased risk for the development of passive and/or mixed forms of PH (Aronson et al., 2011; Taylor, Mojica, et al., 2013).

More recent observations by Williams *et al.* (2001) and Lang *et al.* (2009) provide support for the role of CP in predicting prognosis in HFrEF (Lang et al., 2009; Williams et al., 2001). It was demonstrated in HFrEF that peak CP was significantly different in survivors versus non-survivors, and that when patients were dichotomized at a peak CP cut-off of 1.96 watts, patients above this threshold demonstrated significantly higher

survival compared to patients below this threshold (90.5% versus 43.4%, respectively) (Williams et al., 2001). Moreover, in both univariate and multivariate hazard models, peak CP remained a significant predictor of survival in HF; whereas, peak Q and VO_{2peak} failed to significantly predict survival when put into multivariate models (Williams et al., 2001). Consistent with the observations of Williams *et al.* (2001) (Williams et al., 2001), it has been demonstrated that not only is peak CP related to HF severity, but of all cardiac and cardiovascular factors tested during exercise in HF, CP has been observed to demonstrate the highest area under the receiver operating curve (Lang et al., 2009). Outcomes of the receiver operator curve analyses suggested that a peak CP cut-off of 1.5 watts was most indicative of event-free survival in HF (>1.5 watts = 94% versus <1.5 watts = 69%). Lastly, when patients were dichotomized above or below a common VO_2 threshold of 14 mL/kg/min, results of multivariate hazard modeling produced only peak CP as a significant predictor of outcome in patients with a VO_2 <14 mL/kg/min (Lang et al., 2009).

These recent observations which relate non-traditional measurements of cardiac hemodynamics during exercise with prognosis are important and help to strengthen earlier observations which suggest that peak CP as well as changes in CP from rest to peak exercise are important for the clinical evaluation of HF (Bain, Tan, Murray, Davies, & Littler, 1990). It was observed by Bain *et al.* (1990) that although peak exercise measurements of Q_I correlated with exercise tolerance in HF, peak CP and change in CP were the strongest indicators of exercise tolerance in these patients. Peak exercise measurements of cardiac hemodynamics all related to exercise tolerance to a greater

extent compared to corresponding resting measurements in HF patients (Bain et al., 1990).

Similar to outcomes of CP in HF, it has been suggested that CircP may demonstrate prognostic value while being able to discriminate surviving from non-surviving patients (Cohen-Solal et al., 2002). Cohen-Solal *et al.* (2002) observed that HF patients who did not survive at follow-up demonstrated significantly lower peak CircP (2402 ± 843 versus 3573 ± 1273 mL/kg/min · mm Hg) versus survivors at time of initial testing, respectively (Cohen-Solal et al., 2002). Also, peak measurement of CircP was a significant independent predictor of mortality as well mortality or transplantation, and the median peak CircP was associated with significantly higher survival in HF with a value above 3,047 mL/kg/min · mm Hg (Cohen-Solal et al., 2002). These observations by Cohen-Solal *et al.* (2002) in parallel with CircP validation testing by Williams *et al.* (2005) suggest that cardiac hydraulic power generating capacity in HF may be estimated using cardiovascular-derived factors (i.e. VO_2 and MAP or SBP) while also demonstrating predictive prognostic strength (Cohen-Solal et al., 2002; Williams, Tzeng, et al., 2005).

In summary, the use of non-traditional central hemodynamic indices for predicting prognosis in HFrEF is supported well by the current evidence. However, secondarily, and closely related to predicting prognosis in HF, is the ability to judge the severity of HF based on overt symptomology and signs of this syndrome. This is important because symptoms such as exercise intolerance and signs related to depressed cardiac hemodynamics are commonly considered factors in determining the appropriate course of therapeutic treatment.

Exercise measurements of cardiac function and pulmonary vascular pressures may relate with various parameters of exercise tolerance in HF. In comparison, the body of evidence supporting the use of non-traditional measures of central hemodynamics for predicting exercise capacity in HF is less in number (Bain et al., 1990). In attempts to better understand exercise tolerance as it relates to central hemodynamics, exercise duration and/or exercise workload should be compared with cardiac pumping capability. It remains unclear what relationships exist between exercise duration and/or exercise workload and advanced measures of central hemodynamics in HFrEF and HFpEF.

2.5 Summary of the review of the literature

The anatomy and physiology of the heart in both HFrEF and in HFpEF are paradoxical to the heart of the healthy individual. To further complicate the understanding of HF, it is commonly recognized that differences in heart function between HF and healthy individuals are not the only factors which drive the impairment in functional capacity and higher risk for mortality in patients. Heart failure is a systemic syndrome which, for diagnostic purposes, requires clinical judgment based on a multitude of factors, including signs and symptoms common to this illness. Therefore, it is imperative that our understanding of the underlying mechanisms leading to the signs and symptoms of HF continues to evolve by studying newer non-traditional areas which may also be closely related.

Further, specific research is discussed on non-traditional measurements of cardiac and pulmonary hemodynamics which appear to be promising parameters in adding strength to the clinical classification of HF. Additionally, continued study of neural feedback pathways related to group III and IV sensory nerve fibers appears to be critical in

understanding the disconnect between central dysfunction in HF and symptomology. Lastly, development of new techniques and metrics to study central hemodynamics has resulted in an overall lack of uniformity of measurement techniques and the manner in which indices are being reported in the literature. Although not completely prohibitive in the study of HF, variability in technique utilization in parallel with reporting of data increases the difficulty in interpreting observations across studies in HF.

Chapter 3

Research Projects

3.1 Acetylene Rebreathe and O_2pulse Estimates of Stroke Volume at Peak Exercise in HFpEF

3.2 Influence of Metaboreflex Stimulation on Circulatory Power and Pulmonary Vascular Capacitance in Heart Failure Patients

3.3 Intrathecal fentanyl blockade of neural feedback from skeletal muscle during exercise in heart failure patients: Influence on circulatory power and pulmonary vascular capacitance

3.1 Acetylene Rebreathe and O_2pulse Estimates of Stroke Volume at Peak Exercise in HFpEF

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Summary

Purpose: Direct Fick is the gold standard for measurement of stroke volume (SV), but this technique is invasive and resource dependent. Therefore, this comparison study was designed to examine relationships between non-invasive techniques for estimating SV at rest and peak exercise in heart failure patients with preserved ejection fraction (HFpEF). We hypothesized that SV via echocardiography (SV_{ECHO}), acetylene rebreathe (SV_{ACET}), and O_2pulse would closely relate at peak exercise in HFpEF; and, lung diffusion capacity for carbon monoxide (DL_{CO}), nitric oxide (DL_{NO}), alveolar—capillary membrane conductance (D_M) would not influence SV_{ACET}.

Methods: SV_{ECHO}, SV_{ACET}, and O_2pulse were measured at rest and peak exercise in 18 HFpEF and 26 control participants. Ventilation and gas-exchange measurements via breath-by-breath and 12-lead electrocardiogram were continuous. SV_{ECHO} (LV outflow diameter×velocity-time integral). SV_{ACET}, DL_{CO}, DL_{NO}, and D_M were measured using the rebreathe technique. O_2pulse (oxygen uptake/heart rate) was calculated.

Results: In HFpEF and controls at rest, SV_{ACET} was lower versus SV_{ECHO} ($p>0.05$). In HFpEF at peak exercise, SV_{ACET} was higher versus SV_{ECHO} ($p>0.05$). In controls at peak

exercise, SV_{ACET} was lower versus SV_{ECHO} ($p>0.05$). Linearity between SV_{ACET} with O_2pulse or SV_{ECHO} was significant at rest (R^2=0.65, 0.28; 0.57, 0.66) and peak exercise (R^2=0.67, 0.62; 0.33, 0.28) in HFpEF and controls, respectively. DL_{CO}, DL_{NO}, and D_M were lower at rest and peak exercise in HFpEF versus controls ($p<0.05$). Linearity between SV_{ACET} with DL_{CO}, DL_{NO}, and D_M (R^2=0.25, 0.23, 0.23; 0.59, 0.48, 0.48, respectively; $p<0.05$) were less at peak exercise in HFpEF versus controls, respectively.

Conclusion: Independent of lung diffusion capacity, SV_{ACET} directly related with O_2pulse and SV_{ECHO} at rest and closer at peak exercise in HFpEF.

Introduction

Patients with heart failure with preserved ejection fraction (HFpEF) have impaired cardiac contractility, myocardial relaxation, and loading conditions at rest and during exercise (Borlaug, Olson, et al., 2010; Paulus et al., 2007). During physical activity, the signs and symptoms of central hemodynamic dysfunction are exacerbated in these patients (Borlaug, Olson, et al., 2010; Paulus et al., 2007). More importantly, reduced cardiac pumping capacity is closely related to increased mortality in HFpEF (Bhatia et al., 2006). Therefore, accurate assessment of cardiac output (Q) and/or stroke volume (SV) is critical in these patients.

The most reliable and valid method for assessing cardiac hemodynamics is the direct Fick method which utilizes measures of oxygen uptake (VO_2) and systemic arteriovenous difference in oxygen (O_2) content ($C_{a\text{-}v}O_2$) to estimate Q; yet, because of its invasive nature due to the requirement of arterial and mixed venous blood O_2 samples, this method is time and resource dependent making it impractical for routine use in clinical and research settings. Therefore, other less invasive and time consuming methods are critical for the rapid assessment of Q in these patients.

Echocardiography is a non-invasive alternative to the direct Fick technique for measuring Q in the clinical or research settings. Results from validation and repeatability studies which used direct Fick or thermodilution as standards, indicate that echocardiography can be used to measure Q and SV in healthy individuals or in cardiac patients (e.g. HF) (Holland, Prasad, & Marwick, 2010; Lewis, Kuo, Nelson, Limacher, & Quinones, 1984; Loeppky, Hoekenga, Greene, & Luft, 1984). Importantly, however, as with many techniques, important limitations are associated with echocardiography that should not be

overlooked which include high user dependence related to image acquisition (e.g. view of the heart, sonographer skill, rest vs. exercise, and equipment), reader reliability (e.g. inter- and- intra-observer- and- lab variability), and high technology cost related concerns to name a few (Marwick, Nemec, Pashkow, Stewart, & Salcedo, 1992). Therefore, less user dependent techniques to estimate Q are needed.

The acetylene uptake method for measurement of Q has also been validated against direct Fick at rest and at peak exercise (Hoeper et al., 1999; Johnson et al., 2000). Acetylene, at very small concentrations, is a non-toxic and non-physiological gas with low solubility in lung tissue but a high Bunsen solubility coefficient in blood (0.740, nearly 20 and 40 times that of nitric oxide [0.0364] and carbon monoxide [0.0189], respectively) and low affinity for hemoglobin (Hb) (Cander, 1959; Grollman, 1929; Roughton & Forster, 1957; Tamhane, Johnson, & Hsia, 2001). The acetylene rebreathe technique is considered reliable and accurate for resting and exercise assessment of Q (Q_{ACET}) and SV (SV_{ACET}) in healthy individuals and patients with cardiac or pulmonary diseases such as HF (Olson, Snyder, Beck, & Johnson, 2006), PPH (Hoeper et al., 1999), or cystic fibrosis (Wheatley et al., 2011). However, because accurate estimate of Q using acetylene rebreathe is potentially dependent on near homogenous ventilation of alveoli in parallel with perfusion matching of pulmonary capillaries, as well as the capacity for acetylene gas to freely diffuse across the alveolar—capillary membrane barrier at a rate commensurate with pulmonary capillary blood flow (V_C), it remains unclear if the efficacy of acetylene rebreathe for estimating SV is valid in individuals with low capacity for gas transfer within lungs such as in HFpEF (Olson et al., 2008).

Our group recently demonstrated that measures useful for determining gas transfer capacity within lungs, which include pulmonary diffusion capacity for carbon monoxide (DL_{CO}), nitric oxide (DL_{NO}), alveolar—capillary membrane conductance (D_M), and V_C increase from rest to peak exercise in HFpEF despite being lower compared to healthy individuals (Olson et al., 2008). However, it is unknown of what consequences both low Q and lung gas diffusing capacity have on the capacity for the acetylene rebreathe technique to estimate Q and SV at rest or at peak exercise in HFpEF.

Contrasting acetylene rebreathe, the SV estimate O_2pulse (quotient of oxygen uptake [VO_2] with heart rate [HR]) simply requires standard gas-exchange and HR monitoring systems. However, for VO_2 to remain an estimate for Q, the direct Fick equation ($Q = VO_2/(C_{a\text{-}v}O_2)$) assumes that $C_{a\text{-}v}O_2$ is invariable during steady-state exercise (Beck et al., 2006; Crisafulli, Piras, et al., 2007). Accordingly, consistent with previous reports of O_2pulse in HF with reduced ejection fraction (HFrEF) (Lavie et al., 2004), our group has recently validated O_2pulse via gas-exchange system with direct Fick measurements of SV during submaximal exercise in HFrEF with or without concurrent PH (Taylor, Olson, et al., 2013). Moreover, others have also observed that O_2pulse closely related to SV assessed via carbon dioxide (CO_2) rebreathing during submaximal exercise in healthy individuals (Bhambhani et al., 1994).

Because of the potential utility SV and its surrogate O_2pulse have for prognosis and detection of syndrome severity in HF, specifically during physical activity (Bhatia et al., 2006; Lavie et al., 2004; Paulus et al., 2007), it is important to identify techniques for SV assessment, which are valid, repeatable, non-invasive, and easy to obtain in the clinical setting during peak exercise. This study compared non-invasive measurements of SV

using acetylene rebreathe, O_2pulse, and echocardiography in HFpEF at rest and at peak exercise. We hypothesized that acetylene rebreathe and O_2pulse would compare favorably to echocardiography when measuring SV at rest and at peak exercise in HFpEF. Secondarily, we hypothesized that low gas transfer capacity within lungs would not directly influence SV_{ACET} at peak exercise in HFpEF.

Methods

Participants

Eighteen Caucasian HFpEF patients and 26 Caucasian healthy control participants matched for gender and age were recruited for this study (participant demographics, Table 1). Patients with elevated BNP or N-terminal prohormone BNP (> 200.0 picograms [pg]/milliliters [mL] or > 220.0 pg/mL), significant coronary artery disease (stenosis ≥50.0 %), valvular heart disease (any stenosis, > mild regurgitation), hypertrophic or infiltrative cardiomyopathy, constrictive pericarditis, exercise-induced PH due to vascular disease (mean exercise pulmonary artery pressure >30 millimeters mercury [mm Hg] with pulmonary capillary wedge pressure <15 mm Hg (Tolle, Waxman, Van Horn, Pappagianopoulos, & Systrom, 2008)), or radiographic pulmonary congestion were excluded (Paulus et al., 2007). The Mayo Clinic Heart Failure Service and the Cardiovascular Health Clinic were used to recruit HFpEF. Control participants were recruited through advertisement in the surrounding community. Control participants had normal cardiac function without evidence of exercise-induced ischemia and were without history of hypertension, lung disease, or coronary artery disease. The experimental procedures were approved by the Mayo Clinic Institutional Review Board, all participants provided written informed consent prior to study, and all aspects of the study were performed in accordance with the ethical standards of the Declaration of Helsinki.

Protocol

Upon arrival, participants were fitted with a 12-lead electrocardiogram (Marquette Electronics, Milwaukee, WI) to monitor HR. Participants were seated upright on a cycle ergometer (Corival Lode B.V., Netherlands) where resting simultaneous measures of Q, DL_{CO}, DL_{NO}, VO_2, carbon dioxide production (VCO_2), respiratory exchange ratio (RER), respiratory rate (RR), tidal volume (V_T), minute ventilation (V_E), and end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) were performed. Measures of Q, DL_{CO}, and DL_{NO} were performed at peak exercise, whereas ventilation and gas exchange (Medical Graphics CPX/D, St. Paul, MN) were continuously monitored and averaged every three seconds at rest and throughout exercise. The incremental exercise testing protocol started at 20 watts and increased by 10 watts every three minutes until two of the following occurred: they could no longer maintain a pedal rate between 60 and 80 revolutions per minute, a RER greater than 1.10, or a rating of perceived exertion (Borg scale) of $\geq$17. Ventilatory efficiency (V_E/VCO_2), $C_{a\text{-}v}O_2$ ($VO_2/Q_{ACET}/10$) (Beck et al., 2006), and O_2pulse (VO_2/HR) were calculated as the average of 30 second intervals at the end of each workload (Taylor, Olson, et al., 2013).

Measures of lung gas transfer, cardiac output, and pulmonary capillary blood volume

At each work intensity simultaneous measures of Q, DL_{CO}, and DL_{NO} were performed via standard rebreathe technique as previously described (Reeves & Park, 1992; Snyder, Johnson, & Beck, 2005; Wheatley et al., 2011), and D_M and V_C were calculated (Reeves & Park, 1992; Snyder et al., 2005; Wheatley et al., 2011). Participants breathed into a non-rebreathing three-way automatic pneumatic switching valve (Hans Rudolph, Kansas City, MO) that was connected to a pneumotachometer (Hans Rudolph, Kansas City,

MO), mass spectrometer (Perkin Elmer MGA-1100, Wesley, MA), and NO analyzer (GE Instruments, Boulder, CO), which were all integrated with custom analysis software for the calculation of Q, DL_{CO}, and DL_{NO} (Snyder et al., 2005). The NO analyzer was calibrated prior to each visit using an NO filter and NO gas (45.0 parts per million [ppm]) for the zero and second calibration points, respectively. The inspiratory port of the switching valve allowed for rapid switching for breathing room air or from a 5.0 liter (L) anesthesia rebreathe bag (Hans Rudolph, Kansas City, MO) containing one- to- three L of test gas (0.7% acetylene [C_2H_2], 9.0% helium [He], 0.3% carbon monoxide [$C^{18}O$], 55.0% nitrogen, 40.0 ppm NO (Tamhane et al., 2001), and 35.0% O_2) depending on initial V_T of the subject and the exercise intensity as previously described (Snyder et al., 2005; Wheatley et al., 2011).

Serial measurement of gas concentrations with the mass spectrometer starting at end-expiration of the first breath enabled calculation of measures of lung gas transfer and Q. Because acetylene does not bind to hemoglobin and has low lung tissue and high solubility in blood (Cander, 1959; Grollman, 1929), acetylene disappears in the blood in a linear fashion according to the rate at which a new volume of blood is transported through the lungs and the rate of disappearance of acetylene with each breath to determine Q is calculated from the slope of the exponential disappearance of acetylene with respect to the insoluble gas He (Hsia et al., 1995; Snyder et al., 2005).

According to the Direct Fick model and modified by Grollman (Grollman, 1929), Q_{ACET} and SV_{ACET} using HR can be calculated using C_2H_2 as follows:

$$\mathrm{Q} = \frac{VO_2 \cdot (C_2H_2)_{diff}}{(O_2)_{diff} \cdot \bar{x}C_2H_2 \cdot P_b - 47 \cdot 0.00974}, and \times \frac{1}{HR} = SV$$

Where VO_2 is determined by a standard gas-exchange system, $(C_2H_2)_{diff}$ is the amount of C_2H_2 absorbed per liter of blood during the time of sampling, $(O_2)_{diff}$ is the amount of O_2 absorbed during the time of the sampling, $\bar{x}C_2H_2$ is the average concentration of C_2H_2 during the time of the sampling, P_b is barometric pressure, 47 is the assumed vapor pressure of water in the lungs (mm Hg), and 0.00974 is a numerical constant derived by combining the constants 760 and 100, and 740, the solubility coefficient for C_2H_2 in blood at body temperature (Grollman, 1929).

To calculate D_M and V_C, we used the model of diffusion capacity of the lungs as originally described by Roughton and Forster (Roughton & Forster, 1957) and modified by Reeves and Park (Reeves & Park, 1992):

$$\frac{1}{DL_{CO}} = \frac{1}{DM_{CO}} + \frac{1}{\theta_{CO} \cdot V_C}$$

Relative to DL_{CO}, DL_{NO} reflects predominantly diffusive resistance of the tissue-plasma barrier in man because of the nearly 300 times greater binding affinity for Hb NO has compared to CO, $DL_{NO} \cong DM_{NO}$ (Roughton & Forster, 1957; Tamhane et al., 2001). However, the theoretical DL_{NO}/DM_{CO} ratio (α_{ratio}) equal to 1.93 based on the molecular weights and Bunsen solubilities of NO and CO in blood is suggested to misrepresent a physiological plausible relationship between NO and CO in humans during peak exercise (Reeves & Park, 1992; Tamhane et al., 2001). Accordingly, the corrected α_{ratio} we applied in the calculation of DM_{CO} was the value described by Reeves and Park (Reeves & Park, 1992) equal to 2.26. Moreover, because of dissimilarities in each subject's alveolar partial pressure of oxygen (P_AO_2) as well as Hb concentrations which influence the resistance to the rate of gas uptake per Torr of pressure gradient by whole blood and

binding to hemoglobin ($\frac{1}{\theta}$), we corrected for these differences using the following formula (Reeves & Park, 1992):

$$\frac{1}{\theta_{CO}} = (0.0156 + 0.008 \cdot P_A O_2) \cdot \frac{14.6}{Hb}$$

Echocardiography

Echocardiographic assessment of SV (SV_{ECHO}) was performed at baseline and peak exercise using standard 2-dimensional (2-D) and pulsed wave tissue Doppler imaging (TDI-PW) using the GE Vivid 7® echocardiographic system, and stored for off-line analysis on the GE EchoPac® software, version 5.0. The echocardiographies were read blinded to participant condition (i.e. HFpEF vs. control) and activity state (i.e. rest vs. peak exercise). In the present study, we used the same sonographer and the same echocardiographic reader for all studies. Both of these individuals had extensive experience (>10 years working with stress echocardiography data acquisition and interpretation).

Measurements of SV were obtained according to the methods of Lewis *et al.* (1984) (Lewis et al., 1984) using the left ventricular (LV) outflow method that included views of the apical- five- chamber (A5C) and parasternal- long- axis (PLAX) views. Briefly, in the PLAX view, the left ventricular outflow tract (LVOT) aimed at the aortic annulus was captured using gray-scale 2-D video imaging of five- to- ten consecutive cardiac cycles. Cross-sectional area (CSA) of the LVOT—proximal to the aortic valve points of insertion of the LVOT was assessed at the time point of maximal separation of aortic valve leaflets during systole, with the CSA value used for analysis being the average of five cardiac cycles. Because it is assumed the LVOT is circular, $CSA = \pi \cdot r^2$, where r equals the radius of the LVOT.

In the A5C view using TDI-PW imaging, with the pulsed-wave beam focused at the depth of the LVOT—proximal to the aortic valve points of insertion of the LVOT, the velocity-time integral (VTI) of the flow entering the LVOT during systole was measured using integrated software of the GE EchoPac® system, with the average VTI of five cardiac cycles used for analysis. Stroke volume was calculated as:

$$SV\ (cm^3) = CSA\ (cm^2) \times VTI\ (cm)$$

Statistical Analyses

All data are presented at means ± standard error of mean (SEM). Homogeneity of variance of data was tested using Levene's test. Since it was unclear which technique was the criterion, to nullify this potential bias in our comparisons, analyses of slopes and intercepts between SV_{ECHO}, SV_{ACET}, and O_2pulse were tested using ordinary least products univariate linear regression. Ordinary least squares regression was used for all other tests of relationships. Specific comparison between groups (i.e. controls vs. HFpEF) or conditions (i.e. rest vs. peak exercise) were compared using unpaired and paired two-tailed Student's t-tests, respectively. Multiple comparisons for between- and- within group differences for rest and peak exercise were tested using two-way analysis of variance tests. When the F-statistic was significant for analysis of variance testing, post-hoc testing using the Bonferroni test was used to identify between which comparisons significance occurred. An alpha of 0.05 was set to determine statistical significance. All analyses were conducted using SAS statistical software, version 9.4 (SAS Institute, Cary, North Carolina).

Results

Participant characteristics

Participant characteristics are presented in Table 1. Age, height, weight, and BSA were not significantly different between groups, although HFpEF had a greater BMI versus controls ($p < 0.05$). Ejection fraction percentage was within normal limits for HFpEF (range = 50.0 – 68.0%). However, HFpEF demonstrated significantly lower exercise capacity (VO_{2peak}, predicted percentage range = 30.0 – 95.3% vs. 50.3 – 137.0%). Patients were all of ischemic etiology and on standard optimum pharmacological therapy.

Exercise intensity, symptomology, ventilation, and gas-exchange

Presented in Table 2 are measures of exercise intensity, symptomology, ventilation, and gas-exchange. Overall, HFpEF exercised at a lower workload and duration (17.7 ± 1.0 and 21.3 ± 1.2 minutes; $p < 0.05$) compared to controls despite similar RPE ($p > 0.05$). For baseline measures of ventilation and gas-exchange, there were no differences between HFpEF and controls ($p > 0.05$). At peak exercise, HFpEF generally demonstrated lower function versus controls for ventilation and gas-exchange, showing significance for VO_2, VCO_2, RER, V_E, and V_T.

Not presented in Table 2, linear regression coefficient of determination (R^2) estimates between VO_2 and $C_{a\text{-}v}O_2$ at rest and at peak exercise in HFpEF were negligible ($R^2 = 0.04$ and 0.00, respectively; $p > 0.05$), which were similar to controls at baseline and at peak exercise ($R^2 = 0.09$ and 0.10, respectively; $p > 0.05$).

Heart rate, blood pressure, and cardiac hemodynamics

Presented in Table 3 are baseline and peak exercise HR, blood pressure, and cardiac hemodynamics. At baseline no significant differences were present between groups except for DBP which was less in HFpEF ($p < 0.05$). At peak exercise, HFpEF

demonstrated overall lower HR, blood pressure, and cardiac hemodynamic function versus controls; although, only HR, SBP, DBP, Q, and V_C reached significance.

Not shown in Table 3, linear regression at peak exercise between O_2pulse and Q_{ACET} was similar for HFpEF and controls (R^2 = 0.41 vs. 0.43; $p < 0.05$).

Relationships between techniques for estimating SV

Presented in Figures 1 – 3 (A – D) are linear regression relationships between SV_{ECHO}, SV_{ACET}, and O_2pulse in HFpEF and in controls at rest and at peak exercise. Overall, HFpEF demonstrated a greater magnitude of linearity between techniques at peak exercise versus rest. Linearity between techniques at rest versus peak exercise appeared to be similar in controls.

Figure 1 shows regression equations between SV_{ACET} and SV_{ECHO} in HFpEF and in controls. The common regression equation (i.e. entire sample for rest and at peak exercise) was $f(x) = 0.99x - 4.75$, $p < 0.05$; and goodness of fit for the data was R^2 = 0.44, 95% confidence limits (CL) for R^2 was *0.31, 0.54*. The 95% CL for the slope (0.85, 1.17) and intercept (-17.8, 8.3) indicated the presence of overall fixed bias between SV_{ACET} and SV_{ECHO} in the entire sample, which is supported by goodness of fit lines in panels A, C, and D showing SV_{ACET} consistently underestimated SV versus SV_{ECHO}.

Next, *Figure 2* shows regression equations between O_2pulse and SV_{ECHO} in HFpEF and in controls. The common regression equation was $f(x) = 0.15x - 4.67$, $p < 0.05$; and goodness of fit for the data was R^2 = 0.36, 95% CL for R^2 was *0.22, 0.46*. The 95% CL for the slope (0.13, 0.18) and intercept (-6.8, -2.5) indicated the presence of overall fixed and proportional bias between O_2pulse and SV_{ECHO} in the entire sample. This suggests,

depending on the sample, although O_2pulse may consistently over (HFpEF)- or under (controls)-estimate SV in comparison to SV_{ECHO}, these differences may progressively change as SV increases.

Lastly, presented in *Figure 3* are regression equations between O_2pulse with SV_{ACET} in HFpEF and in controls. The common regression equation was $f(x) = 0.16x - 3.93$, $p < 0.05$; and goodness of fit for the data was $R^2 = 0.55$, 95% CL for R^2 was *0.43, 0.63*. Similar to Figure 2, the 95% CL for the slope (0.13, 0.18) and intercept (-5.6, -2.2) suggests the presence of overall fixed and proportional bias between O_2pulse and SV_{ACET} in the entire sample. Similar to Figure 2, this suggests that although O_2pulse may consistently over (HFpEF)- or under (controls)-estimate SV in comparison to SV_{ACET}, these differences may progressively change as SV increases.

Relationships between cardiac hemodynamics with gas-exchange

Linear regression between Q_{ACET} and VO_2 at rest and peak exercise were significant in HFpEF ($R^2 = 0.59$ and 0.61, respectively). In comparison, linear regression between Q_{ACET} and VO_2 was lower at rest and at peak exercise in controls ($R^2 = 0.25$ and 0.57, respectively). Linear regression at peak exercise between $C_{a\text{-}v}O_2$ and SV_{ACET} was significant for HFpEF ($R^2 = 0.47$) but not significant for controls ($R^2 = 0.08$). Linear regression between O_2pulse and $C_{a\text{-}v}O_2$ ($R^2 = 0.04$ vs. 0.06; $p > 0.05$) was similar for HFpEF versus controls at peak exercise, respectively. Lastly, linear regression between $C_{a\text{-}v}O_2$ and Q_{ACET} at peak exercise in HFpEF ($R^2 = 0.29$, $p < 0.05$) and controls ($R^2 = 0.10$, $p > 0.05$) was less than expected in HFpEF.

Measures of gas transfer capacity within lungs

We have previously reported that HFpEF demonstrate reduced gas transfer in the lungs at rest and at peak exercise (Olson et al., 2008). Briefly, at baseline, DL_{CO}, DL_{NO}, and D_M were less in HFpEF versus controls ($p < 0.05$). These significant differences at baseline persisted at peak exercise for DL_{CO}, DL_{NO}, and D_M in HFpEF in comparison to controls. Also, consistent with DL_{CO}, DL_{NO}, and D_M at baseline and at peak exercise, the increases from baseline to peak exercise in DL_{CO} (6.3 ± 0.5 vs. 10.3 ± 0.8 mL $\cdot$ min^{-1} $\cdot$ mm Hg^{-1}), DL_{NO} (19.2 ± 1.3 vs. 31.8 ± 1.5 mL $\cdot$ min^{-1} $\cdot$ mm Hg^{-1}), and D_M (8.4 ± 0.5 vs. 14.0 ± 0.7 mL $\cdot$ min^{-1} $\cdot$ mm Hg^{-1}) were significantly less for HFpEF compared to controls, respectively.

Despite significant differences in DL_{CO}, DL_{NO}, and D_M between HFpEF and controls at baseline and at peak exercise, the rate of change for DL_{CO} (0.4 ± 0.1 vs. 0.6 ± 0.1), DL_{NO} (1.3 ± 0.2 vs. 1.9 ± 0.3), and D_M (0.8 ± 0.1 vs. 0.6 ± 0.1) were of similar magnitude between HFpEF and controls ($p > 0.05$), respectively. Also, the quotient of DL_{NO} with DL_{CO} was not different between HFpEF and controls at rest or at peak exercise ($p > 0.05$), which was consistent with the direct relationships between DL_{NO} and DL_{CO} at peak exercise for HFpEF ($R^2 = 0.83$) and controls ($R^2 = 0.90$) ($p < 0.05$).

At peak exercise, although significant for HFpEF and controls, the following relationships between DL_{CO} and SV_{ACET} ($R^2 = 0.25$ vs. 0.59), DL_{NO} and SV_{ACET} ($R^2 = 0.23$ vs. 0.48), or D_M and SV_{ACET} ($R^2 = 0.23$ vs. 0.48) were less in HFpEF versus controls, respectively. Whereas, linear regression between D_M and V_C were not significant nor directly related in HFpEF ($R^2 = -0.05$) or in controls ($R^2 = -0.01$). Finally, despite significance for both HFpEF and controls, linear regression between O_2pulse and

DL_{CO} (R^2 = 0.19 vs. 0.34), DL_{NO} (R^2 = 0.23 vs. 0.39), or D_M (R^2 = 0.23 vs. 0.39) at peak exercise was less in HFpEF versus controls, respectively.

Discussion

This study compared three non-invasive techniques for assessing SV at rest and at peak exercise in HFpEF patients and in healthy individuals. Our main observations indicate that SV measured using acetylene rebreathe compared favorably with O_2pulse and echocardiography at rest and to a greater magnitude at peak exercise in participants; but, although linear, the strength of relationships between O_2pulse and echocardiography was less in comparison to relationships between O_2pulse and acetylene rebreathe at rest and at peak exercise in participants. These data also revealed the presence of potential bias associated with these techniques which appeared to be influenced by condition (i.e. HFpEF versus healthy) as well as exercise intensity (i.e. rest versus peak exercise). Our observations indicated the strongest relationships between non-invasive techniques occurred at peak exercise in HFpEF.

A cost-effective, repeatable, and reliable measurement of SV is desirable to clinicians given the dynamic nature of cardiac hemodynamics during exercise. This has important clinical implications since HFpEF demonstrate impaired cardiac function at rest that is exacerbated during exercise, and because the assessment of cardiac function during exercise has been demonstrated to be an excellent measure of syndrome severity and prognosis in these individuals (Bhatia et al., 2006; Lavie et al., 2004; Paulus et al., 2007). Therefore, to the best of our knowledge, the present study is the first to evaluate relationships between SV_{ACET}, O_2pulse, and SV_{ECHO} at rest and at peak exercise in HFpEF. In addition to demonstrating close relationships between SV_{ACET} with O_2pulse

and SV_{ECHO} at peak exercise in HFpEF and in healthy individuals; perhaps, it is of equal importance that the present findings identify SV_{ACET} or O_2pulse as non-invasive, yet reliable techniques for measuring SV at rest and at peak exercise in HFpEF. These data support, while also extend, previous observations which indicate inert gas rebreathing or gas-exchange are valid techniques for assessing cardiac hemodynamics at rest and during exercise in patients with cardiopulmonary abnormalities (Agostoni, Cattadori, Bianchi, & Wasserman, 2003; Olson et al., 2008; Saur et al., 2010; Smith et al., 1999; Taylor, Olson, et al., 2013).

Echocardiography in HFpEF

Although SV can be measured using echocardiography, accurate image capture at rest can be challenging and these difficulties are markedly amplified even during bouts of moderate-intensity exercise (Marwick et al., 1992). Further complicating this technique, irrespective of rest or exercise, no commonly agreed upon protocol or technology (e.g. motion-mode, 2-D, TDI-PW, TDI-continuous wave, or 3-dimensional) has been established and, hence, inconsistencies in the assessment of cardiac hemodynamics are persistent (Christie et al., 1987; Lewis et al., 1984; Marwick et al., 1992). Distortion of the SV jet commonly occurs as a result of calculations which are dependent on image capture (e.g. 2-D and TDI-PW images), heart views (e.g. PLAX vs. A5C), and measurement (e.g. depth in relation to the aortic valve and influence of chest cavity size) which can be variable depending on the sonographer (Christie et al., 1987; Lewis et al., 1984; Marwick et al., 1992). Nevertheless, despite the proposed limitations which may influence the efficacy of echocardiography for SV measurement, these sources of error may potentially be marginalized by an experienced sonographer in combination with a

skilled echocardiographic reader (Christie et al., 1987; Lewis et al., 1984; Marwick et al., 1992; Rowland, Melanson, Popowski, & Ferrone, 1998), and therefore echocardiography can be useful for detecting other potential sources of error in the SV measure such as valvular regurgitation that is suggested to influence techniques such as thermodilution (Christie et al., 1987; Lewis et al., 1984; Marwick et al., 1992; Rowland et al., 1998).

Acetylene rebreathe in HFpEF

Recently it was observed by our group that HFpEF demonstrate low DL_{CO} at rest and at peak exercise which was attributable to a combined attenuation in D_M and V_C both at rest and at peak exercise (Olson et al., 2008). Although demonstrated in HFrEF but still unconfirmed in HFpEF, it is possible that reductions in D_M and V_C could be related to the presence of pulmonary shunt (e.g. exercise-independent interstitial pulmonary edema or obstructive disease) or exercise-related interstitial pulmonary edema (Agostoni et al., 2003; Barbera, Peinado, & Santos, 2003; Friedman, Wilkins, Rothfeld, & Bromberg, 1984; Oelberg et al., 1998; Smith et al., 1999; Tolle et al., 2008), which could potentially influence the accuracy of SV_{ACET} since the rebreathe technique is dependent on the free diffusion acetylene gas across the alveolar–capillary membrane barrier. However, despite lower lung diffusion in comparison to controls, we observed in the present study that lung gas transfer capacity (e.g. DL_{CO}, DL_{NO}, and V_C) was not only higher at peak exercise versus rest in HFpEF, but the magnitude in the rate of increase was comparable to controls (Olson et al., 2008). Hence, these data suggest that low DL_{CO}, DL_{NO}, and V_C demonstrated previously in HFpEF were more likely to be associated with mechanisms influencing the alveolar—capillary membrane relationship related to the pathophysiology of HFpEF and not directly related to lung sarcoidosis, interstitial pulmonary fibrosis,

pulmonary shunt (e.g. exercise-independent interstitial pulmonary edema or obstructive disease), or extrapulmonary restriction for example (Barbera et al., 2003; Friedman et al., 1984; Hughes, Lockwood, Jones, & Clark, 1991; Oelberg et al., 1998; van der Lee, Zanen, Stigter, van den Bosch, & Lammers, 2007). Also, and perhaps equally important, we demonstrate in the present study that despite a lower ratio of D_M, DL_{CO}, and DL_{NO} with SV_{ACET} at peak exercise in HFpEF versus controls, there appears to be a negligible direct influence of lung gas transfer capacity within lungs on SV_{ACET} at peak exercise in HFpEF.

Although we demonstrated direct relationships for measures of SV between SV_{ACET} with O_2pulse or SV_{ECHO} at peak exercise in HFpEF, it is noteworthy that the agreement between these techniques appeared to be influenced by various factors of proportional or systematic bias. As examples of this, the magnitude of relationships between SV_{ACET} and SV_{ECHO} were clearly influenced by exercise intensity (i.e. rest versus peak exercise) in HFpEF but not in controls, whereas exercise intensity did not appear to be influential in the regression of SV_{ACET} with O_2pulse in either group. It is likely that the variability in the comparisons between SV_{ACET} with O_2pulse or SV_{ECHO} could partially be explained by differences in cardiac function (e.g. low cardiac reserve in HFpEF) between groups, in addition to the influence of physiological adjustments which occur during exercise such as pulmonary capillary recruitment and alveoli unfolding, and hence a potential for a rise in alveoli—pulmonary capillary surface area which might influence SV_{ACET} and O_2pulse similarly, while having no influence of SV_{ECHO}.

Comparison of inert gas rebreathing studies

Our current findings most closely support the observations of Saur *et al.* (2010) (Saur et al., 2010) which demonstrated that lung diffusing capacity did not significantly influence estimates of Q measured using inert gas rebreathing in patients with pulmonary disease. Additionally, previous validation studies of Hoeper *et al.* (1999) (Hoeper et al., 1999) (acetylene), Snyder *et al.* (2005) (Snyder et al., 2005) (acetylene), McLure *et al.* (2011) (McLure et al., 2011) (N_2O), and Trinkmann *et al.* (2009) (Trinkmann et al., 2009) (N_2O) in healthy individuals or in patients with cardiac and/or pulmonary disease further support the present study regarding the use of acetylene rebreathe for the measurement of SV during exercise in HFpEF.

O_2pulse in HFpEF

Despite the recent findings from our group and from others (Bhambhani et al., 1994; Crisafulli, Piras, et al., 2007; Lavie et al., 2004; Lim et al., 2005; Taylor, Olson, et al., 2013), O_2pulse during exercise as a surrogate for SV may lose accuracy in the presence of conditions that violate assumptions regarding the relationship of Q with VO_2 or $C_{a\text{-}v}O_2$ during exercise. Persons with unexpected variability in the relationship between cardiac hemodynamics and ventilation and gas-exchange might include clinical patients (e.g. HF, pulmonary disease, mitral valve dysfunction, anemia, and skeletal muscle disorders) (Katz et al., 2000; Lewis & Haller, 1989; Lim et al., 2005; Oelberg et al., 1998; Taylor, Olson, et al., 2013), highly-trained endurance athletes (Beck et al., 2006; Bhambhani et al., 1994; Crisafulli, Piras, et al., 2007), or obese individuals (Lavie et al., 2004). Aberrant variability in $C_{a\text{-}v}O_2$ during exercise could influence the accuracy of O_2pulse by minimizing the direct relationship between VO_2 and Q, and thus misrepresent the quotient of VO_2 with HR as an estimate of SV. Nevertheless, our findings are consistent

with observations from studies examining O_2pulse measures in individuals with the potential for variability in $C_{a-v}O_2$ and relationships between Q and VO_2 during exercise. These samples included diastolic dysfunction, pulmonary disease, valvular regurgitation, and systolic HF patients (Barbera et al., 2003; Katz et al., 2000; Lim et al., 2005; Taylor, Olson, et al., 2013).

The direct relationships between O_2pulse and SV_{ACET} at peak exercise reported in the present study correspond closely to a recent validation study by our group which demonstrated robust relationships between O_2pulse and direct Fick measurements of SV during submaximal exercise in HFrEF (Taylor, Olson, et al., 2013). However, the relationships we demonstrated currently between O_2pulse and SV_{ECHO} suggest low- to-moderate linearity, and appeared to be heavily influenced by proportional or systematic biases associated with either the techniques, exercise status, or differences in participants. Oxygen pulse measurements appeared to consistently overestimate SV in comparison to SV_{ECHO} and SV_{ACET} at rest and at peak exercise in HFpEF, whereas O_2pulse generally underestimated SV at peak exercise in controls.

Several mechanisms may account for differences in SV estimation using O_2pulse between HFpEF and controls. Although HFpEF demonstrated overall low cardiovascular function at rest, it is likely that O_2pulse consistently overestimated SV during exercise because the relative rise in total ventilation or gas-exchange (i.e. VO_2 ~267%) markedly surpassed the relative increase in cardiac function (i.e. SV_{ACET} ~30%, SV_{ECHO} ~8.0%, and HR ~73%) from rest to peak exercise. Whereas, in healthy individuals who are assumed to have normal cardiac function (i.e. both SV and HR), it is likely that because the

relative increase in HR (~106%) was greater than either SV_{ACET} (~33%) or SV_{ECHO} (~44%), an ensuing underestimation of SV via O_2pulse occurred.

Clinical Implications

The data presented in the current study suggests that inert gas rebreathing (i.e. acetylene rebreathe) and other non-invasive gas-exchange (i.e. O_2pulse) techniques can be safely used to reliably estimate SV at rest and during exercise in HFpEF (Hoeper et al., 1999; McLure et al., 2011; Saur et al., 2010; Taylor, Olson, et al., 2013). With these cost-effective, non-invasive, and low user dependent systems available, surrogate estimates of SV during submaximal and/or peak exercise protocols may be readily available to quickly grade disease severity and response to therapy.

Limitations

Although we suggest that exercise-related interstitial pulmonary edema could have occurred but was non-influential to SV measurements in HFpEF, the presence of interstitial pulmonary edema was not directly measured in HFpEF. However, measures of the capacity of lungs for gas transfer (e.g. DL_{CO}, DL_{NO}, and D_M) which are sensitive to the accumulation of interstitial pulmonary edema did not indicate the occurrence of unexpected adjustments in pulmonary vascular hemodynamics at peak exercise (Agostoni et al., 2003; Olson et al., 2006; Smith et al., 1999). Also, our clinical sample included only HFpEF without known pulmonary disease, and despite the close association of this syndrome with secondary pulmonary hypertension, more studies are needed to validate O_2pulse and acetylene rebreathe estimates of SV at peak exercise in patient populations.

Conclusion

In summary, the observations from the present study indicate that there is acceptable agreement between echocardiography, acetylene rebreathe, and O_2pulse as non-invasive techniques for measurement of SV at rest in HFpEF; which, more importantly, increases at peak exercise in these patients. However, because of confounding influences of the pathophysiology of HFpEF concurrent with potential biases associated with these techniques at rest or at peak exercise, these factors should be considered prior to interpreting estimates of SV via these techniques. Nevertheless, we suggest that because of the low user dependence and reliability associated with acetylene rebreathe or O_2pulse, these non-invasive techniques have the capacity to be readily available and efficacious for quick measurement of SV in research or clinical settings.

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Conflict of interest

Author(s) disclose no potential conflicts of interest

Tables

Table 1: Participant characteristics

	CTL	HFpEF
Demographics		
Age, years	65±2	69±2
Gender, male/female	9/17	5/13
Height, m	1.67±0.02	1.65±0.02
Weight, kg	82.0±3.6	93.1±4.9
BMI, $kg \cdot m^{-2}$	29.2±1.1	34.0±1.6*
BSA, m^2	1.9±0.1	2.1±0.1
VO_{2peak}, predicted (%)	92.4±4.2	58.4±4.4*
Left ventricular ejection fraction (%)		62.2±1.3
Heart failure etiology (ischemic)		18
Medications		
ACE inhibitor		8 (44)
Angiotensin II receptor blockers		6 (33)
β-blocker		12 (66)
Digitalis		0 (0)
Aspirin		7 (39)
Diuretics		14 (78)

Data presented as means ± SEM or count (n) and percentage (%). CTL = healthy controls; HFpEF = heart failure with preserved ejection fraction; BMI = body mass index; BSA = body surface area, VO_{2peak} = peak oxygen consumption; *$p < 0.05$.

Table 2: Exercise intensity, symptomology, ventilation, and gas-exchange

	Baseline	Peak exercise
Exercise intensity and symptomology		
Work performed (watts)		
CTL	-	93.8±5.3
HFpEF	-	52.2±5.4*
RPE (Borg 6-20)		
CTL	6.0±0.0	16.1±0.4†
HFpEF	6.0±0.0	15.9±0.4†
Ventilation and gas exchange		
VO_2, $L \cdot min^{-1}$		
CTL	0.3±0.0	1.5±0.1†
HFpEF	0.3±0.0	1.1±0.1*†
VCO_2, $L \cdot min^{-1}$		
CTL	0.2±0.0	1.6±0.1†
HFpEF	0.2±0.0	1.2±0.1*†
RER		
CTL	0.76±0.0	1.1±0.0†
HFpEF	0.81±0.0	1.0±0.0*†
V_E, $L \cdot min^{-1}$		
CTL	8.5±0.4	56.7±2.8†
HFpEF	9.5±0.6	42.9±2.9*†
V_T, $L \cdot min^{-1}$		
CTL	0.6±0.0	1.6±0.1†
HFpEF	0.7±0.1	1.2±0.1*†
RR, $breaths \cdot min^{-1}$		
CTL	14.3±0.6	36.8±1.2†
HFpEF	15.1±0.9	36.4±2.1†
V_E/VCO_2		
CTL	38.6±1.0	34.7±0.7†
HFpEF	40.1±1.5	36.7±1.2
$P_{ET}CO_2$, mm Hg		
CTL	36.5±0.7	35.2±0.6
HFpEF	34.8±0.7	34.2±1.1
$C_{a\text{-}v}O_2$, $mL \cdot 100\ mL^{-1}$		
CTL	7.1±0.3	13.2±0.4†
HFpEF	7.3±0.3	12.2±0.5†
$C_{a\text{-}v}O_2$, slope		
CTL	-	23.8±1.2
HFpEF	-	23.7±1.7

Data presented as means ± SEM. CTL = healthy controls; HFpEF = heart failure with preserved ejection fraction; RPE = rate of perceived exertion; VO_2 = oxygen uptake; VCO_2 = carbon dioxide production; RER = respiratory exchange ratio; V_E = minute ventilation; V_T = tidal volume; RR = respiratory rate; V_E/VCO_2 = ventilatory equivalent for CO_2; $P_{ET}CO_2$ = end-tidal partial pressure CO_2; arteriovenous difference in oxygen content = $C_{a\text{-}v}O_2$; Slope = rate of change from baseline to peak exercise; *HFpEF vs. CTL, $p < 0.05$. †Baseline vs. peak exercise, $p < 0.05$.

Table 3: Heart rate, blood pressure, and cardiac hemodynamics

	Baseline	Peak exercise
HR, beats·min^{-1}		
CTL	70.0±2.1	143.9±3.9†
HFpEF	68.4±3.0	118.5±5.2*†
SBP, mm Hg		
CTL	133.3±3.1	193.8±5.0†
HFpEF	128.8±4.4	158.1±6.5*
DBP, mm Hg		
CTL	77.0±1.4	88.2±3.5†
HFpEF	68.8±2.1*	76.2±2.8*
MAP, mm Hg		
CTL	88.2±5.4	108.7±8.4†
HFpEF	88.6±2.1	103.2±3.6
Cardiac output (acetylene), L·min^{-1}		
CTL	4.2±0.2	11.6±0.6†
HFpEF	4.2±0.3	9.6±0.6*†
V_C, mL		
CTL	65.3±5.5	101.3±7.1†
HFpEF	50.8±5.5	75.3±9.3*†
Stroke volume (ECHO), mL		
CTL	63.4±3.4	91.6±4.3†
HFpEF	74.1±4.9	80.0±5.9
Stroke volume (acetylene), mL		
CTL	61.8±3.5	82.1±4.7†
HFpEF	63.4±4.6	82.7±5.9†
O_2pulse, mL·beat^{-1}		
CTL	4.5±0.2	10.7±0.6†
HFpEF	4.3±0.3	9.8±0.5†

Data presented as means ± SEM. CTL = healthy controls; HFpEF = heart failure with preserved ejection fraction; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; V_C = pulmonary blood volume; ECHO = echocardiography; O_2pulse = oxygen pulse; *HFpEF vs. CTL, $p < 0.05$; †Baseline vs. peak exercise, $p < 0.05$.

Figure Captions

Figure 1. HFpEF = heart failure with preserved ejection fraction; CTL = healthy controls. Solid line represents the line of best fit, dotted line represents the line of identity. Ordinary least product regression equation shown with goodness of fit for the data expressed as the coefficient of determination (R^2). The common regression equation (i.e. combined rest and peak exercise) for the method comparison between SV_{ACET} and SV_{ECHO} in HFpEF was $f(x) = 1.05x - 8.13$, $p < 0.05$; $R^2 = 0.46$, 95% confidence limits (CL) (0.24, 0.59). The 95% CL for the slope (0.82, 1.36) and intercept (-30.0, 13.7) indicates the presence of fixed bias between SV_{ACET} and SV_{ECHO}. **(A)** Linear regression between echocardiographic and acetylene measures of stroke volume (mL) at rest in HFpEF with 95% CL for R^2 (0.02, 0.50) **(B)** Linear regression between echocardiographic and acetylene measures of stroke volume (mL) at peak exercise in HFpEF with 95% CL for R^2 (0.31, 0.75). In panels A and B, 95% CL for the slope at rest (0.61, 1.45) was similar to peak exercise (0.72, 1.37) in HFpEF which suggests there is fixed bias between SV_{ACET} and SV_{ECHO} at rest and at peak exercise. The 95% CL for the intercept at rest (-38.9, 26.4) was similar to peak exercise (-23.9, 30.3) in HFpEF, which indicates no proportional bias was present between techniques at rest or peak exercise.

The common regression equation (i.e. combined rest and peak exercise) for CTL was $f(x) = 0.96x - 2.56$, $p < 0.05$; $R^2 = 0.43$, 95% CL (0.25, 0.55). The 95% CL for the slope (0.78, 1.19) and intercept (-19.4, 14.3) indicate the presence of overall fixed bias between SV_{ACET} and SV_{ECHO} in CTL. **(C)** Linear regression between echocardiographic and acetylene measures of stroke volume (mL) at rest in CTL with 95% CL for R^2 (0.09,

0.51), **(D)** Linear regression between echocardiographic and acetylene measures of stroke volume (mL) at peak exercise in CTL with 95% CL for R^2 (0.06, 0.48). In panels C and D, 95% CL for slope at rest (0.72, 1.42) and at peak exercise (0.76, 1.53) suggests that there was fixed bias between SV_{ACET} and SV_{ECHO}. The 95% CL for intercept at rest (-26.4, 20.3) and at peak exercise (-52.7, 20.0) indicate that there was no proportional bias between techniques at rest or at peak exercise in CTL.

Figure 2. HFpEF = heart failure with preserved ejection fraction; CTL = healthy controls. Solid line represents the line of best fit, dotted line represents the line of identity. Ordinary least product regression equation shown with goodness of fit for the data expressed as the coefficient of determination (R^2). The common regression equation (i.e. combined rest and peak exercise) for HFpEF is $f(x) = 0.14x - 3.49$, $p < 0.05$; R^2 = 0.13, 95% confidence limits (CL) (0.01, 0.30). The 95% CL for the slope (0.10, 0.19) and intercept (-7.15, 0.17) indicate the presence of overall fixed bias between O_2pulse and SV_{ECHO} in HFpEF. **(A)** Linear regression between echocardiographic (mL) and O_2pulse (mL/beat) measures of stroke volume at rest in HFpEF with 95% CL for R^2 (0.05, 0.55). **(B)** Linear regression between echocardiographic (mL) and O_2pulse (mL/beat) measures of stroke volume at peak exercise in HFpEF with 95% CL for R^2 (0.00, 0.43). In panels A and B, 95% CL for slope at rest (0.04, 0.08) and at peak exercise (0.05, 0.13) both suggest the presence of fixed bias between O_2pulse and SV_{ECHO} in HFpEF. The 95% CL for intercept at rest (-1.5, 2.2) and peak exercise (-0.12, 6.52) suggests no presence of proportional bias between techniques at rest or at peak exercise in HFpEF.

The common regression equation (i.e. combined rest and peak exercise) for CTL was *f(x)* = 0.16*x* – 5.30, $p < 0.05$; $R^2 = 0.52$, 95% CL (0.35, 0.63). The 95% CL for the slope (0.14, 0.20) and intercept (-7.9, -2.7) suggests the presence of overall fixed and proportional bias between O_2pulse and SV_{ECHO} in CTL. **(C)** Linear regression between echocardiographic (mL) and O_2pulse (mL/beat) measures of stroke volume at rest in CTL with 95% CL for R^2 (0.11, 0.54). **(D)** Linear regression between echocardiographic (mL) and O_2pulse (mL/beat) measures of stroke volume at peak exercise in CTL with 95% CL for R^2 (0.07, 0.49). In panels C and D, the 95% CL for slope at rest (0.05, 0.09) and at peak exercise (0.10, 0.20) indicate the presence of fixed bias between O_2pulse with SV_{ECHO}. The 95% CL for intercept at rest (-1.24, 1.62) and at peak exercise (-7.0, 2.4) suggests no evidence of proportional bias was present between techniques at rest or at peak exercise.

Figure 3. HFpEF = heart failure with preserved ejection fraction; CTL = healthy controls. Solid line represents the line of best fit, dotted line represents the line of identity. Ordinary least product regression equation shown with goodness of fit for the data expressed as the coefficient of determination (R^2). The common regression equation (i.e. combined rest and peak exercise) for HFpEF was *f(x)* = 0.13*x* – 2.43, $p < 0.05$; R^2 = 0.53, 95% confidence limits (CL) (0.32, 0.65). The 95% CL for the slope (0.10, 0.17) and intercept (-4.80, -0.02) indicate the presence of fixed and proportional bias between O_2pulse and SV_{ACET} in HFpEF. **(A)** Linear regression between acetylene (mL) and O_2pulse (mL/beat) measures of stroke volume at rest in HFpEF with 95% CL for R^2 (0.35, 0.76). **(B)** Linear regression between acetylene (mL) and O_2pulse (mL/beat)

measures of stroke volume at peak exercise in HFpEF with 95% CL for R^2 (0.38, 0.78). In panels A and B, 95% CL for slope at rest (0.04, 0.08) and at peak exercise (0.06, 0.11) both suggest fixed bias between O_2pulse and SV_{ACET} in HFpEF. The 95% CL for intercept at rest (-0.54, 1.94) and at peak exercise (0.76, 5.12) both suggest no presence of proportional bias between O_2pulse and SV_{ACET} in HFpEF.

The common regression equation (i.e. combined rest and peak exercise) for CTL was $f(x) = 0.17x - 4.86$, $p < 0.05$; $R^2 = 0.58$, 95% CL (0.42, 0.67). The 95% CL for the slope (0.14, 0.21) and intercept (-7.3, -2.5) indicate the presence of overall fixed and proportional bias between O_2pulse and SV_{ACET} in CTL. **(C)** Linear regression between acetylene (mL) and O_2pulse (mL/beat) measures of stroke volume at rest in CTL with 95% CL for R^2 (0.32, 0.70). **(D)** Linear regression between acetylene (mL) and O_2pulse (mL/beat) measures of stroke volume at peak exercise in CTL with 95% CL for R^2 (0.44, 0.76). In panels C and D, 95% CL for slope at rest (0.05, 0.08) and at peak exercise (0.10, 0.17) both indicate fixed bias between O_2pulse and SV_{ACET}. The 95% CL for intercept at rest (-0.74, 1.50) and at peak exercise (-2.9, 2.6) suggests that there was no proportional bias between O_2pulse with SV_{ACET} at rest or at peak exercise.

Figures

Figure 1.

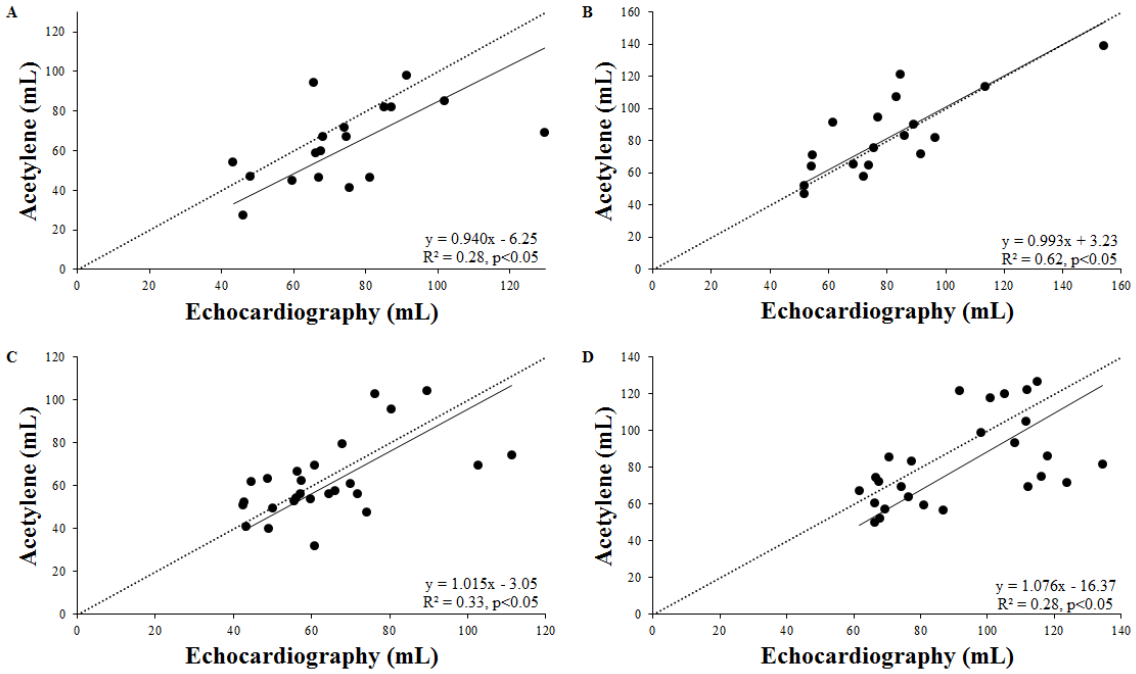


Figure 2.

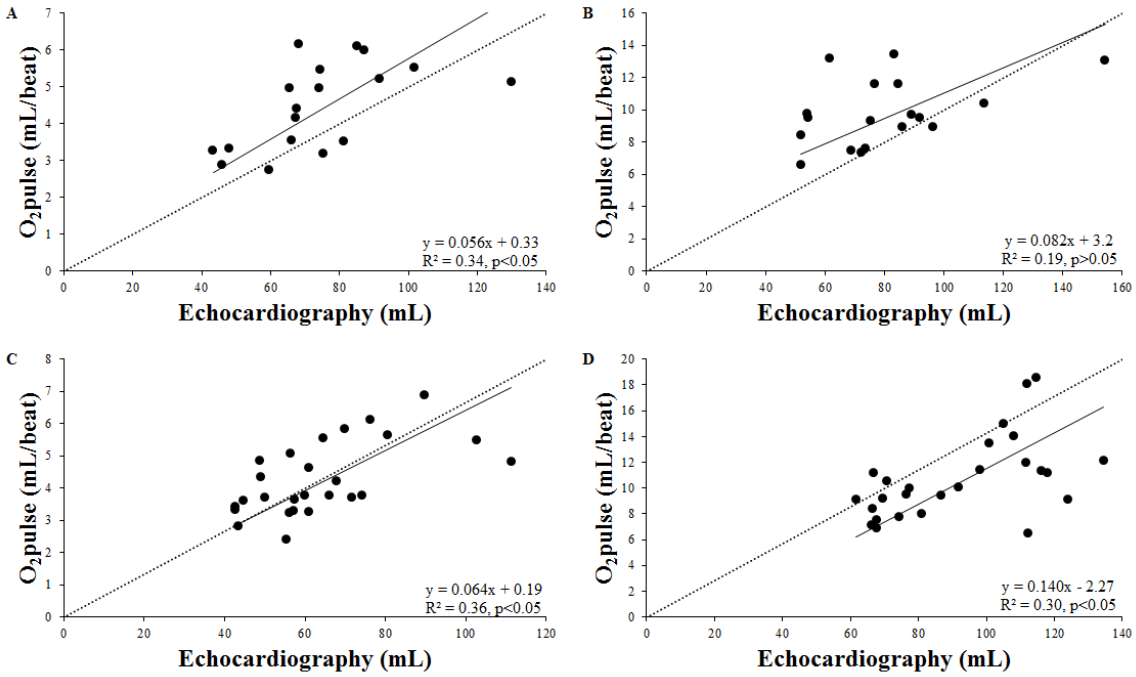
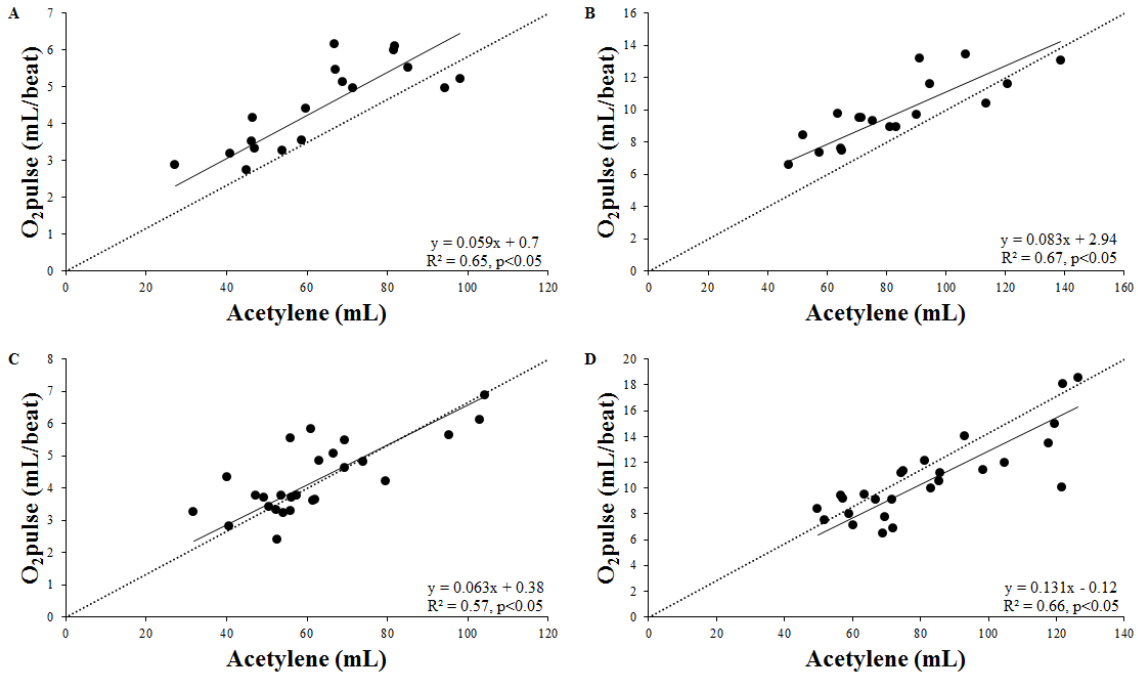


Figure 3.


A
O2pulse (mL/beat)
Acetylene (mL)
y = 0.059x + 0.7
R² = 0.65, p<0.05
B
O2pulse (mL/beat)
Acetylene (mL)
y = 0.083x + 2.94
R² = 0.67, p<0.05
C
O2pulse (mL/beat)
Acetylene (mL)
y = 0.063x + 0.38
R² = 0.57, p<0.05
D
O2pulse (mL/beat)
Acetylene (mL)
y = 0.131x - 0.12
R² = 0.66, p<0.05

3.2 Influence of Metaboreflex Stimulation on Circulatory Power and Pulmonary Vascular Capacitance in Heart Failure Patients

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Summary

Background: An impaired metaboreflex is associated with abnormal ventilatory and peripheral vascular function in heart failure (HF), whereas its influence on cardiac function or pulmonary vascular pressure remain unclear. This study investigated whether stimulating metabolite-sensitive neural feedback (metaboreflex) from locomotor muscles via post-exercise regional circulatory occlusion (RCO) would attenuate non-invasive estimates of central hemodynamics in HF patients.

Methods: Eleven HF patients (NYHA class: I/II; ages, 51±15; EF: 32±9%) and 11 age and gender matched controls (ages, 43±9) completed three cycling sessions (four-minutes, 60% VO_{2peak}). Session one: control trial with normal recovery (NR). Sessions two or three: bilateral upper-thigh pressure tourniquets inflated suprasystolic at end-exercise (RCO) for two-minutes recovery with or without inspired CO_2 (RCO+CO_2) (randomized). Mean arterial pressure (MAP), HR, and VO_2 were continuously measured. Estimates of central hemodynamics; circulatory power (CircP=(VO_2×MAP)/weight),

oxygen pulse index (O_2pulse_I=(VO_2/HR)/BSA), and pulmonary vascular capacitance (GX_{CAP}= $O_2pulse_I \times P_{ET}CO_2$) were calculated.

Results: At rest and end-exercise, CircP and GX_{CAP} were lower in HF versus controls (P<0.05), but no between treatment differences (P>0.05). At two-minutes recovery, GX_{CAP} was lower during RCO versus NR in HF and controls (72±23 versus 98±20 and 73±34 versus 114±35 mL·beat^{-1}·mm Hg·m^{-2}, respectively; P<0.05); whereas, CircP did not differ between recovery treatments (P>0.05). O_2pulse_I at rest, end-exercise, or two-minutes recovery for HF or controls did not differ across treatments (P>0.05).

Conclusion: The present observations suggests metaboreflex stimulation did not influence CircP or O_2pulse_I in HF or controls. Whereas, metaboreflex stimulation may have evoked decreases in GX_{CAP} (increased pulmonary vascular pressures) in these same individuals.

Introduction

Patients with chronic heart failure (HF) frequently develop pulmonary system abnormalities which are commonly associated with symptoms of dyspnea and exercise intolerance (Guazzi et al., 2005; Kleber et al., 2000; Olson et al., 2014; Olson, Joyner, & Johnson, 2010; Rubin & Brown, 1984). Recently it has become evident that measures of both cardiac and ventilatory function are clear markers of syndrome severity and prognosis in HF patients, especially during physical activity when signs and symptoms of HF are exacerbated (Guazzi et al., 2005; Kleber et al., 2000; Lang et al., 2009; Lavie et al., 2004; Rubin & Brown, 1984). Further, abnormalities of pulmonary vascular hemodynamics related to increases in pulmonary vascular pressures and pulmonary vascular resistance (i.e. pulmonary hypertension [PH]) are common to HF patients (Aronson et al., 2011; Butler et al., 1999; Moraes et al., 2000; Taylor, Olson, et al., 2013); and, also closely linked to increased mortality (Aronson et al., 2011; Moraes et al., 2000).

Recent evidence in support of the "muscle hypothesis" suggests that neural feedback from group III/IV skeletal muscle afferents contributes to altered arterial pressure and ventilatory control during exercise in HF (Keller et al., 2014; Olson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999; Scott, Francis, Coats, & Piepoli, 2003). Post-exercise regional circulatory occlusion (RCO) is a validated non-invasive technique used to selectively stimulate metabolite-sensitive neural feedback from skeletal muscle (i.e. metaboreflex) in both humans and animals (Scott, Francis, et al., 2003; Spranger et al., 2013). Activation of the metaboreflex leads to sympathetically mediated

vasoconstriction and increased arterial pressure as well as augmented ventilation leading to ventilatory inefficiency in HF (Keller et al., 2014; Olson, Joyner, & Johnson, 2010). Although generally believed to be a favorable mechanism for improving peripheral vascular conductance and ventilatory and cardiac function during exercise in healthy individuals (Amann et al., 2011; Crisafulli et al., 2011; Crisafulli et al., 2006; Crisafulli et al., 2003; Fisher et al., 2010), recent observations suggest that the metaboreflex may contribute to unexpectedly high mean pulmonary arterial pressure (mPAP) during and following exercise in healthy individuals (Lykidis et al., 2008; White et al., 2013). However, it is unclear whether this mechanism contributes to secondary PH frequently observed in HF.

Consistent with the beneficial influences of the metaboreflex observed in healthy individuals (Amann et al., 2011; Crisafulli et al., 2011; Crisafulli et al., 2006; Crisafulli et al., 2003; Fisher et al., 2010), invasive studies in healthy canines help confirm that the metaboreflex evokes increases in cardiac function (e.g. heart rate [HR], stroke volume [SV], and cardiac output [Q]) during and immediately following submaximal exercise (Ichinose et al., 2010; Sala-Mercado et al., 2006; Spranger et al., 2013). Further, as a result of these observations it has been suggested that increases in arterial pressure during or following exercise are primarily facilitated by metaboreflex-mediated increases in cardiac hemodynamics; and, that the metaboreflex does not provoke significant peripheral vasomotor pressor responses when maintenance of arterial pressure is flow-mediated via adjustments in myocardial contractility and SV (Ichinose et al., 2010; Sala-Mercado et al., 2006; Spranger et al., 2013). In contrast, extended studies in canines with

HF indicate that the metaboreflex contributes to augmented arterial pressure but does so primarily in the absence of contributions from increases in cardiac function during exercise (Hammond et al., 2000; Ichinose et al., 2008; Ichinose et al., 2010; Iellamo et al., 2007; O'Leary et al., 2004; Sala-Mercado et al., 2007; Sala-Mercado et al., 2006; Spranger et al., 2013).

Encouraging observations suggest that cardiac power, the product of Q and mean arterial pressure (MAP), determined invasively or with inert-gas breathing is a robust index of cardiac function and prognosis in HF (Lang et al., 2009; Tan, 1986). More recently, it has been demonstrated that the indirect estimate of cardiac power, circulatory power (CircP), closely correlates with cardiac power and similarly shows prognostic strength in HF (Cohen-Solal et al., 2002; Williams, Tzeng, et al., 2005). Moreover, our group recently demonstrated that oxygen pulse (O_2pulse) during exercise strongly related with invasive determination of SV in HF patients with or without secondary PH; and, equally noteworthy, it was also shown that mPAP was related to end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) (Taylor, Olson, et al., 2013). Together, we previously demonstrated that the indirect determination of pulmonary vascular capacitance (PV_{CAP}), which shows prognostic power in PH patients (Mahapatra, Nishimura, Sorajja, et al., 2006), using O_2pulse and $P_{ET}CO_2$ (i.e. GX_{CAP}) closely associated with invasive determination of PV_{CAP} (Taylor, Olson, et al., 2013).

Therefore, because it is known that systolic HF patients are afterload sensitive and that there is an association between metaboreflex activation with increased systemic arterial pressure in these patients (Keller et al., 2014; Piepoli et al., 1999); the aim of this study

was to examine the influence of the skeletal muscle metaboreflex, via post-exercise RCO, on central hemodynamics estimated using CircP and GX_{CAP} in HF. We hypothesized that stimulation of the skeletal muscle metaboreflex during post-exercise recovery would attenuate both CircP and GX_{CAP} in HF patients.

Methods

Subjects

Eleven HF patients were recruited through the Mayo Clinic Heart Failure Service and the Cardiovascular Health Clinic. Eleven healthy control participants were recruited through advertisement in the surrounding community with attempts to match the HF group for age and gender (participant demographics, Table 1). Inclusion criteria for HF patients included diagnosis of ischemic or dilated cardiomyopathy with duration of HF symptoms >one-year; stable HF symptoms (>three-months); left ventricular (LV) ejection fraction percentage (EF%) ≤35% (from clinical records within three months); body mass index (BMI) <35.0 kg/m^2 (at enrolment); and current non-smokers with a past smoking history <15 pack-years (at enrolment). All HF patients were on standard optimum pharmacological therapy at the time of the study. Control participants had normal cardiac function without evidence of exercise-induced ischemia and were without history of hypertension, lung disease, or coronary artery disease. The Mayo Clinic Institutional Review Board approved all experimental procedures. Prior to study, all participants provided written informed consent, and all aspects of the study were performed in accordance with the ethical standards of the Declaration of Helsinki.

Protocol Overview

The study consisted of two separate days of exercise testing procedures, in an environmentally controlled physiological laboratory, separated by ≥48-hours. For all study visits, participants were asked to avoid strenuous physical activity for 24-hours and refrain from eating or consuming caffeine for three hours prior to arrival at the physiological laboratory for testing. Day one of testing consisted of a peak exercise test to volitional fatigue (peak oxygen consumption [VO_{2peak}]). Day two consisted of three separate and randomized submaximal exercise sessions at 60% of the previously determined VO_{2peak}.

For each testing day, upon arrival, participants were fitted with a 12-lead electrocardiogram (Marquette Electronics, Milwaukee, WI) to monitor heart rate (HR) and rhythm. Participants were seated on a recumbent cycle ergometer and fitted with a nose clip and standard mouthpiece attached to a PreVent Pneumotach (Medical Graphic, St Paul, MN) connected to a metabolic measurement system (MedGraphics CPX/D; Medical Graphics) which was calibrated for volume (3.0 liter [L] syringe) and gases immediately prior to each test (Olson, Joyner, & Johnson, 2010). Resting simultaneous measures of gas-exchange and ventilation included: VO_2, carbon dioxide production (VCO_2), respiratory exchange ratio (RER), respiratory rate (RR), tidal volume (V_T), minute ventilation (V_E), ventilatory equivalent for carbon dioxide production (V_E/VCO_2), and end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) were performed. Blood pressure was measured via manual sphygmomanometer at rest and the end of each stage during peak exercise testing and each minute during constant-load submaximal exercise sessions. For all exercise testing, measures of gas-exchange and flow analysis as well as HR and

oxygen saturation were continuously monitored and averaged every three-seconds at rest and throughout exercise sessions. For analysis and data reporting, at rest we averaged the entire three min period for all measures, for exercise we averaged the final 30-seconds of each exercise stage. During recovery, data were averaged in 10-second intervals. Additional calculations included the SV estimate O_2pulse adjusted for body surface area (BSA) (VO_2/HR)/BSA) (Taylor, Olson, et al., 2013), the non-invasive surrogate for PV_{CAP} (GX_{CAP} = $O_2pulse_I \times P_{ET}CO_2$) (Taylor, Olson, et al., 2013), and cardiac power estimated by CircP (VO_2/weight)×MAP) (Cohen-Solal et al., 2002; Scharf et al., 2002; Williams, Tzeng, et al., 2005). We calculated O_2pulse_I, GX_{CAP}, and CircP at rest, end-exercise, and at two-minutes (min) post-exercise.

During day two of testing, each participant performed three separate and randomized bouts of constant-load submaximal exercise at 60% of VO_{2peak} (measured on day one of testing). Each of the three exercise sessions were identical in procedure and consisted of three-min of resting data collection, followed by four-min of constant-load cycle ergometry, and five-min of passive recovery which was randomized between cuffing conditions. Session one was the control trial which included a normal recovery at end-exercise (NR). Sessions two and three were randomized and included, immediately at cessation of exercise, RCO via inflation of bilateral upper-thigh pressure tourniquets to ≈20 mm Hg above peak exercise arm systolic blood pressure (SBP) measured during the VO_{2peak} test conducted on day one. Session two or three also included addition of CO_2 (RCO+CO_2) to the inspired air to clamp end-exercise $P_{ET}CO_2$ in attempt to account for reduced venous return of CO_2 due to RCO and its potential influence on central

chemoreceptor activity. The cuffing protocol has been validated in HF and has not been indicated to induce significant pain or discomfort associated with biasing measures of cardiovascular function (Olson, Joyner, & Johnson, 2010; Scott, Francis, et al., 2003).

Statistical Analyses

No dropouts or test failures occurred during the collection of study data and all data were included in the analyses. Where appropriate, all data are presented at means±standard deviation (SD). Normality of data was tested using Levene's test. Two-tailed Student's t-tests were conducted for comparison between groups. Multiple comparisons for between-and- within group differences for treatment condition as well as control vs. HF were tested using the repeated measures one-way analysis of variance test. When the F-test statistic was significant from the analysis of variance test, Bonferroni post-hoc analysis was used to correct for multiple comparisons and to identify between which comparisons significance occurred. Statistical significance was determined using an alpha level of 0.05. All computations were made using SAS statistical software, v.9.4 (SAS Institute, Cary, NC).

Results

Participant demographics

Participant characteristics are presented in Table 1. The age range for participants was 22- to- 69 years with males accounting for 64% in each group. Heart failure patients weighed approximately 10% more than controls ($P>0.05$); however, the lack of difference in height resulted in non-significant differences in BSA between groups.

End-exercise intensity and symptomology; and, basic cardiovascular measures at baseline, end-exercise, and two-minutes post-exercise

Presented in Table 2 is exercise intensity and symptomology at end-exercise. Heart failure patients did not perceive their level of exertion to be significantly different versus controls despite a significantly less workload at end-exercise.

Our laboratory has previously demonstrated HR, blood pressure, ventilation, and gas-exchange responses for HF and controls at baseline, end-exercise, and at two-min post-exercise with or without RCO (Keller et al., 2014; Olson, Joyner, & Johnson, 2010). Therefore, for the purposes of the aims of the present study, only HR, VO_2, $P_{ET}CO_2$, and MAP at baseline, end-exercise, and at two-min post-exercise are presented in Table 2 because these metrics are directly related to the estimation of central hemodynamics.

Briefly, previous data indicated that overall HR and blood pressure (mm Hg) were reduced at baseline, end-exercise, and at two-min post-exercise in HF versus controls (P>0.05) (Keller et al., 2014). No significant between treatment differences were present at baseline or end-exercise for HF or controls. Although, at two-min post-exercise with RCO or RCO+CO_2 in HF, absolute differences for SBP (12.7±0.5 or 14.7±5.0, both P>0.05), diastolic blood pressure (DBP) (10.2±2.9 or 10.9±2.0, both P<0.05), and MAP (11.0±2.2 and 12.2±0.1, both P>0.05) were elevated compared to NR, respectively. Also, during RCO or RCO+CO_2 in HF, absolute differences for SBP (5.2±3.2 or 4.9±4.0, P>0.05) and MAP (6.5±0.2 or 6.5±0.1, P>0.05) were greater at two-min post-exercise compared to end-exercise measures, respectively.

For measurements related to ventilation or gas-exchange at baseline or end-exercise, no significant within group differences were present; but, overall, HF demonstrated significantly worse ventilation and gas-exchange versus controls.(Olson, Joyner, & Johnson, 2010) Significant differences at end-exercise (i.e. V_E, V_T, V_E/VCO_2, or $P_{ET}CO_2$) between HF and controls persisted to two-min post-exercise. Also, within group differences were demonstrated for both HF and controls at two-min post-exercise. For HF, V_E (RCO+CO_2 higher vs. NR), V_E/VCO_2 (RCO+CO_2 higher vs. NR or RCO), and $P_{ET}CO_2$ (RCO lower vs. NR or RCO+CO_2) were different (all $P<0.05$). For controls, V_E (RCO lower vs. NR or RCO+CO_2), V_E/VCO_2 (RCO+CO_2 higher vs. NR or RCO), and $P_{ET}CO_2$ (RCO lower vs. NR or RCO+CO_2) differed (all $P<0.05$).

Estimates of central hemodynamics at baseline, end-exercise, and two-minutes post-exercise

Indices directly related to the estimation of central hemodynamics including O_2pulse_I, CircP, and GX_{CAP} at baseline, end-exercise, and at two-min post-exercise are presented in Table 3. At baseline, no significant between treatment condition differences were present for HF or controls. Circulatory power was less in HF versus controls at baseline ($P>0.05$), and reached significance during the RCO+CO_2 session. At baseline, lower O_2pulse_I for RCO and RCO+CO_2 sessions in HF contributed to significantly lower GX_{CAP} versus controls.

For all treatment conditions at end-exercise, lower O_2pulse_I and $P_{ET}CO_2$ contributed to significantly lower GX_{CAP} in HF versus controls. However, at end-exercise there were no between treatment condition differences present for O_2pulse_I, CircP, or GX_{CAP} in HF or

controls (P>0.05). Consistent with lower VO_2 (P<0.05) and MAP (P>0.05) at end-exercise for all treatment conditions, CircP was significantly lower in HF versus controls for all treatment conditions.

At two-min post-exercise despite no differences in O_2pulse_I between or within groups, because of significantly lower $P_{ET}CO_2$ in RCO versus NR in both HF and controls, GX_{CAP} was also lower in both HF and controls in RCO versus NR by magnitudes of -26.1±2.5 and -41.1±0.7 mL/beat · mm Hg/m^2, respectively; P<0.05. Whereas, consistent with no between or within group differences for VO_2 and MAP at two min post-exercise (P>0.05), CircP did not differ between or within groups at two min post-exercise (P>0.05).

Taking into consideration the elevation of O_2pulse_I, CircP, and GX_{CAP} at end-exercise in controls versus HF, but the attenuation of these differences at two min post-exercise in controls versus HF, there was a lower rate of reduction from end-exercise to two-min post-exercise for these measures in all three treatments in HF versus controls (Table 3, P<0.05 for all). Also, in comparing the slopes (i.e. rate of change, end-exercise to two-min post-exercise) for O_2pulse_I, CircP, or GX_{CAP} between RCO and NR for each in HF versus controls, the absolute difference in slopes (i.e. RCO minus NR) for O_2pulse_I in HF was lower versus controls (3.3±2.0 and 5.7±4.7 mL/beat/m^2, respectively); for CircP, HF was lower versus controls (8.8±26.4 and 136.7±76 mL/kg/min · mm Hg, respectively), and GX_{CAP}, HF was lower versus controls (10.3±0.1 and 23.5±5.7 mL/beat · mm Hg/m^2, respectively) (all P>0.05).

Absolute change in CircP and GX_{CAP} from end-exercise to two-min post-exercise are shown in Figure 1 (A and B), respectively. Figure 2 (A and B) shows CircP and GX_{CAP} at two-min post-exercise as a percentage of CircP and GX_{CAP} at end-exercise, respectively. Absolute mean values from rest to end-exercise to two-min post-exercise for CircP and GX_{CAP} are presented in Figure 3 (A and B), respectively.

Discussion

The main findings of this study suggests that metabolically mediated neural feedback from skeletal muscle, which is increased during constant-load submaximal exercise and maintained during post-exercise RCO, contributes to reductions in GX_{CAP} in HF patients and healthy individuals. In contrast, CircP or O_2pulse were not appreciably altered by metaboreflex stimulation or by decreased GX_{CAP} suggesting that there was a direct influence of the metaboreflex on the pulmonary vasculature perhaps related to increased pulmonary vascular pressures.

Physical activity results in increased autonomic nervous system activity and augmented adrenergic drive to cardiac and peripheral tissues in an attempt to maintain or restore hemodynamic homeostasis by increasing perfusion while also matching oxygen supply with demand (O'Leary, Augustyniak, Ansorge, & Collins, 1999); however, the "muscle hypothesis" suggests that the mechanisms of this pathway are deranged in HF (Keller et al., 2014; O'Leary et al., 2004; Olson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999). The unencapsulated nerve endings of group III/IV neural afferents are dispersed within skeletal muscle and are sensitized by mechanical and/or chemical stimuli (Adreani & Kaufman, 1998; Piepoli et al., 1995; Scott, Wensel, et al., 2003;

Smith, Williams, et al., 2005). It is suggested that neural feedback from group III/IV skeletal muscle afferents influences changes in arterial pressure via primary adjustments in cardiac function in healthy individuals and animals (Amann et al., 2011; Crisafulli et al., 2011; Sala-Mercado et al., 2006; Spranger et al., 2013). Whereas, it is unclear whether neural feedback from skeletal muscle group III/IV fibers evokes adjustments in central hemodynamics in HF patients (Amann et al., 2014; Coutsos et al., 2013; O'Leary et al., 2004; Sala-Mercado et al., 2007).

Stimulation of the metaboreflex using RCO or comparable muscle ischemia techniques have been validated for use in promoting neural feedback from skeletal muscle group IV fibers during or immediately following exercise in HF and healthy models (Crisafulli et al., 2011; Sala-Mercado et al., 2007; Scott, Francis, et al., 2003; Spranger et al., 2013). The RCO technique during post-exercise is particularly useful for examining metabolite-sensitive neural feedback from skeletal muscle and its influence on inotropy and SV since central command and mechanical deformation from muscle are not present at this time, and because increases in HR (i.e. chronotropy) are not present during post-exercise RCO likely due to a robust baroreflex effector presence in response to pronounced metaboreflex sensitization (Crisafulli et al., 2011; Iellamo et al., 2007; Sala-Mercado et al., 2006). However, recent observations would suggest that although activation of the metaboreflex during or immediately following exercise in HF or healthy models contributes to increased arterial pressure (Crisafulli et al., 2011; Ichinose et al., 2010; Sala-Mercado et al., 2006; Spranger et al., 2013), it is likely that the mechanisms underlying these adjustments in peripheral vascular hemodynamics are not similar in HF

patients compared to healthy individuals. For example, based on the observations of Ichinose et al. (2010) and Spranger et al. (2013) in healthy canines (Ichinose et al., 2010; Spranger et al., 2013), it could be suggested that if hemodynamics generated from metaboreflex mediated increases in myocardial contractility cannot adequately overcome parallel sympathetically mediated increases in afterload, the metaboreflex could be expected to provoke substantial lowering of systemic vascular conductance and increased vascular resistance to compensate for the low contribution of cardiac hemodynamics to the generation of arterial pressure. Thus, because HF patients are known to have severely depressed cardiac reserve related to impaired inotropy, the observations in the present study indicate that stimulation of the metaboreflex likely influenced adjustments in GX_{CAP} via pressor responses in pulmonary vascular tone with little contribution from the recruitment of myocardial contractile reserve and SV as indicated by null adjustments in CircP and O_2pulse in these patients (Cohen-Solal et al., 2002; Keller et al., 2014; Lang et al., 2009; O'Leary et al., 2004; Sala-Mercado et al., 2007).

Although it has been demonstrated by us and others previously that hyperpnea concurrent with attenuated gas-exchange function occur during exercise in HF in comparison to healthy individuals (Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999), the parallel impairment of both ventilation and gas-exchange demonstrated during exercise amongst our three treatment conditions in HF compared to controls did not persist at two-min post-exercise recovery. As noted by our present and previous observations (Olson, Joyner, & Johnson, 2010), at two-min post-exercise there were marked differences in gas-exchange (i.e. $P_{ET}CO_2$) in the absence of ventilation (i.e. V_E, RR, and V_T) and other

gas-exchange (i.e. VO_2) in RCO versus NR in HF; whereas, during exercise these measures were similar between RCO and NR. This is important as it suggests that although ventilation – perfusion mismatch concordant with attenuated Q and increased afterload could have occurred during exercise in HF as has been demonstrated previously (Buller & Poole-Wilson, 1990; Reindl et al., 1998), our post-exercise observations of changes in $P_{ET}CO_2$ during RCO in comparison to NR were not likely due to mechanical adjustments in ventilatory function or ability to deliver blood to the pulmonary circulation caused by RCO-metaboreflex activation. Indeed, because others have demonstrated that increased V_E/VCO_2 slope may be related to increased mPAP during exercise in HF (Reindl et al., 1998), and because we have previously demonstrated an association between $P_{ET}CO_2$ and mPAP during exercise in HF (Taylor, Olson, et al., 2013), we suggest that attenuated $P_{ET}CO_2$ observed at two-min post-exercise during RCO in HF was the consequence of increased mPAP related to metaboreflex stimulation. Moreover, although the adjustments in $P_{ET}CO_2$ and GX_{CAP} in response to metaboreflex sensitization were similar between HF and controls, these data are consistent with previous observations which suggested that metaboreflex activation leads to increased mPAP during and following exercise in healthy individuals (Lykidis et al., 2008; White et al., 2013). However, the present observations extend these previous findings by being the first to examine this mechanism in HF and by demonstrating that the metaboreflex influence on GX_{CAP} was independent of adjustments in the SV estimate O_2pulse in both controls and HF (Lykidis et al., 2008; Taylor, Olson, et al., 2013; White et al., 2013).

We did observe that CircP and O_2pulse were comparable at two-min post-exercise with RCO or RCO+CO_2 versus NR in controls which was similar to the pattern shown in HF. This finding occurred despite preserved cardiac function in controls, in addition to previous observations which suggested that the metaboreflex contributed to increased cardiac function (e.g. Q and SV) in healthy individuals (Amann et al., 2011; Crisafulli et al., 2011; Crisafulli et al., 2003). However, it is important to point out that HR responses in the present study were consistent with previous observations of HR during post-exercise recovery with RCO in healthy individuals (Crisafulli et al., 2011). Also, it is of interest that Coutsos et al. (2013) recently demonstrated in both canines with or without HF during exercise with metaboreflex stimulation that Q but not HR or MAP were significantly higher with alpha adrenergic (α_1) receptor blockade versus without (Coutsos et al., 2013). As such, it is important to consider, although augmented cardiac adrenergic drive is known to stimulate myocardial beta adrenergic ($\beta_{1,2}$) receptors which subsequently leads to increased myocardial metabolic activity and metabolite mediated hyperemia within coronary arteries, coronary vascular α_1 receptors associated with a vasoconstrictor capabilities are also stimulated. Thus, in the event that α_1 reserves exceeded $\beta_{1,2}$ reserves; or, $\beta_{1,2}$ receptors had a blunted sensitivity compared to α_1 receptors in individuals of the present study, it is possible that a restrained coronary arterial vasodilatory ability could attenuate myocardial perfusion and inotropy despite the presence of augmented cardiac adrenergic drive facilitate by RCO-metaboreflex activation (Coutsos et al., 2013; O'Leary et al., 2007). Moreover, this mechanism could be further exacerbated in HF when combined with additional myocardial abnormalities such as an exhausted Frank—Starling mechanism, as well as dysfunctional cardiac $\beta_{1,2}$

receptors (e.g. attenuated reuptake or degradation of catecholamines at cardiac $\beta_{1,2}$ receptor sites (Eisenhofer et al., 1996; Rose, Burgess, & Cousineau, 1983)) which may also help to explain the attenuated return of CircP to baseline levels during recovery with or without RCO in HF patients.

Lastly, that the present study suggests a potential synergistic interaction between feedback from central chemoreceptors with feedback from ergoreceptors as GX_{CAP} responses during RCO+CO_2, although not significantly different versus RCO, were noticeably higher in HF and in controls. Thus, reaffirming the critical role of the regions within the dorsal medulla (e.g. caudal nucleus tractus solitarius) in receiving, organizing, and integrating neural feedback from the viscera of central and peripheral origins.

Clinical Implications

While cardiac and pulmonary system abnormalities are most evident during physical activity in HF, to date, controversy remains regarding the specific mechanisms underlying the etiology of exertional symptoms and functional limitation of these patients. The present study suggests neural feedback from metabolite-sensitive skeletal muscle afferents may partially explain chronic increases in pulmonary vascular pressures which contribute to impaired pulmonary vascular reactivity and permeability and pulmonary vascular resistance in HF. This is critically important because common complications of HF includes ventilatory dysfunction, dyspnea, and exercise intolerance which may be related to tonic increases in pulmonary vascular resistance (Butler et al., 1999; Moraes et al., 2000; Taylor, Olson, et al., 2013); but, perhaps more importantly due

to the relationship between increased pulmonary vascular pressures and increased mortality risk (Aronson et al., 2011; Moraes et al., 2000).

Limitations

Although the post-exercise RCO technique has been well validated in multiple populations (Scott, Francis, et al., 2003), our current study did not directly measure intramuscular metabolite concentration and thus it was not possible to identify specific metabolites that mediated stimulation of metaboreceptors. Further, we did not assess α- or β-adrenergic receptor density or function within the heart or vasculature and therefore our conjecture regarding α- and β-adrenergic receptors is aimed to be thought provoking. Lastly, we did not directly measure Q or mPAP and therefore do not know whether there was unanticipated bias that accompanied our surrogate estimates. Nevertheless, CircP, O_2pulse, and GX_{CAP} have been well validated as accurate surrogates for their respective invasive counterparts (Cohen-Solal et al., 2002; Lavie et al., 2004; Scharf et al., 2002; Taylor, Olson, et al., 2013; Williams, Tzeng, et al., 2005), and therefore use of indirect estimates of central hemodynamics were considered adequate as the intent of this study design was to assess relative changes in central hemodynamics in response to metaboreflex augmentation, not absolute measurements.

Summary

Administrating RCO immediately at cessation of submaximal constant-load exercise in HF patients and healthy individuals facilitates stimulation of metabolite-sensitive neural feedback from skeletal muscle nerve fibers. This neural feedback pathway contributes to decreased GX_{CAP} (i.e. increased pulmonary vascular pressures) in low- to- moderate

severity HF patients and in older healthy individuals; whereas, because of null changes in CircP and O_2pulse, it is unlikely that this pathway influences adjustments in cardiac function in these same individuals.

Acknowledgments

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Disclosures

None.

Tables

Table 1. Participant Characteristics

	Healthy Control	Heart Failure	*P*
Demographics			
Age, years	43 ± 9	51 ± 15	0.21
Gender, male/female	7/4	7/4	1.00
Height, m	1.76 ± 0.06	1.73 ± 0.08	0.35
Weight, kg	78.3 ± 10.9	87.4 ± 18.5	0.18
BMI, $kg \cdot m^{-2}$	25.2 ± 3.6	29.1 ± 6.1	0.10
BSA, m^2	2.0 ± 0.1	2.0 ± 0.2	0.33
VO_{2peak}, $mL \cdot kg^{-1} \cdot min^{-1}$	36.3 ± 9.2	17.5 ± 4.8	<0.001
LVEF, %		32.1 ± 9.2	
HF etiology (ischemic/idiopathic)		4/7	
NYHA class		1.6 ± 0.5	
I		4	
II		7	
Medications			
ACE inhibitor		6 (55)	
Angiotensin II receptor blockers		4 (36)	
β-blocker		10 (91)	
Digitalis		4 (36)	
Aspirin		7 (64)	
Diuretics		7 (64)	

Data are mean ± SD or as n. BMI, body mass index; BSA, body surface area; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; ACE, angiotensin converting enzyme.

Table 2: Workload, symptomology, heart rate, VO_2, $P_{ET}CO_2$, and MAP

	NR	RCO	RCO+CO_2
Baseline			
HR, beats · min^{-1}			
CTL	64±13	68±11	68±11
HF	76±14	77±14	77±14
VO_2, L · min^{-1}			
CTL	0.4±0.1	0.4±0.2	0.5±0.1
HF	0.4±0.1	0.4±0.1	0.4±0.1*
$P_{ET}CO_2$, mm Hg			
CTL	36.6±4.3	35.7 ± 3.8	36.0±3.6
HF	34.1±3.3	33.7 ± 4.0	33.5±3.7
MAP, mm Hg			
CTL	90±12	93±12	92±10
HF	91±14	91±15	92±14
End-exercise			
Workload (watts)			
CTL	115±16	-	-
HF	36±7*	-	-
RPE (Borg 6-20)			
CTL	11.4±1.2	11.4±1.4	11.2±1.7
HF	10.7±1.8	11.3±1.8	11.6±2.0
Dyspnea (Borg 0-10)			
CTL	1.8±1.2	1.7±1.1	1.6±1.1
HF	1.9±1.0	2.1±1.1	2.5±0.8
HR, beats · min^{-1}			
CTL	116±10	115±11	117±13
HF	105±16	104±15	106±16
VO_2, L · min^{-1}			
CTL	1.7±0.4	1.8±0.5	1.8±0.5
HF	1.0±0.3*	1.0±0.3*	1.1±0.3*
$P_{ET}CO_2$, mm Hg			
CTL	41.4±5.2	40.6±4.3	40.3±3.8
HF	35.4±4.7*	35.3±4.7*	34.7±4.8*
MAP, mm Hg			
CTL	111±14	112±14	111±14
HF	99±14	100±13	101±16
Post-exercise (2 min)			
HR, beats · min^{-1}			
CTL	75±14	77±11	76±11
HF	80±16	87±18	85±17
VO_2, L · min^{-1}			
CTL	0.4±0.1	0.3±0.1	0.4±0.1
HF	0.5±0.1	0.4±0.1	0.4±0.1
$P_{ET}CO_2$, mm Hg			
CTL	36.4±4.0	33.2±4.9†	38.4±3.9‡
HF	33.8±4.5*	30.2±3.4†	34.8±4.0*‡
MAP, mm Hg			
CTL	98±12	97±34	108±13
HF	96±15	107±13	108±16

Data are mean ± SD. NR, normal recovery; RCO, regional circulatory occlusion; RCO+CO_2, added CO_2 during RCO; HF, heart failure; CTL, control; RPE, rate of perceived exertion; HR, heart rate; VO_2, oxygen uptake; $P_{ET}CO_2$, end-tidal partial pressure CO_2; MAP, mean arterial pressure; *$p<0.05$ compared to CTL after Bonferroni correction; †$p<0.05$ compared with NR after Bonferroni correction; ‡$p<0.05$ compared with RCO after Bonferroni correction.

Table 3: Estimates of central hemodynamics

	NR	RCO	RCO+CO_2
Baseline			
O_2pulse_I, mL/beat/m^2			
CTL	3.3±0.8	3.4±1.6	3.6±1.1
HF	2.5±0.5*	2.6±0.8	2.4±0.5*
CircP, mL/kg/min · mm Hg			
CTL	465±102	510±199	542±105
HF	391±76	418±152	397±87*
GX_{CAP}, mL/beat · mm Hg/m^2			
CTL	122±38	122±59	128±43
HF	84±16*	88±30	80±15*
End-exercise			
O_2pulse_I, mL/beat/m^2			
CTL	7.6±1.6	7.9±1.7	7.8±1.7
HF	4.9±1.1*	4.8±1.0*	5.0±1.2*
CircP, mL/kg/min · mm Hg			
CTL	2451±572	2579±750	2527±673
HF	1217±367*	1187±323*	1241±301*
GX_{CAP}, mL/beat · mm Hg/m^2			
CTL	318±86	323±86	315±79
HF	175±51*	170±43*	172±50*
Post-exercise (2 min)			
O_2pulse_I, mL/beat/m^2			
CTL	3.1±0.9	2.2±0.8‡	2.6±1.0‡
HF	2.9±0.7	2.4±0.7	2.5±0.7
CircP, mL/kg/min · mm Hg			
CTL	557±132	453±233	510±189
HF	505±83‡	492±138	520±105‡
GX_{CAP}, mL/beat · mm Hg/m^2			
CTL	114±35	73±34†‡	100±47
HF	98±20	72±23†	86±22
Slope			
O_2pulse_I, mL/beat/m^2			
CTL	16.1±3.1	21.8±7.8	19.3±6.3
HF	12.5±6.6	15.8±4.6*	16.1±5.4
CircP, mL/kg/min · mm Hg			
CTL	-947±263	-1084±339	-1009±289
HF	-356±187*	-348±161*	-361±134*
GX_{CAP}, mL/beat · mm Hg/m^2			
CTL	-102±28	-125±34	-108±25
HF	-39±24*	-49±24*	-43±19*

Data are mean±SD. CTL, control; CircP, circulatory power; GX_{CAP}, pulmonary vascular capacitance; HF, heart failure; NR, normal recovery; O_2pulse_I, oxygen pulse indexed to body surface area; RCO, regional circulatory occlusion; RCO+CO_2, added carbon dioxide during RCO; Slope, rate of change from end-exercise to 2-min post-exercise; *p<0.05, compared to CTL after Bonferroni correction; †p<0.05, RCO compared to NR; ‡p<0.05, compared to baseline.

Figure captions

Figure 1. Data presented as means±SD. (A) Circulatory power (CircP) absolute change (Δ) from end-exercise to two-min post-exercise. (B) Pulmonary vascular capacitance (GX_{CAP}) absolute Δ from end-exercise to two-min post-exercise. *Significant between groups, heart failure vs. control (P<0.05).

Figure 2. Data presented as means±SD. (A) Circulatory power (CircP) at two-min post-exercise as a percentage (%) of CircP at end-exercise. (B) Pulmonary vascular capacitance (GX_{CAP}) at two-min post-exercise as a % of GX_{CAP} at end-exercise. †Significant across control conditions NR vs. RCO or RCO+CO_2 (P<0.05). *Significant between groups, heart failure vs. control (P<0.05).

Figure 3. Data presented as means±SD. (A) CircP at rest, end-exercise, two-min post-exercise. At rest, CircP was less in heart failure for RCO+CO_2 compared to controls (P<0.05). Heart failure showed less of an increase in CircP compared to control at end-exercise for all conditions (P<0.05). At two-min post-exercise no significant differences were present between or within groups. (B) GX_{CAP} at rest, end-exercise, and two-min post-exercise. At rest GX_{CAP} was significantly less in heart failure for NR and RCO+CO_2 compared to controls. At end-exercise GX_{CAP} was significantly less for heart failure compared to controls for all conditions. At two-min post-exercise, RCO differed compared to NR for heart failure and controls (P<0.05). CircP=circulatory power, GX_{CAP}=pulmonary vascular capacitance, NR=normal recovery, RCO=regional circulatory occlusion, RCO+CO_2=regional circulatory occlusion+carbon dioxide.

Figures

Figure 1

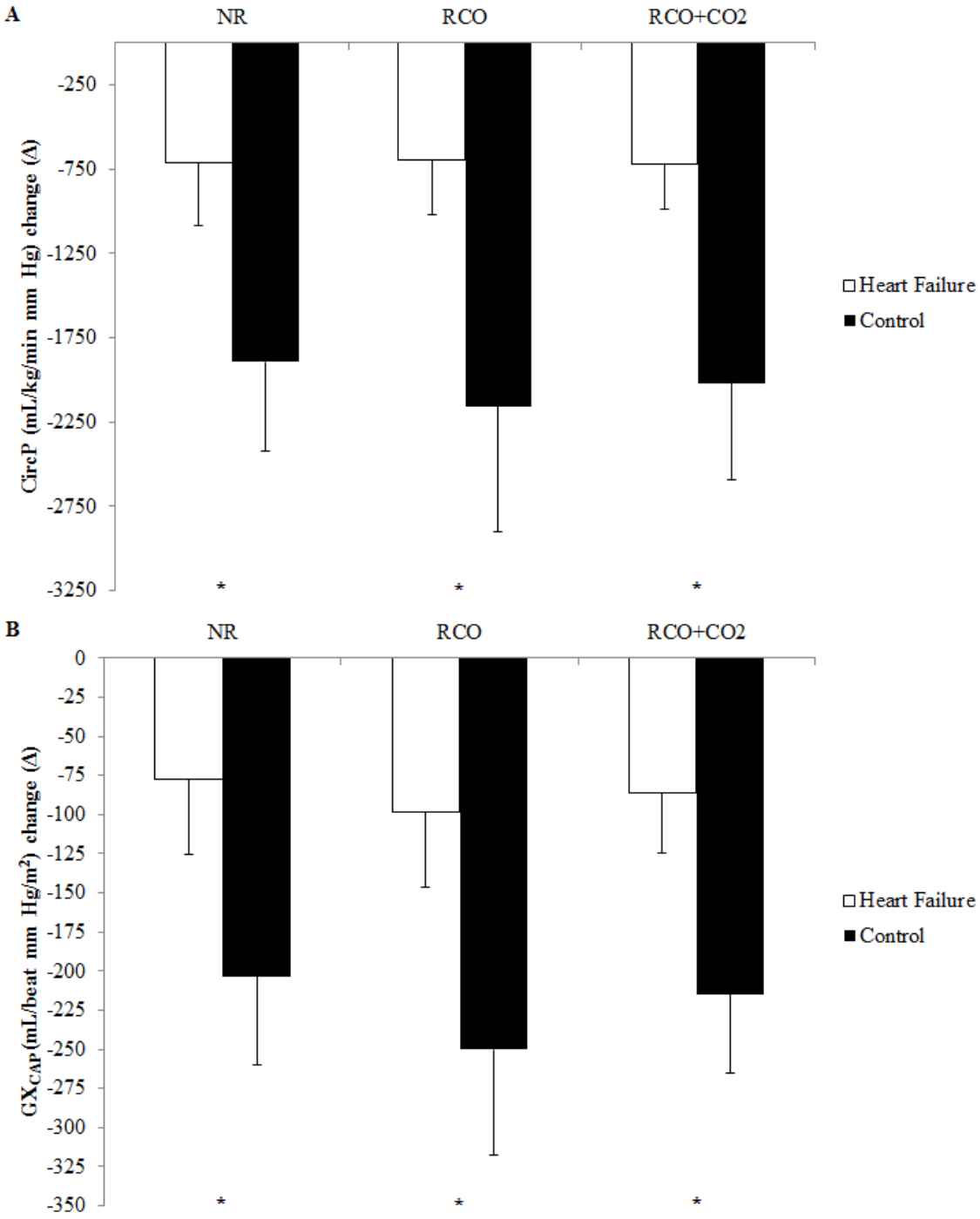


Figure 2

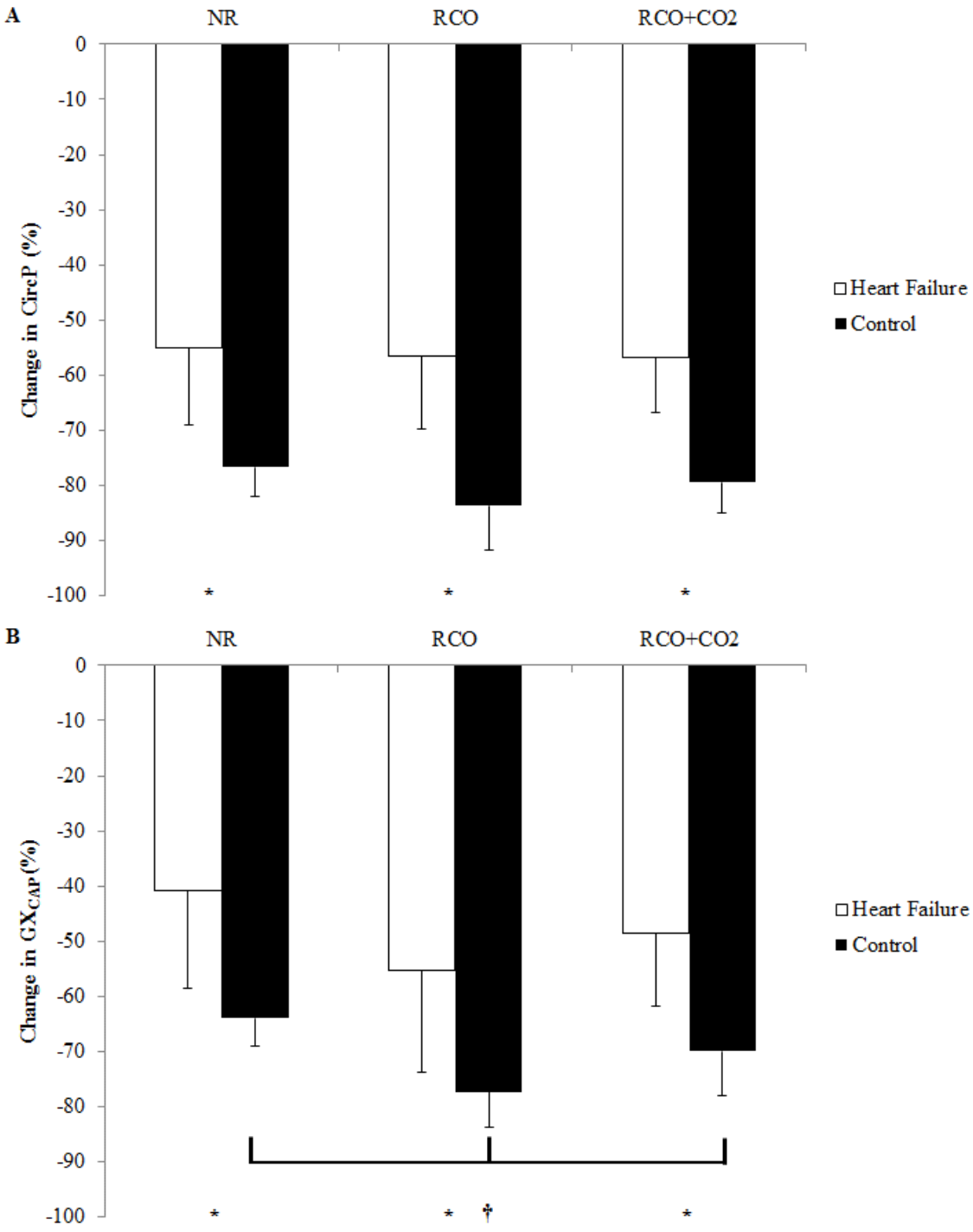
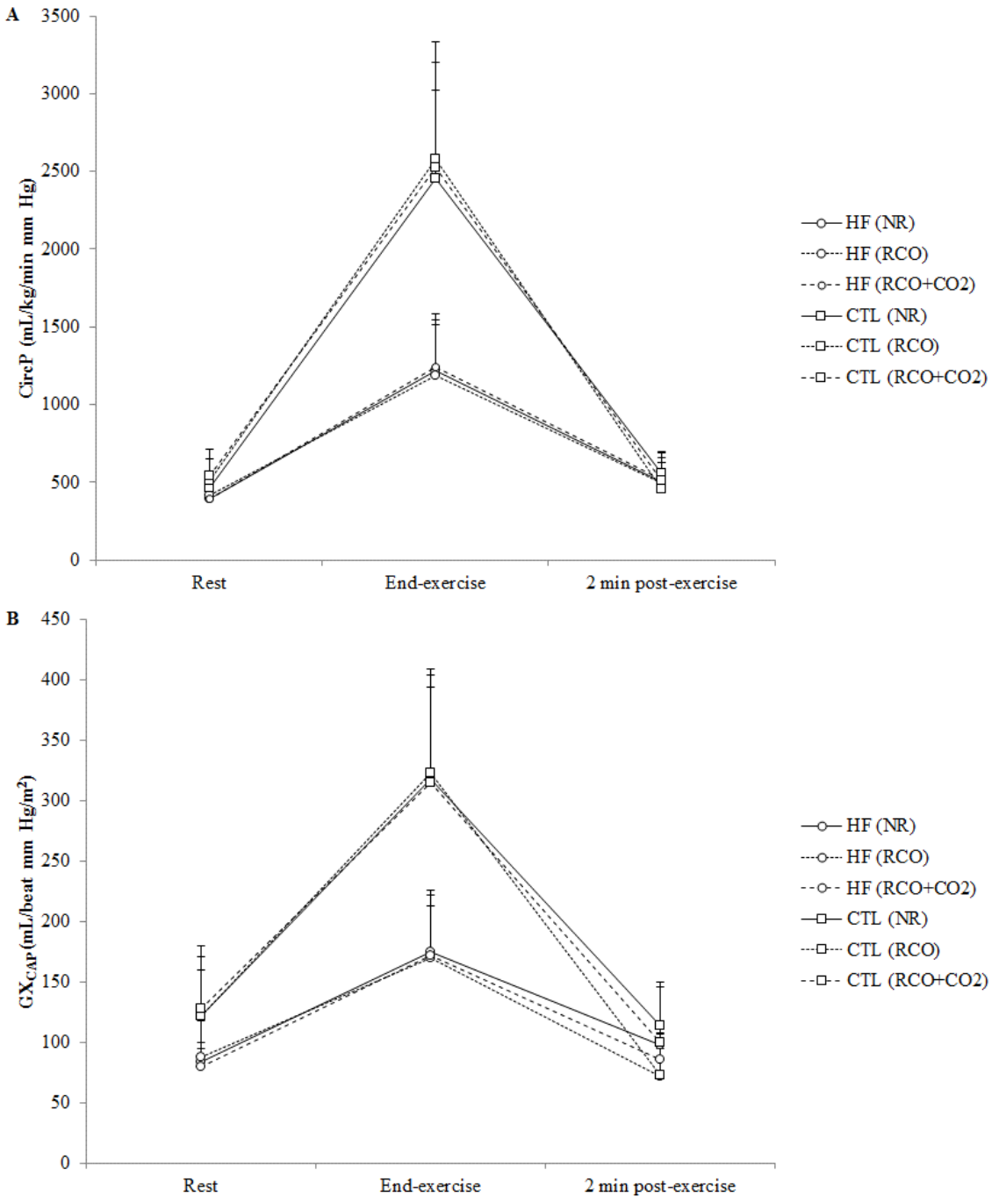

A
NR
RCO
RCO+CO2
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
Change in CircP (%)
*
*
*
Heart Failure
Control
B
NR
RCO
RCO+CO2
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
Change in GXCAP (%)
*
*
†
*
Heart Failure
Control

Figure 3


A
CircP (mL/kg/min mm Hg)
3500
3000
2500
2000
1500
1000
500
0
Rest
End-exercise
2 min post-exercise
HF (NR)
HF (RCO)
HF (RCO+CO2)
CTL (NR)
CTL (RCO)
CTL (RCO+CO2)
B
GXCAP (mL/beat mm Hg/m2)
450
400
350
300
250
200
150
100
50
0
Rest
End-exercise
2 min post-exercise
HF (NR)
HF (RCO)
HF (RCO+CO2)
CTL (NR)
CTL (RCO)
CTL (RCO+CO2)

3.3 Intrathecal fentanyl blockade of neural feedback from skeletal muscle during exercise in heart failure patients: Influence on circulatory power and pulmonary vascular capacitance

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Summary

Background: Secondary pulmonary hypertension is common in heart failure (HF) patients. We hypothesized that inhibition of neural feedback from locomotor muscle group III/IV neurons contributes to reduced pulmonary vascular pressures independent of changes in cardiac function during exercise in HF.

Methods: 9 HF patients (ages, 60±2; EF, 26.7±1.9%; New York Heart Association class, I-III) and 9 age/gender matched controls (ages, 63±2) completed five-minutes of constant-load cycling (65% $Workload_{peak}$) with intrathecal fentanyl or placebo, on separate days (randomized). Mean arterial pressure (MAP), heart rate (HR), end-tidal partial pressure of CO_2 ($P_{ET}CO_2$), and oxygen consumption (VO_2) were measured at rest and exercise. Non-invasive surrogates for cardiac power (circulatory power, $CircP=VO_2 \times MAP$), stroke volume (oxygen pulse, $O_2pulse=VO_2/HR$), and pulmonary arterial pressure ($GX_{CAP}=O_2pulse \times P_{ET}CO_2$) were calculated.

Results: At rest and end-exercise, differences between fentanyl versus placebo were not significant for CircP in HF or controls. Differences between fentanyl versus placebo for GX_{CAP} were not significant at rest in HF or controls. At end-exercise, GX_{CAP} was significantly higher with fentanyl versus placebo in HF (691±59 versus 549±38 mL/beat×mm Hg), but not controls (536±59 versus 474±43 mL/beat×mm Hg). Slopes (rest to end-exercise) for GX_{CAP} were significantly higher with fentanyl versus placebo in HF (95.1±9.8 versus 71.6±6.0 mL/beat×mm Hg), but not controls (74.3±9.5 versus 60.8±6.5 mL/beat×mm Hg). CircP slopes did not differ between fentanyl versus placebo in HF or controls ($p>0.05$).

Conclusion: We conclude that neural feedback from locomotor muscle group III/IV neurons may evoke increased pulmonary vascular pressures independent of changes in cardiac function during exercise in HF.

Introduction

Secondary pulmonary hypertension (PH) is a frequent consequence of chronic heart failure (HF) (Abramson et al., 1992; Aronson et al., 2011; Ghio et al., 2001; Moraes et al., 2000; Taylor, Olson, et al., 2013). Initially, the elevation in pulmonary pressures (mPAP) is caused by passive hemodynamic backup due to increased left ventricular and/or left atrial pressures because of impaired left ventricular output (passive PH) (Galie et al., 2009). In some cases, however, despite reduction in the hemodynamic volume of the pulmonary circulation, the transpulmonary gradient between mPAP and pulmonary capillary wedge pressure remain elevated (i.e. >15 mm Hg) and high pulmonary vascular resistance (PVR) persists (Galie et al., 2009; Rich & Rabinovitch, 2008). The latter form of secondary PH has been termed "reactive" or "mixed" although the pathogenic mechanisms are not fully understood (Aronson et al., 2011; Butler et al., 1999; Galie et al., 2009).

Another debilitating consequence of HF is a dysregulated autonomic nervous system (ANS) with chronically elevated sympathetic nervous system activity. Sympathoexcitation contributes to increased vascular resistance in the periphery (Amann et al., 2014; Keller et al., 2014; O'Leary et al., 2004); although, its contribution to the pulmonary vascular system remains unclear in HF. Because of the close link between secondary PH in HF and increased morbidity and mortality it is critical to improve the understanding of mechanisms linking elevated adrenergic drive, impaired cardiac function, and pulmonary vascular health in these patients (Aronson et al., 2011; Butler et al., 1999; Ghio et al., 2001; Moraes et al., 2000).

Recently, it has been suggested that a major contributor to sympathoexcitation during exercise in HF originates from mechanically (group III mechanoreceptors) and/or metabolically (group IV metaboreceptors) sensitive skeletal muscle neurons (Amann, Proctor, Sebranek, Pegelow, & Dempsey, 2009; O'Leary et al., 1999; Olson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999). Group III/IV unencapsulated nerve fibers are embedded near venules, capillaries, and within collagenous tissue of skeletal muscle and have been shown to influence both peripheral cardiovascular and ventilatory control in humans (Amann et al., 2009; Coote et al., 1971; McCloskey & Mitchell, 1972; O'Leary et al., 1999; Olson et al., 2014; Piepoli et al., 1999). In healthy individuals, an ample body of evidence now suggests that neural feedback from group III/IV nerve fibers contributes to favorable adjustments in ventilation, cardiac function, and hemodynamic circulation which leads to improved homeostatic regulation during exercise (Amann et al., 2009; Crisafulli et al., 2011; Fisher et al., 2010). In contrast, observations in HF suggest that neural feedback from skeletal muscle group III/IV nerve fibers contribute to impaired ventilatory and increased peripheral vascular resistance which are closely associated with reduced functional capacity and exertional symptoms (Keller et al., 2014; Olson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999).

Assessment of the influence of neural feedback from group III/IV nerve fibers during exercise in humans remains challenging. However, μ-opioid receptors which can be found superficially located on the lumbar dorsal horn of the spinal cord where group III/IV nerve fibers synapse represent a feasible target for pharmacological manipulation of afferent neural signaling (Besse, Lombard, & Besson, 1991; Besse, Lombard, Zajac,

Roques, & Besson, 1990; Meintjes, Nobrega, Fuchs, Ally, & Wilson, 1995; Pomeroy, Ardell, & Wurster, 1986). Inhibition of group III and IV neural transmission via a selective μ-opioid receptor agonist has been shown to inhibit the cortical projection of group III/IV neural feedback in human and animal models (Amann et al., 2011; Amann et al., 2009; Meintjes et al., 1995). Indeed, intrathecal administration of fentanyl at the lower-lumbar level prior to exercise in healthy individuals has been associated with reduced exercise ventilation, improved ventilatory efficiency, and reduced vascular resistance without affecting efferent neuromuscular control or the force-generating capacity of skeletal muscle related to central command (Amann et al., 2011; Amann et al., 2009).

Intrathecal injection of fentanyl at the lower-lumbar level prior to submaximal exercise has been shown to improve minute ventilation (V_E), respiratory rate (RR), the ventilatory equivalent for carbon dioxide production (V_E/VCO_2), and mean arterial pressure (MAP) in HF (Amann et al., 2014; Olson et al., 2014). While the influence of locomotor muscle neural feedback inhibition during exercise on peripheral vascular function and ventilatory control appear clear, it's influence on cardiac hemodynamics in human HF remains open to question (Amann et al., 2014); and, the influence of locomotor muscle neural inhibition during exercise on pulmonary vascular pressures (i.e. GX_{CAP})(Taylor, Olson, et al., 2013) has not been investigated previously in this population.

A number of studies have suggested that non-invasive gas-exchange based indices including oxygen pulse (O_2pulse) (Taylor, Olson, et al., 2013), circulatory power (CircP) (Scharf et al., 2002; Williams, Tzeng, et al., 2005), and GX_{CAP}(Taylor, Olson, et al.,

2013) are robust surrogates of direct measures of stroke volume, cardiac power, and pulmonary vascular pressures during exercise in HF, respectively. In particular, cardiac power has shown to be a robust indicator of cardiac hemodynamic and pressure generating capability during exercise in HF (Lang et al., 2009). In contrast, pulmonary vascular capacitance describes the interaction between pulmonary vascular pressures and hemodynamics (Taylor, Olson, et al., 2013), and when measured during exercise has been shown to discriminate between HF patients with and without secondary PH.

The present study sought to test the hypothesis that selective μ-opioid inhibition of neural feedback from group III/IV nerve fibers using intrathecal fentanyl at the lower-lumbar level during submaximal constant-load exercise would improve CircP (Scharf et al., 2002; Williams, Tzeng, et al., 2005) and GX_{CAP} (Taylor, Olson, et al., 2013) in HF patients.

Methodology

Participants

Nine Caucasian systolic HF patients along with nine Caucasian healthy control participants matched for gender and age were recruited and participated in this study (participant demographics, Table 1). Inclusion criteria for HF patients included diagnosis of ischemic or dilated cardiomyopathy with duration of HF symptoms >one year; stable HF symptoms (>three months); left ventricular ejection fraction percentage ≤35.0% (from clinical records within three months); body mass index (BMI) <35.0 kilograms/meter^2 (at enrolment); and current non-smokers with a past smoking history <15 pack-years (at enrolment). All patients were on standard optimum pharmacological

therapy for HF at the time of the study. Heart failure patients were recruited through the Mayo Clinic Heart Failure Service and the Cardiovascular Health Clinic. Control participants were recruited through advertisement in the surrounding community. Control participants had normal cardiac function without evidence of exercise-induced ischemia and were without history of hypertension, lung disease, or coronary artery disease. The experimental procedures were approved by the Mayo Clinic Institutional Review Board, all participants provided written informed consent prior to study, and all aspects of the study were performed in accordance with the ethical standards of the Declaration of Helsinki. Parts of these data have been reported previously (Olson et al., 2014); however, the data presented in the current manuscript represent a new analysis based on an a priori novel hypothesis.

Protocol Overview

Each participant underwent three days of testing, separated by ≥48-hours, in an environmentally controlled physiology laboratory. For all study visits, participants were asked to avoid strenuous physical activity for 24-hours prior to the visit and refrain from eating or consuming caffeine for three hours prior to arrival at the physiological laboratory for testing. Upon arrival, participants were fitted with a 12-lead electrocardiogram (Marquette Electronics, Milwaukee, WI) to continuously monitor heart rate (HR) and rhythm. Participants were seated on a recumbent cycle ergometer and fitted with a nose clip and mouthpiece attached to a PreVent Pneumotach (Medical Graphics, St Paul, MN) connected to a breath-by-breath gas-exchange system (MedGraphics CPX/D; Medical Graphics), which was calibrated for volumes (3.0 liter [L] syringe) and gases

immediately prior to each protocol (Olson, Joyner, & Johnson, 2010). Resting measures of oxygen uptake (VO_2), VCO_2, respiratory exchange ratio (RER), breathing frequency (f_B), tidal volume (V_T), V_E, and $P_{ET}CO_2$ were performed. Blood pressure was measured using manual sphygmomanometry at rest and near the end of each stage during the peak exercise test and continuously by indwelling radial artery catheter during constant-load submaximal exercise sessions. Measures of all gas-exchange and flow analysis as well as HR and oxygen saturation were continuously monitored and averaged every three seconds at rest and throughout exercise sessions. Oxygen pulse was calculated as, $O_2pulse = VO_2/HR$ (Taylor, Olson, et al., 2013); and the non-invasive surrogate for pulmonary vascular capacitance was calculated as, $GX_{CAP} = O_2pulse \times P_{ET}CO_2$ (Taylor, Olson, et al., 2013).

The first study day included a peak exercise test beginning at 20 watts (W) and increased by 20 W (HF) and 40 W (controls) every three min while maintaining a cadence of 65 repetitions per minute (rpm) until volitional fatigue (i.e. rating of perceived exertion ≥17 [Borg Scale = 6–20] or RER of ≥1.10). Study days two- and- three were randomized to either intrathecal injection of fentanyl at the lumbar level (fentanyl) to produce blockade of neural feedback from locomotor muscles or sham injection (placebo) in a single-blind fashion, where each exercise session then consisted of constant-load exercise at 65% of previously determined peak work.

Study days two- and- three, irrespective of randomization order, were identical except for intrathecal injection of fentanyl or placebo. Briefly, within two- to- three min following placement of the radial intra-arterial catheter and intrathecal injection techniques

(described below), participants were seated on a recumbent cycle ergometer and ventilation and gas-exchange measures were collected for five min at rest with the last min being used for analysis. Immediately following rest, at a cadence of 65 rpm participants cycled at their previously determined 65% of peak workload for five min. Non-active post-exercise recovery commenced for five min immediately following each exercise session. After recovery, but within ten min following cessation of exercise, assessment of central chemoreceptor sensitivity using a CO_2 rebreathe technique occurred (described below).

Radial intra- arterial blood pressure and gases assessment

During study days two- and- three, blood pressures and blood gases were measured using radial intra-arterial catheterization. Briefly, a 20-guage Teflon catheter (FA-04020; Arrow International Inc., Reading, PA) was placed into a radial artery after induction of local anesthesia (1.0% lidocaine). Real-time blood sampling and measurement of arterial pressure was recorded from a pressure transducer (PXMK099; Edwards Lifesciences, Irvine, CA) and exported to a digital oscilloscope for offline analysis of SBP, diastolic blood pressure (DBP), and MAP. Partial pressure of arterial oxygen (P_aO_2) and CO_2 (P_aCO_2) were also measured from anaerobically drawn arterial blood samples from the same catheter over a period of 10–15 seconds at rest and immediately before cessation of exercise (ABL825 Flex Blood Gas/CO-ox analyzer; Radiometer America Inc., Westlake, OH). From these blood pressure measures with our ventilatory gas-exchange measures we calculated the non-invasive estimate of cardiac power (Lang et al., 2009), circulatory

power, adjusted for body weight as, CircP = VO_2×MAP/weight (Cohen-Solal et al., 2002; Scharf et al., 2002; Williams, Tzeng, et al., 2005).

Intrathecal injection to influence neural feedback from locomotor muscles, fentanyl versus placebo

The following approach used in the present study was similar to the approach used in a variety of clinical anesthesiology settings and research laboratories, as described previously (Amann et al., 2009; Olson et al., 2014). Briefly, on study days two- and-three participants were seated in an upright position. Immediately following arterial catheter placement, using aseptic technique, the skin and subcutaneous tissue were anaesthetized at the L3—L4 vertebral interspace with 2.0–4.0 milliliters (mL) of 1.0% lidocaine. A 22-guage Whitaker needle was advanced to the subarachnoid space between L3—L4, with placement confirmed by visualization of free-flowing cerebrospinal fluid 1.0 mL of fentanyl (0.05 milligram/mL) was injected into the subarachnoid space following aspiration of a small amount of cerebrospinal fluid. The participants remained seated in the upright position to minimize cephalad migration of fentanyl. During the placebo visit, with the exception of the advancement of the 22-guage Whitaker needle into the subarachnoid space for fentanyl injection, all procedures were identical to the fentanyl injection visit.

Central chemosensitivity assessment

To assess for cephalad migration of fentanyl in the cerebrospinal fluid we measured central chemosensitivity using a CO_2 rebreathing method optimally timed within ten min following cessation of exercise. This timing, as opposed to immediately following

injection, was chosen because if cephalad migration in cerebrospinal fluid was to occur it would be most readily detectable post-exercise when sufficient time for migration had elapsed.

In brief, participants breathed into a non-rebreathing three-way pneumatic switching valve (Hans Rudolph, Kansas City, MO) that was connected to a pneumotachometer (Hans Rudolph, Kansas City, MO) and gas-exchange system (MedGraphics CPX/D; Medical Graphics). The inspiratory port of the switching valve allowed for rapid switching from breathing room air to the 6.0 L anesthesia rebreathe bag (Hans Rudolph, Kansas City, MO) containing a gas-mixture volume of 5.0% CO_2 and 95.0% O_2 depending on initial V_T of the subject. Participants breathed room air for two min that was followed by a slow maximal expiration to residual volume where the participants were then switched into the rebreathe bag. Participants began with a maximal full inspiration of rebreathe bag air that was followed with relaxed breathing for four min. Continuous measures of $P_{ET}CO_2$, end-tidal partial pressure of oxygen ($P_{ET}O_2$), and V_E occurred. The test was stopped prior to the conclusion of the four min maneuver period if one of the following occurred: $P_{ET}CO_2$ of 65.0 mm Hg; $P_{ET}O_2$ of 160.0 mm Hg; V_E of 100 L/min; or participant wished to stop. The sensitivity to CO_2 was assessed using the slope of the relationship between measures of V_E and $P_{ET}CO_2$. On study days two- and-three, the CO_2 rebreathe test was performed three separate times with three- to- five min of exposure to room air to allow V_E and $P_{ET}CO_2$ to return to baseline between measures.

Statistical analysis

All data are presented at means ± standard deviation (SD). Normality and homogeneity of variance of the data was assessed and met using Levene's test. Specific comparison between groups (i.e. controls vs. HF) or conditions (i.e. rest vs. exercise or fentanyl vs. placebo) were compared using unpaired and paired two-tailed Student's t-tests, respectively. Multiple comparisons for time, group, and condition effects were tested using the repeated measures two-way analysis of variance test. In the occurrence of a significant F-test statistic, a correction for multiple comparisons using the post-hoc Bonferroni test was used to identify significance between specific comparisons. The alpha level was set at 0.05 to determine significance. Computations were made using SAS statistical software, v.9.4 (SAS Institute, Cary, NC).

Results

Participant characteristics

Participant characteristics are presented in Table 1. Participants were predominantly male in both groups with an overall age range of 52- to-73. Height did not differ between groups, whereas controls weighed on average ~18.0% less than HF which contributed to the differences in BMI and body surface area between groups. Ejection fraction percentage was characteristic of systolic HF (range = 20.0–35.0%). Exercise capacity was significantly reduced in HF compared to controls (peak oxygen consumption [VO_{2peak}], range = 14.0–23.1 vs. 22.0–36.7 mL/kg/min, respectively; $P<0.05$). Heart failure patients were of New York Heart Association (NYHA) classification range I–III, ischemic or idiopathic etiology, and optimally medicated for HF using standard pharmacological therapy.

Baseline ventilation and hemodynamics

Baseline measures of ventilation and hemodynamics for both groups and experimental conditions are presented in Table 2. There was no difference in V_E, V_T, f_B, or $P_{ET}CO_2$ between HF and controls (P>0.05, for all). Heart rate did not differ significantly between or within groups, whereas SBP, DBP, and MAP were lower in HF versus controls (P<0.05, for all). Because of the predominately lower blood pressure in HF, CircP was significantly lower in HF versus controls.

Exercise ventilation and hemodynamics

Exercise intensity, ventilation, and hemodynamic measures averaged over the final 30 seconds of exercise for both groups and experimental conditions are presented in Table 3. Although there was a significant difference in exercise workload between groups, percentage of VO_{2peak} between groups was not different (P>0.05). Lack of significant differences between groups and experimental conditions for VO_2 and VCO_2 were also reflected in RER. This lack of difference demonstrates that the groups were well matched for metabolic intensity. Whereas, although no significant differences appeared for VCO_2 and V_T, there were significant differences for V_E in HF with fentanyl versus controls with fentanyl. The average difference for $P_{ET}CO_2$ between experimental conditions for HF was 7.2±0.1 mm Hg (P<0.05), whereas the average difference for controls was 2.1±0.8 mm Hg (P>0.05).

The significantly lower HR for HF versus controls contrasted non-significant differences in O_2pulse, but shared a similar pattern for significant differences in CircP before adjustment for workload. Although, differences in O_2pulse at end-exercise were

consistent with baseline O_2pulse differences between HF and controls (P>0.05). Significant reductions in SBP and DBP in HF with fentanyl versus placebo likely contributed to significant reductions in MAP in this same comparison. Unexpectedly, after adjustment for workload, CircP was similar across groups and experimental conditions (P>0.05). Whereas, O_2pulse and GX_{CAP} for HF were greater versus controls after adjustment for workload (P<0.05). Differences in unadjusted CircP for HF between experimental conditions was 48±76 mL/kg/min · mm Hg (P>0.05), and in controls was 247±55 mL/kg/min · mm Hg (P>0.05). Unadjusted differences in GX_{CAP} between experimental conditions in HF was 142±62 mL/beat · mm Hg (P<0.05), and in controls this difference was 62±47 mL/beat · mm Hg (P>0.05). The significantly greater $P_{ET}CO_2$ in HF with fentanyl versus placebo appeared to contribute to the baseline to end-exercise rate of change difference for GX_{CAP} in HF with fentanyl versus placebo (P<0.05). There was no significant rate of change difference for GX_{CAP} between experimental conditions in controls.

The absolute change (Δ) from baseline to end-exercise for CircP and GX_{CAP} comparing groups and experimental conditions are shown in Figure 1 (A and B). The percentage increase from baseline to end-exercise for CircP and GX_{CAP} comparing groups and experimental conditions are shown in Figure 2 (A and B). Presented in Figure 3 (A and B) are the averages for CircP and GX_{CAP} during the exercise period in 30 second intervals, while also comparing group differences as well as differences between experimental conditions for HF and controls.

Discussion

In this randomized, single-blind, placebo-controlled crossover study, it was observed that inhibition of centrally projecting neural feedback from locomotor muscle group III/IV nerve fibers using intrathecal fentanyl during constant-load submaximal exercise improved GX_{CAP} (i.e. pulmonary vascular pressures) (Taylor, Olson, et al., 2013) in HF. In contrast, despite an apparent influence on both pulmonary and peripheral vascular pressures, inhibition of neural feedback from locomotor muscle group III/IV nerve fibers during exercise did not appear to evoke increases in myocardial contractility and/or hemodynamics (i.e. O_2pulse and CircP) (Cohen-Solal et al., 2002; Scharf et al., 2002; Taylor, Olson, et al., 2013; Williams, Tzeng, et al., 2005) in HF.

In an attempt to restore or maintain central hemodynamic homeostasis during exercise, the cardiovascular system relies on the ANS for augmented adrenergic drive. This acute feedback response of the sympathetic nervous system during times of hemodynamic instability is influenced by neural feedback from group III/IV nerve fibers found dispersed within skeletal muscle, and observed to contribute to the sustained maintenance of cardiac and cardiovascular function during exercise in healthy individuals (Amann et al., 2011; Amann et al., 2009; Crisafulli et al., 2011; Fisher et al., 2010). In HF, this integrated physiology regulating the interaction between neural feedback from group III/IV nerve fibers and the ANS is impaired and contributes to persistently elevated adrenergic drive at rest which is exacerbated during exercise (Amann et al., 2014; Keller et al., 2014; Olson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999). Moreover, and of clinical significance, our group and others have demonstrated that this impaired neural feedback pathway contributes to detrimental changes in ventilatory and

peripheral vascular control in patients with HF (Amann et al., 2014; Keller et al., 2014; Olson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999). To date, however, limited data exists on the impact of neural feedback from skeletal muscle group III/IV nerve fibers on changes in central hemodynamics during exercise in human HF (Amann et al., 2014). Therefore, a major finding of the present study suggests that GX_{CAP} (i.e. pulmonary vascular pressures) (Taylor, Olson, et al., 2013) is improved by neural inhibition during exercise in HF. This extends findings from two previous studies which demonstrate neural feedback from skeletal muscle contribute to increased mPAP during and immediately following submaximal exercise in healthy individuals (Lykidis et al., 2008; White et al., 2013).

Because of close relationships that pulmonary blood flow shares with mixed venous CO_2 content, mPAP, PVR, and $P_{ET}CO_2$ (Shibutani et al., 1994; Taylor, Olson, et al., 2013), the present observations in HF suggest that exercise with intrathecal fentanyl decreased mPAP as reflected by an increase in $P_{ET}CO_2$ measured with gas-exchange. The higher GX_{CAP} observed during exercise with intrathecal fentanyl in HF during the present study was likely attributable to attenuations in both mPAP and PVR since O_2pulse (i.e. product of stroke volume and arteriovenous oxygen difference) (Cohen-Solal et al., 2002; Taylor, Olson, et al., 2013) remained unchanged compared to the placebo condition. In further support of this mechanism in HF, occurring in parallel with increases in $P_{ET}CO_2$ during exercise with intrathecal fentanyl were significant reductions in systemic arterial pressure.

Although healthy individuals in the present study did not demonstrate a change in GX_{CAP} during exercise with intrathecal fentanyl similar to the observations of Lykidis et al. (2008) and White et al. (2013) during exercise in healthy individuals, it is important to point out that the techniques utilized in these previous studies differed considerably from the current study (Lykidis et al., 2008; White et al., 2013). Lykidis et al. (2008) and White et al. (2013) stimulated neural feedback responses from group IV (and perhaps group III) afferents during exercise using regional circulatory occlusion (RCO) of exercising limbs (Lykidis et al., 2008; White et al., 2013). Additionally, previous studies comparing mPAP responses between exercise with RCO versus RCO not associated with exercise (either during or after), with the latter condition known to not generate a significant pressor response (Lykidis et al., 2008; White et al., 2013). In contrast, the present cross-over study used neural feedback inhibition during exercise which were compared to a matched exercise session with placebo. Hence, comparing the influence of neural feedback from locomotor muscle afferents on vascular responses between RCO with exercise and RCO independent of exercise may overestimate the influence of neural feedback from skeletal muscle group III/IV nerve fibers on vascular pressures relative to the observations of the present study (Lykidis et al., 2008; White et al., 2013). Nevertheless, observations during post-exercise+RCO by Lykidis et al. (2008) and White et al. (2013) are consistent with the present exercise+placebo hemodynamic circulatory findings (Lykidis et al., 2008; White et al., 2013), in addition to recently reported evidence by our group which suggests that stimulation of the metaboreflex using RCO immediately following exercise in HF patients contributes to increased peripheral arterial pressure (Keller et al., 2014).

Although not measured directly, we assume based on the observations from other studies of HF during exercise that peripheral vascular reactivity and permeability was not completely impaired in the present HF sample (Wilson, Martin, Schwartz, & Ferraro, 1984; Zelis, Mason, & Braunwald, 1969). It is likely that increased vascular conductance and decreased systemic vascular resistance during exercise with intrathecal fentanyl lead to a reduction in afterload and mPAP in HF. These changes would be expected to facilitate an improvement in cardiac output and contribute to the maintenance of arterial pressure. However, inhibition of locomotor muscle neural feedback did not influence significant inotropic and/or chronotropic increases which is suggested by null increases in O_2pulse (Cohen-Solal et al., 2002; Taylor, Olson, et al., 2013), CircP (Scharf et al., 2002; Williams, Tzeng, et al., 2005), or HR during exercise in HF; which, also considering blood pressure responses (i.e. ↓ afterload) further suggests that myocardial contractile reserve is not influenced by neural feedback from locomotor muscle group III/IV nerve fibers in patients. The comparable O_2pulse between HF and control participants at baseline was likely caused by the presence of larger ventricular cavities related to dilated cardiomyopathy, and not because of preserved cardiac function since patients also demonstrated significantly attenuated CircP at rest and at end-exercise as well as reduced HR at end-exercise in comparison to control participants.

Cardiac pumping capacity is impaired in HF at rest and during exercise despite heightened cardiac adrenergic drive (Bristow et al., 1982; Dickstein et al., 2008; Lang et al., 2009). This is suggested to be related to impaired mechanisms intrinsic to HF such as a failing Frank—Starling mechanism (related to ischemic wall segments and/or

dysfunctional cardiac β-adrenergic receptors) (Bristow et al., 1982; Eisenhofer et al., 1996; Komamura et al., 1993; Rose et al., 1983; Schwinger et al., 1994). Therefore, because HF patients are commonly regarded as being afterload dependent (i.e. limited myocardial contractile reserve), this suggests that augmented afterload associated with exercise should attenuate the ability of cardiac hemodynamics to contribute to the increase of systemic arterial pressure. However, our present findings suggest cardiac function is less dependent on the response of the systemic vasculature to the influences of neural feedback from locomotor muscle group III/IV nerve fibers during exercise in HF. The lack of increase in cardiac hemodynamics despite a decrease in afterload during exercise with intrathecal fentanyl was likely related to mechanisms associated with limited myocardial contractile reserve in HF, which could also explain why elevated adrenergic drive associated with neural feedback from locomotor muscle group III/IV nerve fibers had little influence on inotropy and SV during exercise.

Clinical Implications

The present findings suggest that neural feedback from locomotor muscle group III/IV nerve fibers contributes to pulmonary vascular pressures independent of cardiac hemodynamic status during submaximal exercise in HF; and, perhaps more importantly, may provide further insight into the mechanisms contributing to exertion-related symptomology at work intensities equivalent to those of daily living. These observations are also important because it is well-accepted that HF patients commonly develop secondary PH which is closely linked to increased morbidity and mortality (Abramson et al., 1992; Aronson et al., 2011; Ghio et al., 2001; Moraes et al., 2000). The current

findings extend those of others by highlighting a novel contributory mechanism of secondary PH in HF.

Limitations

The patients in the present study are characterized as NYHA class I-III, whereas NYHA class IV patients exhibit more severe cardiac and/or pulmonary impairment in parallel with diminished vascular health which are commonly associated with signs and symptoms at rest and during low-level activity. Therefore, while it might be expected that the findings of the present study would be exacerbated in patients exhibiting increased exertional symptoms, these data are only generalizable to the patients studied. Further research is necessary to confirm our findings in a larger patient population. Also, although we did not directly assess sympathetic nervous system activity during exercise, the intrathecal fentanyl technique has been used previously to inhibit group III/IV neural feedback during exercise in healthy individuals and HF patients (Amann et al., 2009; Amann et al., 2014; Olson et al., 2014). Finally, although O_2pulse is a validated surrogate of SV (Taylor, Olson, et al., 2013), and CircP for cardiac power in HF (Scharf et al., 2002; Williams, Tzeng, et al., 2005), we did not directly measure SV or Q in the current study. Nevertheless, cardiac power and its estimate CircP are suggested to be robust indicators of cardiac hemodynamic function (Cohen-Solal et al., 2002; Lang et al., 2009; Scharf et al., 2002; Williams, Tzeng, et al., 2005), and therefore it is important to point out that our use of indirect indices were adequate to address our study objectives since we aimed to study relative changes in central hemodynamics between experimental conditions as opposed to absolute capacities.

Conclusion

In conclusion, the findings of the present study suggest that inhibition of neural feedback from group III/IV nerve fibers originating within locomotor muscles using intrathecal fentanyl during submaximal exercise improves pulmonary vascular pressures independent of changes in cardiac function.

Tables

Table 1: Participant characteristics

	Healthy Control	Heart Failure	*P*
Demographics			
Age, years	63±8	60±6	0.37
Gender, male/female	7/2	7/2	1.00
Height, cm	175.7±9.8	175.9±9.5	0.97
Weight, kg	80.1±12.4	97.8±9.2	0.003
BMI, kg/m^2	25.9±3.4	31.9±4.4	0.005
BSA, m^2	2.0±0.2	2.2±0.1	0.02
VO_{2peak}, mL/kg/min	26.8±5.2	18.4±2.8	0.001
LVEF, %		26.7±5.7	
Etiology (ischemic/idiopathic)		5/4	
NYHA class		1.9±0.8	
I		3	
II		3	
III		3	
Medications			
ACE inhibitor		6 (67)	
Angiotensin II receptor blockers		3 (33)	
β-blocker		9 (100)	
Aspirin		5 (56)	
Diuretics		6 (67)	

Data are mean±SD or as n (%).ACE, angiotensin converting enzyme; BMI, body mass index; BSA, body surface area; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

Table 2: Resting measures

	Healthy Control		Heart Failure	
	Placebo	Fentanyl	Placebo	Fentanyl
Ventilation and gas exchange				
VO_2, L/min	0.34±0.03	0.33±0.07	0.36±0.03	0.38±0.05
VCO_2, L/min	0.31±0.03	0.30±0.05	0.34±0.04	0.36±0.05
RER	0.87±0.08	0.85±0.04	0.94±0.09	0.94±0.09*
V_E, L/min	12±2	12±2	14±2	13±2
V_T, L	0.76±0.11	0.72±0.14	0.74±0.12	0.75±0.07
f_B, breaths/min	17±3	18±4	19±2	18±2
$P_{ET}CO_2$, mm Hg	34.9±2.2	35.2±3.0	36.5±8.2	38.8±4.5
Hemodynamics				
HR, beats/min	73±13	72±9	70±10	70±17
SBP, mm Hg	148±17	145±18	122±9*	115±8*
DBP, mm Hg	68±10	64±8	59±8*	57±9*
MAP, mm Hg	95±11	91±10	80±6*	76±8*
O_2pulse, mL/beat	4.9±1.1	4.7±1.2	5.3±0.8	5.6±1.1
CircP, mL/kg/min · mm Hg	411±70	381±80	299±38*	300±53*
GX_{CAP}, mL/beat · mm Hg	170±41	165±45	191±47	216±35*

Data are presented as means±SD. CircP, circulatory power; DBP, diastolic blood pressure; VO_2, oxygen consumption; f_B, breathing frequency; GX_{CAP}, pulmonary vascular capacitance; HR, heart rate; MAP, mean arterial pressure; O_2pulse, oxygen pulse; $P_{ET}CO_2$, end-tidal partial pressure CO_2; RER, respiratory exchange ratio; SBP, systolic blood pressure; VCO_2, carbon dioxide production; V_E, minute ventilation; V_T, tidal volume. *$p<0.05$ compared with control after Bonferroni correction.

Table 3: End-exercise measures

	Healthy Control		Heart Failure	
	Placebo	Fentanyl	Placebo	Fentanyl
Exercise intensity				
Workload (watts)	120±37	-	78±23*	-
Percent of VO_{2peak}	77±13	77±13	82±14	84±15
Ventilation and gas exchange				
VO_2, L/min	1.7±0.4	1.7±0.5	1.5±0.3	1.5±0.3
VCO_2, L/min	2.0±0.5	2.0±0.5	1.5±0.3	1.6±0.3
RER	1.09±0.06	1.08±0.05	1.05±0.02	1.04±0.04
V_E, L/min	63±16	63±19	51±9	45±10*
V_T, L	2.1±0.6	2.1±0.6	1.7±0.4	1.7±0.3
f_B, breaths/min	30±4	30±5	33±12	26±5
$P_{ET}CO_2$, mm Hg	37.1±2.7	39.2±3.5	36.3±3.6	43.5±3.7*†
Hemodynamics				
HR, beats/min	131±15	125±21	99±19*	97±21*
SBP, mm Hg	239±20	225±26	161±24*	152±29*†
DBP, mm Hg	75±9	66±6	62±9*	56±11*†
MAP, mm Hg	130±6	119±8	95±11*	88±15*†
O_2pulse, mL/beat	12.8±3.5	13.9±4.9	15.1±2.5	16.0±4.3
O_2pulse, watts^{-1}	0.11±0.02	0.11±0.02	0.22±0.1*	0.22±0.06*
CircP, mL/kg/min · mm Hg	2721±618	2475±673	1413±257*	1365±333*
CircP, watts^{-1}	23.3±6.0	21.0±4.2	20.0±8.4	19.9±9.4
GX_{CAP}, mL/beat · mm Hg	474±130	536±177	549±115	691±176†
GX_{CAP}, watts^{-1}	3.9±0.6	4.3±0.8	7.9±3.4*	9.5±3.0*
	Slope			
O_2pulse, mL/beat	1.59±0.53	1.83±0.80	1.96±0.39	2.08±0.66
CircP, mL/kg/min · mm Hg	462±125	419±127	223±50*	212±66
GX_{CAP}, mL/beat · mm Hg	61±19	74±29	72±18	95±30†

Data are presented as means±SD. CircP, circulatory power; DBP, diastolic blood pressure; VO_2, oxygen consumption; f_B, breathing frequency; GX_{CAP}, pulmonary vascular capacitance; HR, heart rate; MAP, mean arterial pressure; O_2pulse, oxygen pulse; $P_{ET}CO_2$, end-tidal partial pressure CO_2; RER, respiratory exchange ratio; SBP, systolic blood pressure; VCO_2, carbon dioxide production; V_E, minute ventilation; V_T, tidal volume; slope = rate of change baseline to end-exercise; *$p<0.05$ compared with control after Bonferroni correction; †$p<0.05$ compared with placebo after Bonferroni correction.

Figure Captions

Figure 1: Data are presented as means±SD (A) Absolute change (Δ) circulatory power (CircP) from rest to end-exercise. *Significant between groups, heart failure vs. control, $p<0.05$. (B) Δ in GX_{CAP} from rest to end-exercise. *Significant between fentanyl and placebo for heart failure, $p<0.05$.

Figure 2: Data are presented as means±SD. (A) Circulatory power (CircP) at end-exercise as a percentage (%) of CircP at baseline. *Significant between groups, heart failure vs. control, $p<0.05$. (B) Pulmonary vascular capacitance (GX_{CAP}) at end-exercise as a % of GX_{CAP} at baseline. *Significant between fentanyl and placebo for control, $p<0.05$.

Figure 3: Data are presented as means±SD. (**A**) HF, heart failure; CTL, control. Pattern of change for circulatory power (CircP) as increments of 30 second averages from rest to end-exercise. *$p<0.05$, HF-Fentanyl vs. CTL-Fentanyl and HF-Placebo vs. CTL-Placebo. (**B**) Pattern of change for pulmonary vascular capacitance (GX_{CAP}) as increments of 30 second averages from rest to end-exercise *$p<0.05$, HF-Fentanyl vs. CTL-Fentanyl. †$p<0.05$, HF-Fentanyl vs. HF-Placebo.

Figures

Figure 1

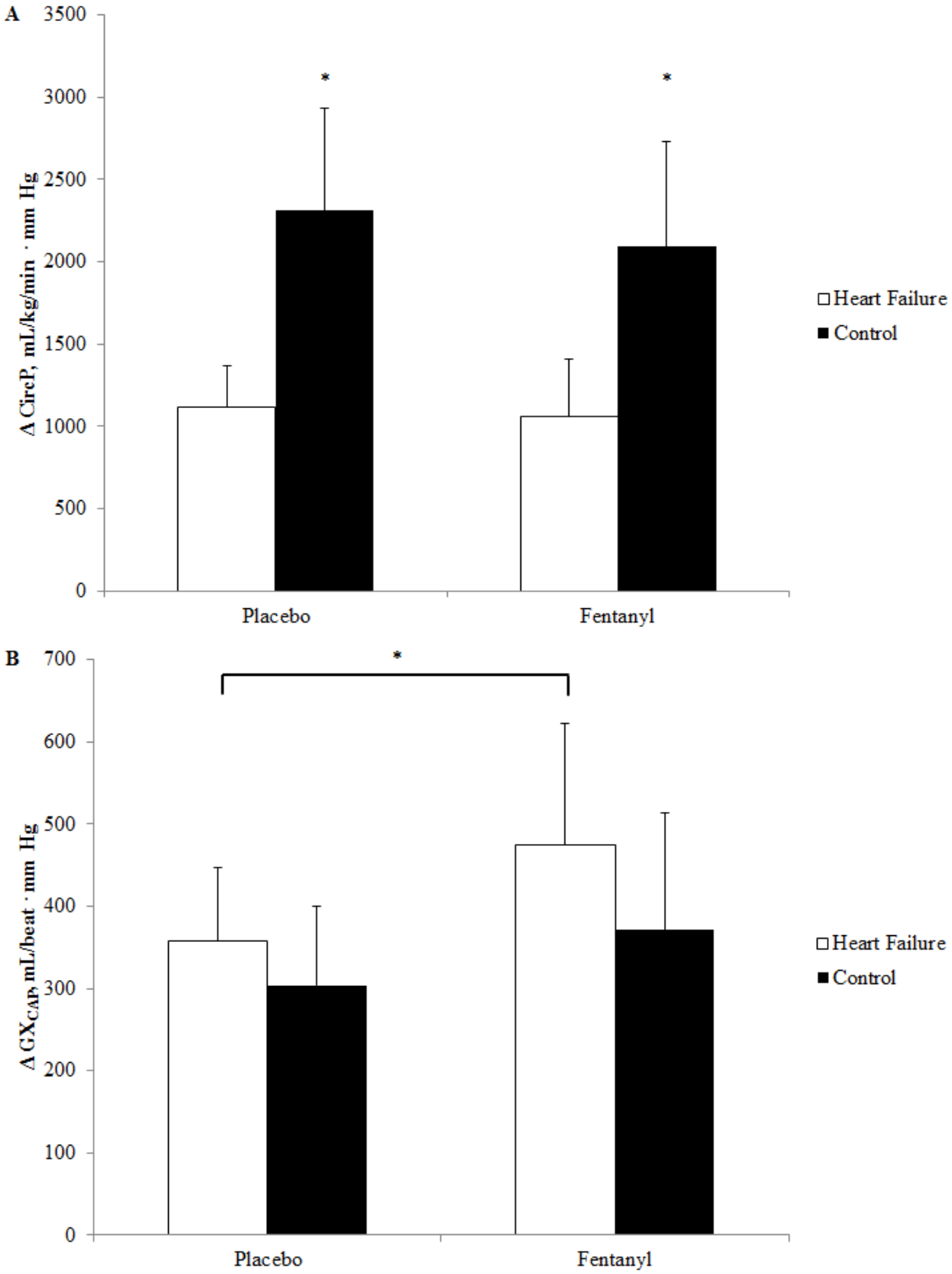


Figure 2

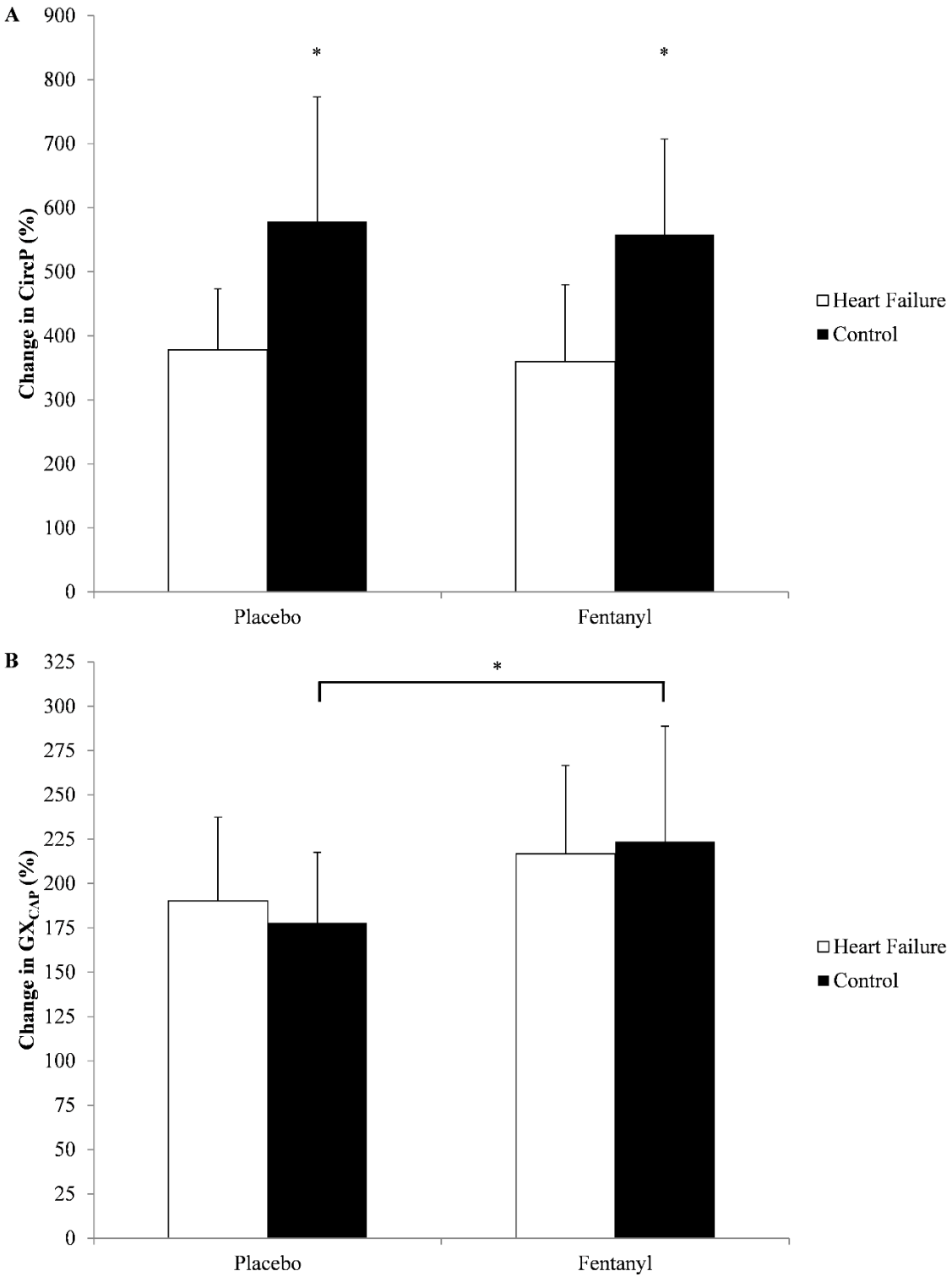
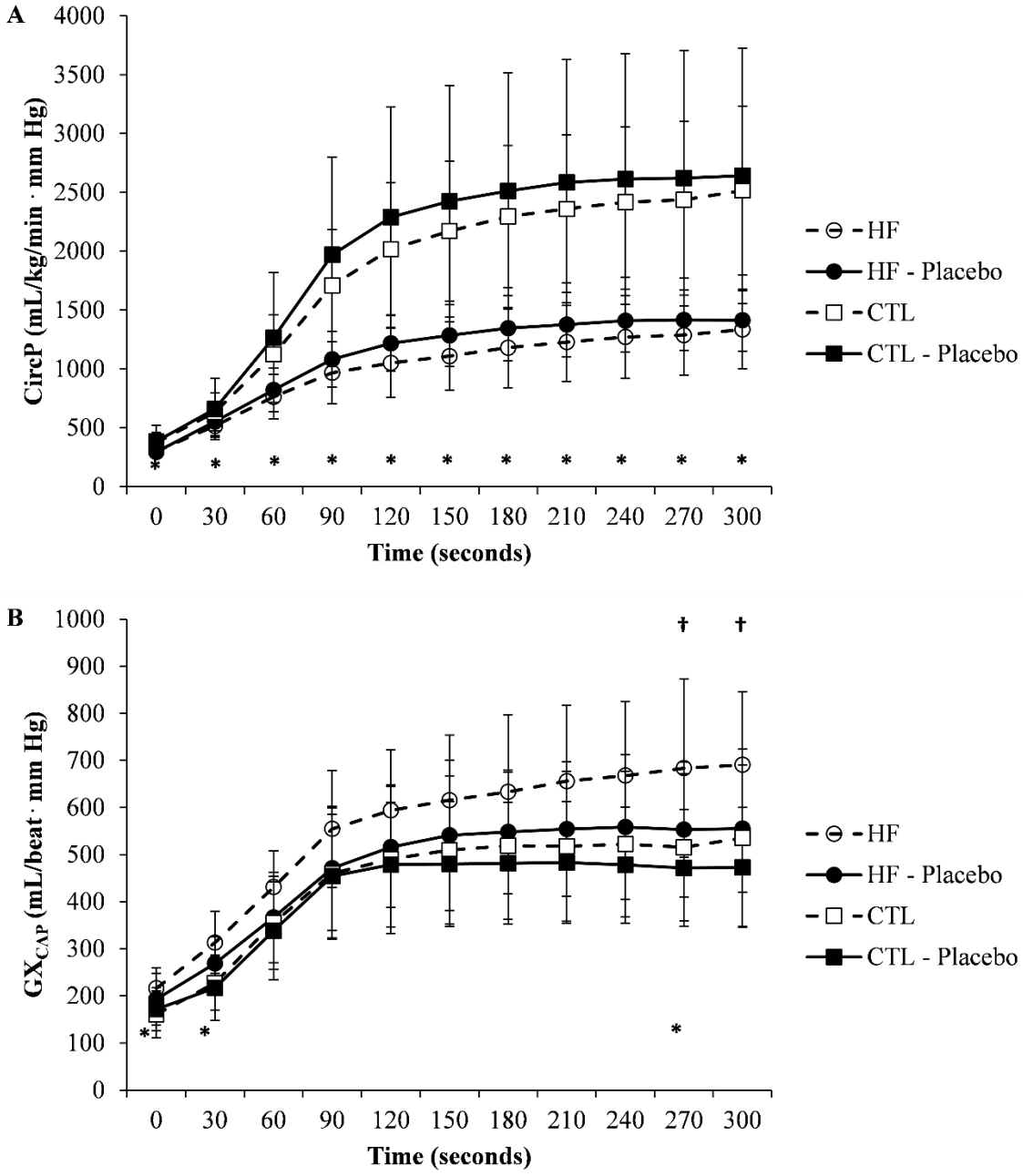

A
900
800
700
600
500
400
300
200
100
0
Change in CircP (%)
*
*
Placebo
Fentanyl
Heart Failure
Control
B
325
300
275
250
225
200
175
150
125
100
75
50
25
0
Change in GX$_{CAP}$ (%)
*
Placebo
Fentanyl
Heart Failure
Control

Figure 3


A
CircP (mL/kg/min · mm Hg)
4000
3500
3000
2500
2000
1500
1000
500
0
0 30 60 90 120 150 180 210 240 270 300
Time (seconds)
HF
HF - Placebo
CTL
CTL - Placebo
B
GXCAP (mL/beat · mm Hg)
1000
900
800
700
600
500
400
300
200
100
0
0 30 60 90 120 150 180 210 240 270 300
Time (seconds)
HF
HF - Placebo
CTL
CTL - Placebo

Chapter 4

Overall discussion

Heart failure is a debilitating syndrome that influences all organ systems and is overtly expressed with both signs and symptoms unique to this illness. Importantly, this syndrome is associated with an increased risk for morbidity and premature mortality (Dickstein et al., 2008; Paulus et al., 2007; Roger et al., 2012). The consequences of HF are multifactorial and complicated. Included amongst the generally recognized symptoms of HF are dyspnea and fatigue, whereas objective signs of HF include abnormal cardiac hemodynamics (e.g. low Q and SV) (Dickstein et al., 2008; "Heart Failure Society of America (HFSA) practice guidelines. HFSA guidelines for management of patients with heart failure caused by left ventricular systolic dysfunction--pharmacological approaches," 1999; Paulus et al., 2007; Remme, Swedberg, Task Force for the, & Treatment of Chronic Heart Failure, 2001). However, despite the current knowledge of HF, it remains unclear what primarily mediates exercise intolerance, as well as the magnitude of relationships between cardiac pumping capability and syndrome severity and prognosis in HF. As such, although it is known that no single factor mediates the signs, symptoms, or prognosis in HF, a more comprehensive understanding of specific factors (e.g. exercise intolerance and central hemodynamics) pertaining to their contribution to the clinical classification of HF is critical because of the impact this knowledge could have on diagnosis and therapeutic regimen of patients. Accordingly, the measurement of central hemodynamic function during exercise provides invaluable

information about the magnitude of impairment of cardiac pumping capability as well as overall functional capacity in HF; but, equally important, this information is closely related to prognosis in these patients (Bain et al., 1990; Franciosa et al., 1981; Lang et al., 2009; Szlachcic, Massie, et al., 1985; Williams, Jackson, et al., 2005).

The first of several objectives outlined in this dissertation was to provide a comprehensive review of the traditional and non-traditional measurements used to describe central hemodynamics in humans. There is considerable variability in the body of literature concerning how to best describe the blood flow and pressure generating capability of the heart, which is also closely related to the issue of how to describe the hemodynamic relationship the heart shares with pulmonary vasculature. Due to of the complexity of factors known to influence human central hemodynamics, many efforts were made to identify and study those that are not only influential in adjusting central hemodynamics, but also functionally contrasting in HF compared to healthy individuals. Thus, it could be concluded that although each index is not without its own assumptions, non-traditional parameters of central hemodynamics add valuable information about the blood flow and pressure generating capability of the heart in HF and in healthy individuals (Bain et al., 1990; Cohen-Solal et al., 2002; Jakovljevic et al., 2012; Lang et al., 2009; Taylor, Olson, et al., 2013; Williams, Tzeng, et al., 2005). Non-traditional central hemodynamic indices appear to be sensitive to the integrative influence that hemodynamics share with pressure and loading of the central and peripheral circulation in HF, particularly during exercise.

Because less is known about the usefulness of certain techniques in HFpEF compared to HFrEF, the aim of the first project sought to examine specific non-invasive techniques for the quantification of cardiac hemodynamics during exercise in HFpEF. Observations from this project suggested that SV measured using ECHO, acetylene rebreathe, and O_2pulse methods could be adequately accomplished during peak exercise testing in HFpEF. Moreover, because the efficacy of the acetylene rebreathe technique is potentially dependent on the ability of acetylene gas to diffuse freely across the alveolar-capillary membrane barrier, and because HFpEF demonstrate low gas transfer capacity within lungs (Olson et al., 2008), we sought to study relationships between gas transfer capacity within lungs and acetylene rebreathe measurements of SV in these patients. We observed that there was no direct association between gas transfer capacity within lungs and acetylene rebreathe measurements of SV at peak exercise in HFpEF. These were important observations because of the information that can be garnered from exercise cardiac hemodynamics for the clinical classification of HF; and, because of the clinical implications that non-invasive techniques have in terms of offering widely available and reliable methods for cardiac hemodynamic assessment in both clinical and research settings.

The second and third projects in Chapter three of this dissertation focused in on the "Muscle Hypothesis" in HF (Piepoli, 1998; Piepoli et al., 2008; Piepoli et al., 1999). The "Muscle Hypothesis" suggests that peripheral neural factors are highly influential in contributing to abnormal ANS function and ventilatory and peripheral hemodynamic control and, hence, exercise intolerance in HF (Middlekauff et al., 2004; Olson et al.,

2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1999; Scott, Davies, et al., 2002). However, while the evidence is convincing that neural feedback from group III and IV sensory fibers could be responsible for ventilatory inefficiency and hypertension during exercise in HF, it is controversial whether this peripheral neural feedback pathway contributes to exacerbated central hemodynamic function during exercise in these patients (Amann et al., 2014; Ansorge et al., 2005; O'Leary et al., 2004).

We observed using two separate but previously used techniques for manipulating neural feedback from skeletal muscle ergoreceptors (i.e. RCO or intrathecal fentanyl) that the cardiac hemodynamic response to this peripheral neural mechanism was in contrast to previous observations of cardiovascular responses to skeletal muscle ergoreceptor activation in HF (Amann et al., 2011; Amann et al., 2014; Olson et al., 2014; Olson, Joyner, & Johnson, 2010; Piepoli et al., 1996; Piepoli et al., 2008; Scott, Francis, et al., 2003). As such, the observations from projects 3.2 and 3.3 suggest that sympathoexcitation associated with the activation of skeletal muscle metabo- or mechano-reflexes are not facilitatory in the cardiac hemodynamic responses observed during exercise in HF.

However, it is conceivable that the perceived lack of response of cardiac hemodynamics to ergoreceptor-mediated sympathoexcitation was not necessarily absent altogether. The ergoreceptor pathway may have influenced cardiac function at the onset of exercise and assisted in maintaining cardiac function throughout the remainder of exercise as well as during recovery in HF. The myocardium likely was still receiving ergoreceptor-mediated sympathetic drive, but because of cardiac impairment that is common to HF such as

failure of the Frank—Starling mechanism (attributable to a combination of cardiac β-adrenergic receptor dysfunction as well as ischemic/necrotic tissue), the heart was incapable of increasing pumping capability beyond its intrinsic reserve capacity irrespective of augmented cardiac adrenergic drive (Bristow et al., 1982; Bristow et al., 1986; Kitzman et al., 1991; O'Leary et al., 2004). Although it also suggested that energetics of the myocardium are inefficient and potentially influential in lowering the working capacity of the heart in HF (Eichhorn et al., 1990), there is no strong support to suggest that acute augmentation of adrenergic drive to the heart (e.g. via EPR or inotropic therapy) would lower cardiac pumping capability in patients (Maskin, Forman, Sonnenblick, Frishman, & LeJemtel, 1983; O'Leary et al., 2004). Lastly, although still unclear amongst scientists, it remains possible that metaboreceptor sensitivity is not blunted in HF (Li et al., 2004; Smith et al., 2003; Smith, Williams, et al., 2005); but, in contrast, metaboreceptors may be hyper-excitable and could explain the elevated CircP during normal recovery in HF patients in project 3.2 (Piepoli et al., 1996; Piepoli et al., 2008; Piepoli et al., 1999). Whereas, similarities in CircP during exercise with or without intrathecal fentanyl in project 3.3 likely occurred because of the influence of tonic sympathoexcitation that is native to HF. Thus, for reasons mentioned previously, blocking neural feedback from locomotor muscles likely had little influence on global cardiac adrenergic drive in HF, which is supported by observations of null differences in HR between exercise conditions as well.

Based on the pulmonary hemodynamic responses demonstrated by HF and healthy individuals in projects 3.2 and 3.3 we suggest that peripheral neural feedback from group

IV and perhaps also group III afferents are be instrumental in adjusting pulmonary hemodynamics during exercise in humans. However, despite HF and healthy individuals demonstrating adjustments in GX_{CAP}, it was unclear based on these observations what the functional purpose was of the ergoreceptor pathway potentially influencing increases in pulmonary pressures (e.g. mPAP). Although, increases in pulmonary pressures due to activated ergoreceptors could possibly be protective to pulmonary vasculature since these vessels are known to have high compliance in healthy individuals (e.g. ↓ vessel loading/distention) (Lykidis et al., 2008; White et al., 2013).

Lastly, the pulmonary hemodynamic response to exercise with intrathecal fentanyl as well as during post-exercise recovery with RCO suggests that there may be an important synergistic interaction between neural feedback from ergoreceptors with neural feedback from central chemoreceptors as suggested previously by observations in healthy individuals during ergoreceptor activation concurrent with hypercapnia (Bruce & White, 2012; Lykidis, Kumar, Vianna, White, & Balanos, 2010). Minor hypocapnia which may occur during exercise or RCO without clamping $P_{ET}CO_2$ could be enough to attenuate the activity of the central chemoreflex that in-turn could augment the influence of neural feedback from ergoreceptors on reflexes of the entire pulmonary system (i.e. ventilation and hemodynamics) (Keller et al., 2014; Olson, Joyner, & Johnson, 2010). This could help to support observations suggesting that the sensitivity of skeletal muscle metaboreceptors are blunted in HF and hence require an integrating influence from other mechanisms in order to contribute to abnormal cardiovascular responses (Sterns et al., 1991). Thus, given the anatomical and physiological relationship that terminating neural

feedback from chemoreceptors and ergoreceptors share within the NTS region of the medullary center this may well be a conceivable relationship (Donoghue, Felder, Jordan, & Spyer, 1984; Nattie & Li, 2002; Paton, Deuchars, Li, & Kasparov, 2001; Potts et al., 1999). The potential synergistic relationship between central chemoreceptors and muscle ergoreceptors is supported currently by the post-exercise recovery GX_{CAP} response during RCO+CO_2 (versus NR and RCO) as well as during exercise with intrathecal fentanyl in both HF and healthy individuals. Although the magnitude of influence that neural feedback from central chemoreceptors would appear less in healthy individuals versus HF, it is reasonable to suggest that even a slight elevation in the activity of central chemoreflex could have profound effects on the influence of neural feedback from ergoreceptors on pulmonary ventilatory and hemodynamic function in HF.

Chapter 5

Limitations

5.1 Introduction

The research encompassing dissertation requires highly technical methodology in addition to in-depth knowledge of unique factors related to human physiology and pathophysiology. Accordingly, during the course of research and interpretation of data a number of standard assumptions were made which may have influenced the outcomes of this dissertation. However, every attempt was made to minimize the influence of known and unknown confounding factors which could have had an impact on the results the three studies included in this dissertation. Nevertheless, several of the factors which could potentially limit the applicability and/or generalizability of the present findings may be related to the participants tested in these studies. For each of these studies we did not include in the experimental sample HF from all four NYHA class groups (i.e. NYHA I/II display no or limited symptomology during low- to- moderate levels of physical activity compared to NYHA III/IV patients who exhibit more severe signs and symptoms at rest and during exercise), nor did we include both HFrEF and HFpEF in each study. These shortcomings influence the generalizability of the present observations across all HF conditions and therefore future study designs should consider this limitation.

Other considerations that could have influenced the interpretability of the present findings are more specific to each study and/or methodology utilized to test study objectives. Still, despite potential limitations unique to each study, it was believed that

during inception of individual study designs and during execution of each protocol that all considerations were made in order to minimize imposing challenges to the interpretability of resulting observations.

5.2 Validity of non-traditional indices

The observations from studies which have tested non-traditional indices of central hemodynamics during exercise are encouraging that these metrics are indeed robust indicators of cardiac pumping capability (Bain et al., 1990; Bhella et al., 2011; Cohen-Solal et al., 2002; Jakovljevic et al., 2012; Lang et al., 2009; Williams, Tzeng, et al., 2005). However, despite promising findings in both healthy individuals and in HF, there is currently a limited body of evidence to support consistent agreement that these indices are the best descriptors of cardiac hemodynamics in HF. Therefore it is unclear if measurements such as CP or CircP are valid indicators of cardiac pump function in this population.

Pertinent to projects 3.2 and 3.3 in HFrEF, although it has been demonstrated that CircP could be an estimate for CP (and CP relates closely with Q) (Williams, Tzeng, et al., 2005), based on the availability of resources, we did not perform more direct assessments of cardiac hemodynamics such as Q, SV, or CP in these individuals. Therefore, it cannot be ascertained with certainty whether CircP in these studies was indeed descriptive of cardiac pumping capability in HF and in healthy individuals. However, the relative and absolute differences that we observed between HF and healthy individuals suggest that CircP is descriptive of cardiovascular function in these patients; and, perhaps cardiac pump function as well.

Apart from the single study from our group which tested the non-invasive measurement of PV_{CAP} (Taylor, Olson, et al., 2013), it is relatively unknown whether GX_{CAP} consistently serves as a robust surrogate estimate for PV_{CAP} in HF. However, if the basic assumptions of the factors underlying this measure are consistent (Baraka et al., 2004; Isserles & Breen, 1991; Maslow et al., 2001; Taylor, Olson, et al., 2013), it is reasonable to suggest that GX_{CAP} is indeed a good estimate PV_{CAP} at rest and during exercise. More testing of GX_{CAP} and PV_{CAP} is necessary to better understand the potential value of these measurements in describing the health of the heart–lung hemodynamic interaction during exercise in HF.

5.3 Central hemodynamic techniques

5.3.1 Echocardiography

Although it was demonstrated in the present dissertation and by others that ECHO can be used to measure SV at rest and during exercise in HF, accurate and precise image capture at rest can be challenging and these difficulties are markedly amplified during moderate-intensity bouts of exercise (Dittmann, Voelker, Karsch, & Seipel, 1987; Grothues et al., 2002; Lu et al., 1992; Marwick et al., 1992). Also, there is no commonly agreed upon protocol or technology (e.g. M-mode, 2-D, P-wave TDI, C-wave TDI, or 3-dimensional) and inconsistencies in the assessment of cardiac hemodynamics are persistent (Christie et al., 1987; Lewis et al., 1984; Marwick et al., 1992). Mismatches of image capture, heart views (e.g. PLAX versus A5C), and measurement (e.g. depth in relation to the aortic valve and influence of chest cavity size) commonly influence the calculation of the

product of CSA and VTI that results in a distortion of the SV jet (Christie et al., 1987; Lewis et al., 1984; Marwick et al., 1992).

Nevertheless, for studies in this dissertation, both the sonographer and ECHO reader had extensive experience (>10 years working with stress echocardiography data acquisition and interpretation) and were the same for all ECHO analyses. As such, it is suggested that experience and a high level of technical skill can overcome the proposed limitations influential to the efficacy of ECHO for SV measurement (Christie et al., 1987; Lewis et al., 1984; Marwick et al., 1992; Rowland et al., 1998).

5.3.2 Acetylene rebreathe

The use of acetylene rebreathe to assess cardiac hemodynamics during exercise has the potential for superiority versus ECHO because it is less user dependent and can be performed easily by the participant during high-levels of exercise. Still, this technique is not without limitations, which if not fully understood can influence the accuracy and reproducibility of the technique. The main concerns of this technique have to do with the homogenous mixing of acetylene gas within the lungs, the ability of acetylene gas to diffuse freely across the alveolar-capillary membrane barrier, and the potential presence of pulmonary shunt (e.g. exercise-independent interstitial pulmonary edema or obstructive disease) (Barbera et al., 2003; Friedman et al., 1984; Oelberg et al., 1998). These factors may have an impact on perfusion of acetylene gas into pulmonary arterial blood and thus lead to misrepresentation of the disappearance of acetylene gas. Also potentially influential to this technique would be the presence of exercise-related interstitial pulmonary edema which is suggested to occur in HFrEF (Agostoni et al.,

2003; Smith et al., 1999; Tolle et al., 2008). This occurrence could influence alveolar-capillary recruitment via abnormal V_C and D_M and hence prohibit the ability for acetylene gas to diffuse freely across the alveolar—capillary membrane barrier.

Nonetheless, the HFpEF patients in the present dissertation were without diagnosed pulmonary disease and demonstrated no signs which would indicate the presence of pulmonary shunt or exercise-related interstitial pulmonary edema. Moreover, it was also demonstrated in the present dissertation that gas transfer capacity within lungs influence acetylene rebreathe measurements of SV at peak exercise in HFpEF.

5.3.3 Oxygen pulse

As discussed, the accuracy of O_2pulse is highly dependent on the physiology of the cardiovascular system meeting specific assumptions at rest and during exercise. Most notably, based on the direct Fick equation, if there is considerable variability in $C_{a\text{-}v}O_2$ during steady state exercise then it is likely that VO_2 could not be assumed to be a direct estimate of Q. This is a potential concern in a certain subset of individuals including clinical patients (e.g. HF, pulmonary disease, mitral valve dysfunction, anemia, and skeletal muscle disorders) (Katz et al., 2000; Lewis & Haller, 1989; Lim et al., 2005; Oelberg et al., 1998; Taylor, Olson, et al., 2013), highly-trained endurance athletes (Beck et al., 2006; Bhambhani et al., 1994; Crisafulli, Piras, et al., 2007), or obese individuals (Lavie et al., 2004). However, no observations were made in the present studies which suggested that the relationships depicted in the direct Fick equation were violated in HF. Moreover, it was observed in the first project (3.1) of this dissertation that O_2pulse at peak exercise demonstrated close relationships with acetylene rebreathe measurements of

SV in HFpEF. Others before us and our group have also previously demonstrated that O_2pulse closely relates with invasive and non-invasive measurements of SV in healthy individuals and HF during exercise (Bhambhani et al., 1994; Crisafulli, Piras, et al., 2007; Lavie et al., 2004; Lim et al., 2005; Taylor, Olson, et al., 2013).

5.4 Manipulation of neural feedback from skeletal muscle afferents

The influence of skeletal muscle afferents on efferent sympathetic activity has been demonstrated repeatedly; however we did not directly assess MSNA nor did we measure blood or plasma catecholamine levels in participants. Nevertheless, it has been observed in numerous animal and human HF studies that both the metabo- and mechano-reflexes are associated with abnormal firing of these neural afferents which are closely associated with sympathoexcitation (Amann et al., 2014; Dibner-Dunlap & Thames, 1992; Negrao et al., 2001; Silber et al., 1998; Sterns et al., 1991).

5.4.1 Intrathecal fentanyl injection

The intrathecal fentanyl injection technique used in project 3.3 has been used previously by our group and others in HF and in healthy individuals (Amann et al., 2010; Amann et al., 2008; Amann et al., 2009; Amann et al., 2014; Olson et al., 2014). Therefore, assumptions were made in the present study based on observations of others.

Multiple methods have been used to block neural feedback from skeletal muscle including opiate narcotics and Caine anesthetics (Amann et al., 2010, 2011; Amann et al., 2009; Besse et al., 1991; Meintjes et al., 1995; Schadt, McKown, McKown, & Franklin, 1984). However, Caine anesthetics have been shown to reduce neuromuscular facilitation

whereas opiates do not appear to have an impact of efferent neuromuscular control (Amann et al., 2010; Amann et al., 2008; Amann et al., 2009). Thus, we suggest that intrathecal fentanyl (μ-opioid agonist) blocked group III and IV neural feedback to the brainstem without influencing central motor command associated with skeletal muscle force production.

5.4.2 Regional circulatory occlusion

The RCO technique used in project 3.3 has been used in HF and in healthy individuals during and immediately following exercise in these individuals (Crisafulli et al., 2011; Crisafulli et al., 2006; Olson, Joyner, & Johnson, 2010). Moreover, the RCO technique has been tested for validity in HF and in healthy individuals and it is suggested to adequately stimulate the metaboreflex in humans (Scott, Francis, et al., 2003). However, even when using RCO during static recovery in humans, it remains unclear to what magnitude that metabolite-specific factors stimulate metaboreceptors versus mechanoreceptors. Also unclear, despite the identification of numerous metabolites associated with sensitization of metaboreceptors (e.g. H^+, pH, capsaicin, bradykinin, prostaglandins, K^+, lactic acid, or arachidonic acid) (Clark et al., 1995; Hanna et al., 2002; Kaufman et al., 1982; Kaufman et al., 1983; Kaufman & Rybicki, 1987; Kaufman, Rybicki, et al., 1984; Li & Sinoway, 2002; McCord et al., 2009; Piepoli et al., 1995; Rotto et al., 1989; Rybicki et al., 1984; Scott, Wensel, et al., 2003; Scott, Wensel, et al., 2002; Stebbins et al., 1986; Victor et al., 1988), which metabolites are directly responsible for stimulating the metaboreflex in humans. Accordingly, in the present study, we did not study specific metabolic factors that could have contributed to the

stimulation of metaboreceptors nor did we quantify the activity of group III versus IV afferents in these individuals. Nevertheless, based on observations from our group and others previously (Crisafulli et al., 2011; Crisafulli et al., 2006; Negrao et al., 2001; Olson, Joyner, & Johnson, 2010; Scott, Wensel, et al., 2003; Sterns et al., 1991), the present observations indicate that it was primarily the metaboreflex that was activated during post-exercise recovery with RCO.

Chapter 6

Future directions and concluding remarks

Future directions

The observations from the projects included in this dissertation although providing useful information regarding factors pertaining to the importance of central hemodynamic evaluation during exercise in HF, also indicate that there is clearly more opportunity to expand our understanding of this area of study. It would appear critical to proceed with continued testing of non-traditional indices of central hemodynamics using non-invasive techniques during exercise in HFrEF and HFpEF. Further, it would be useful in strengthen our understanding of the value of hydraulic power measurements their impact on converting venous return into hydraulic and subsequently mechanical power in HF. Closely related to this would be to use this additional information to better understand relationships between cardiac hemodynamics and pulmonary vascular hemodynamics, particularly during exercise in HF. The concept of PV_{CAP} has demonstrated usefulness in the clinical evaluation of PH (Mahapatra, Nishimura, Oh, et al., 2006; Mahapatra, Nishimura, Sorajja, et al., 2006), and based on the findings from this dissertation and observations from our group previously it would appear that non-invasive measurement of PV_{CAP} may demonstrate value in HF (Taylor, Olson, et al., 2013). Accordingly, for the highest impact, it is important that the next direction for the concepts outlined and studied in this dissertation be aimed at the clinical and risk stratification (i.e. morbidity and mortality) of a large sample of HFrEF and HFpEF (NYHA class I-IV). This information,

although critical for all HF, could be particularly valuable in HFpEF since less is known about the relationships between central hemodynamics and mortality risk in this population.

Our observations from projects 3.2 and 3.3 provided thought provoking ideas for mechanistic studies which could potentially help us and others to better understand relationships between neural feedback from skeletal muscle ergoreceptors and central hemodynamics during exercise in HF. We suggest that in future studies using the techniques used currently that additional conditions of exercise and recovery be added to potentially elucidate influences of group III versus IV neural feedback as well as potential influences from neural feedback from chemoreceptors on central hemodynamics in HF.

Additional study conditions which might help to address questions left by the present observations would be to include separate clamped conditions of normoxia and hypercapnia, exercise with and without concurrent RCO of contractile-active musculature, and intra-arterial injection of sodium bicarbonate or saline during exercise with intrathecal fentanyl. In addition to these added factors to the current ergoreceptor specific techniques, hemodynamic assessment could potentially be improved by adding ECHO in order to non-invasively but directly estimate pulmonary hemodynamics and pressures. Lastly, it could be valuable to have participants perform exercise bouts of constant-load as well as progressive loading under the above conditions.

The above added study conditions could strengthen our understanding of the impact of afferent neural feedback on central hemodynamics in HF. First, clamping $P_{ET}CO_2$ in

normoxic and hypercapnic exercise conditions would help to address the potential influence of the central chemoreflex on the strength that the ergoreflex could possibly have on pulmonary hemodynamics. Second, with RCO during exercise concurrent with hypercapnia or normoxia, because of the possible augmentation of the metaboreflex in the presence of the chemoreflex, this could potentially help to quantify the contribution of mechanical versus metabolic specific neural feedback on pulmonary function (i.e. hemodynamic and ventilatory). Third, intra-arterial injection of sodium bicarbonate (because it is a buffer and lowers H^+) versus saline could help to better understand the role of H^+ in sensitizing ergoreceptors. Fourth, it is possible that we might get a better understanding of whether the sensitivity of metaboreceptors are indeed heightened in HF, or whether the influence of the central chemoreflex charges neural feedback from metaboreceptors upon integration within the medulla at the NTS. Fifth, having participants perform a progressive exercise test under these conditions could allow us to examine hemodynamic responses at anaerobic threshold, which with blood sampling could improve our understanding of the association of certain metabolites (e.g. lactic acid and H^+) and the EPR in HF. Lastly, if these conditions persisted into post-exercise recovery, with the elimination of the mechanoreflex and central command, it is possible for further clarification of the possible relationship between the metaboreflex and the central chemoreflex on pulmonary hemodynamics.

Concluding remarks

In summary, observations from the projects in this dissertation suggest that non-invasive assessment of central hemodynamics during exercise in HF is feasible yet also adequate

to provide additional value to the process of the clinical evaluation of these patients. It could also be suggested now more than ever before that the common assessment of central hemodynamic function using Q, SV, and mPAP should be complemented with non-traditional measurements of central hemodynamics including CP, CircP, or GX_{CAP} which can be assessed non-invasively while also potentially being of high prognostic value in HF. Finally, it is also apparent that although possibly influential in contributing to abnormal pulmonary hemodynamics and exercise intolerance via pathways of ventilatory inefficiency and hypertension in HF, the aberrant metabo- and mechano-reflex pathways that HF patients generally demonstrate do not appear to readily influence adjustments in cardiac hemodynamics associated with exercise in HFrEF.

Bibliography

Abramson, S. V., Burke, J. F., Kelly, J. J., Jr., Kitchen, J. G., 3rd, Dougherty, M. J., Yih, D. F., . . . Phiambolis, T. P. (1992). Pulmonary hypertension predicts mortality and morbidity in patients with dilated cardiomyopathy. *Ann Intern Med, 116*(11), 888-895.

Adreani, C. M., & Kaufman, M. P. (1998). Effect of arterial occlusion on responses of group III and IV afferents to dynamic exercise. *J Appl Physiol (1985), 84*(6), 1827-1833.

Agostoni, P., Cattadori, G., Bianchi, M., & Wasserman, K. (2003). Exercise-induced pulmonary edema in heart failure. *Circulation, 108*(21), 2666-2671. doi: 10.1161/01.CIR.0000097115.61309.59

Alam, M., & Smirk, F. (1937). Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *J Physiol, 89*(4), 372-383.

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2010). Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans. *J Appl Physiol (1985), 109*(4), 966-976. doi: 10.1152/japplphysiol.00462.2010

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2011). Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans. *J Physiol, 589*(Pt 21), 5299-5309. doi: 10.1113/jphysiol.2011.213769

Amann, M., Proctor, L. T., Sebranek, J. J., Eldridge, M. W., Pegelow, D. F., & Dempsey, J. A. (2008). Somatosensory feedback from the limbs exerts inhibitory influences on central neural drive during whole body endurance exercise. *J Appl Physiol (1985), 105*(6), 1714-1724. doi: 10.1152/japplphysiol.90456.2008

Amann, M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2009). Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J Physiol, 587*(Pt 1), 271-283. doi: 10.1113/jphysiol.2008.163303

Amann, M., Venturelli, M., Ives, S. J., Morgan, D. E., Gmelch, B., Witman, M. A., . . . Richardson, R. S. (2014). Group III/IV muscle afferents impair limb blood in patients with chronic heart failure. *Int J Cardiol, 174*(2), 368-375. doi: 10.1016/j.ijcard.2014.04.157

Ansorge, E. J., Augustyniak, R. A., Perinot, M. L., Hammond, R. L., Kim, J. K., Sala-Mercado, J. A., . . . O'Leary, D. S. (2005). Altered muscle metaboreflex control of coronary blood flow and ventricular function in heart failure. *Am J Physiol Heart Circ Physiol, 288*(3), H1381-1388. doi: 10.1152/ajpheart.00985.2004

Aronson, D., Eitan, A., Dragu, R., & Burger, A. J. (2011). Relationship between reactive pulmonary hypertension and mortality in patients with acute decompensated heart failure. *Circ Heart Fail, 4*(5), 644-650. doi: 10.1161/CIRCHEARTFAILURE.110.960864

Asmussen, E., & Nielsen, M. (1964). Experiments on Nervous Factors Controlling Respiration and Circulation during Exercise Employing Blocking of the Blood

Flow. *Acta Physiol Scand, 60*(1-2), 103-111. doi: 10.1111/j.1748-1716.1964.tb02873.x

Bain, R. J., Tan, L. B., Murray, R. G., Davies, M. K., & Littler, W. A. (1990). The correlation of cardiac power output to exercise capacity in chronic heart failure. *Eur J Appl Physiol Occup Physiol, 61*(1-2), 112-118.

Banchero, N., Sime, F., Peñaloza, D., Cruz, J., Gamboa, R., & Marticorena, E. (1966). Pulmonary pressure, cardiac output, and arterial oxygen saturation during exercise at high altitude and at sea level. *Circulation, 33*(2), 249-262.

Baraka, A. S., Aouad, M. T., Jalbout, M. I., Kaddoum, R. N., Khatib, M. F., & Haroun-Bizri, S. T. (2004). End-tidal CO2 for prediction of cardiac output following weaning from cardiopulmonary bypass. *J Extra Corpor Technol, 36*(3), 255-257.

Barbera, J. A., Peinado, V. I., & Santos, S. (2003). Pulmonary hypertension in chronic obstructive pulmonary disease. *Eur Respir J, 21*(5), 892-905.

Batman, B. A., Hardy, J. C., Leuenberger, U. A., Smith, M. B., Yang, Q. X., & Sinoway, L. I. (1994). Sympathetic nerve activity during prolonged rhythmic forearm exercise. *J Appl Physiol (1985), 76*(3), 1077-1081.

Beck, K. C., Randolph, L. N., Bailey, K. R., Wood, C. M., Snyder, E. M., & Johnson, B. D. (2006). Relationship between cardiac output and oxygen consumption during upright cycle exercise in healthy humans. *Journal of Applied Physiology, 101*(5), 1474-1480.

Becklake, M. R., Frank, H., Dagenais, G. R., Ostiguy, G. L., & Guzman, C. A. (1965). Influence of age and sex on exercise cardiac output. *J Appl Physiol, 20*(5), 938-947.

Benge, W., Litchfield, R. L., & Marcus, M. L. (1980). Exercise capacity in patients with severe left ventricular dysfunction. *Circulation, 61*(5), 955-959.

Besse, D., Lombard, M. C., & Besson, J. M. (1991). Autoradiographic distribution of mu, delta and kappa opioid binding sites in the superficial dorsal horn, over the rostrocaudal axis of the rat spinal cord. *Brain Res, 548*(1-2), 287-291.

Besse, D., Lombard, M. C., Zajac, J. M., Roques, B. P., & Besson, J. M. (1990). Pre- and postsynaptic distribution of mu, delta and kappa opioid receptors in the superficial layers of the cervical dorsal horn of the rat spinal cord. *Brain Res, 521*(1-2), 15-22.

Bhambhani, Y., Norris, S., & Bell, G. (1994). Prediction of stroke volume from oxygen pulse measurements in untrained and trained men. *Can J Appl Physiol, 19*(1), 49-59.

Bhatia, R. S., Tu, J. V., Lee, D. S., Austin, P. C., Fang, J., Haouzi, A., . . . Liu, P. P. (2006). Outcome of heart failure with preserved ejection fraction in a population-based study. *N Engl J Med, 355*(3), 260-269. doi: 10.1056/NEJMoa051530

Bhella, P. S., Prasad, A., Heinicke, K., Hastings, J. L., Arbab-Zadeh, A., Adams-Huet, B., . . . Levine, B. D. (2011). Abnormal haemodynamic response to exercise in heart failure with preserved ejection fraction. *Eur J Heart Fail, 13*(12), 1296-1304. doi: 10.1093/eurjhf/hfr133

Borlaug, B. A., Melenovsky, V., Russell, S. D., Kessler, K., Pacak, K., Becker, L. C., & Kass, D. A. (2006). Impaired chronotropic and vasodilator reserves limit exercise

capacity in patients with heart failure and a preserved ejection fraction. *Circulation, 114*(20), 2138-2147. doi: 10.1161/CIRCULATIONAHA.106.632745

Borlaug, B. A., Nishimura, R. A., Sorajja, P., Lam, C. S., & Redfield, M. M. (2010). Exercise hemodynamics enhance diagnosis of early heart failure with preserved ejection fraction. *Circ Heart Fail, 3*(5), 588-595. doi: 10.1161/CIRCHEARTFAILURE.109.930701

Borlaug, B. A., Olson, T. P., Lam, C. S., Flood, K. S., Lerman, A., Johnson, B. D., & Redfield, M. M. (2010). Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction. *J Am Coll Cardiol, 56*(11), 845-854. doi: 10.1016/j.jacc.2010.03.077

Brandfornbrener, M., Landowne, M., & Shock, N. W. (1955). Changes in cardiac output with age. *Circulation, 12*(4), 557-566.

Braunwald, E., & Ross, J., Jr. (1963). The ventricular end-diastolic pressure. Appraisal of its value in the recognition of ventricular failure in man. *Am J Med, 34*, 147-150.

Braunwald, E., Ross, J., Jr., Gault, J. H., Mason, D. T., Mills, C., Gabe, I. T., & Epstein, S. E. (1969). NIH clinical staff conference. Assessment of cardiac function. *Ann Intern Med, 70*(2), 369-399.

Bristow, M. R., Ginsburg, R., Minobe, W., Cubicciotti, R. S., Sageman, W. S., Lurie, K., . . . Stinson, E. B. (1982). Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N Engl J Med, 307*(4), 205-211. doi: 10.1056/NEJM198207223070401

Bristow, M. R., Ginsburg, R., Umans, V., Fowler, M., Minobe, W., Rasmussen, R., . . . Jamieson, S. (1986). Beta 1-and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. *Circulation research, 59*(3), 297-309.

Bristow, M. R., Hershberger, R. E., Port, J. D., Gilbert, E. M., Sandoval, A., Rasmussen, R., . . . Feldman, A. M. (1990). Beta-adrenergic pathways in nonfailing and failing human ventricular myocardium. *Circulation, 82*(2 Suppl), I12-25.

Broek, v. d., van Veldhuisen, D. J., de Graeff, P. A., Landsman, M. L., Hillege, H., & Lie, K. I. (1992). Comparison between New York Heart Association classification and peak oxygen consumption in the assessment of functional status and prognosis in patients with mild to moderate chronic congestive heart failure secondary to either ischemic or idiopathic dilated cardiomyopathy. *The American journal of cardiology, 70*(3), 359-363.

Bromley, P. D., Hodges, L. D., & Brodie, D. A. (2006). Physiological range of peak cardiac power output in healthy adults. *Clin Physiol Funct Imaging, 26*(4), 240-246. doi: 10.1111/j.1475-097X.2006.00678.x

Bruce, R. M., & White, M. J. (2012). Muscle afferent activation causes ventilatory and cardiovascular responses during concurrent hypercapnia in humans. *Exp Physiol, 97*(2), 208-218. doi: 10.1113/expphysiol.2011.061606

Buller, N. P., & Poole-Wilson, P. A. (1990). Mechanism of the increased ventilatory response to exercise in patients with chronic heart failure. *Br Heart J, 63*(5), 281-283.

Butler, J., Chomsky, D. B., & Wilson, J. R. (1999). Pulmonary hypertension and exercise intolerance in patients with heart failure. *J Am Coll Cardiol, 34*(6), 1802-1806.

Cander, L. (1959). Solubility of inert gases in human lung tissue. *J Appl Physiol, 14*(4), 538-540.

Carlsson, M., Andersson, R., Bloch, K. M., Steding-Ehrenborg, K., Mosen, H., Stahlberg, F., . . . Arheden, H. (2012). Cardiac output and cardiac index measured with cardiovascular magnetic resonance in healthy subjects, elite athletes and patients with congestive heart failure. *J Cardiovasc Magn Reson, 14*, 51. doi: 10.1186/1532-429X-14-51

Chomsky, D. B., Lang, C. C., Rayos, G. H., Shyr, Y., Yeoh, T. K., Pierson, R. N., 3rd, . . . Wilson, J. R. (1996). Hemodynamic exercise testing. A valuable tool in the selection of cardiac transplantation candidates. *Circulation, 94*(12), 3176-3183.

Christie, J., Sheldahl, L. M., Tristani, F. E., Sagar, K. B., Ptacin, M. J., & Wann, S. (1987). Determination of stroke volume and cardiac output during exercise: comparison of two-dimensional and Doppler echocardiography, Fick oximetry, and thermodilution. *Circulation, 76*(3), 539-547.

Chua, T. P., Clark, A. I., Amadi, A. A., & Coats, A. J. (1996). Relation between chemosensitivity and the ventilatory response to exercise in chronic heart failure. *Journal of the American College of Cardiology, 27*(3), 650-657.

Chua, T. P., Ponikowski, P., Harrington, D., Anker, S. D., Webb-Peploe, K., Clark, A. L., . . . Coats, A. J. (1997). Clinical correlates and prognostic significance of the ventilatory response to exercise in chronic heart failure. *J Am Coll Cardiol, 29*(7), 1585-1590.

Ciriello, J., & Calaresu, F. R. (1977). Lateral reticular nucleus: a site of somatic and cardiovascular integration in the cat. *Am J Physiol, 233*(3), R100-109.

Clark, A. L., Piepoli, M., & Coats, A. J. (1995). Skeletal muscle and the control of ventilation on exercise: evidence for metabolic receptors. *Eur J Clin Invest, 25*(5), 299-305.

Clark, A. L., Poole-Wilson, P. A., & Coats, A. J. (1996). Exercise limitation in chronic heart failure: central role of the periphery. *J Am Coll Cardiol, 28*(5), 1092-1102. doi: 10.1016/S0735-1097(96)00323-3

Cleland, J. G., Dargie, H. J., & Ford, I. (1987). Mortality in heart failure: clinical variables of prognostic value. *Br Heart J, 58*(6), 572-582.

Coats, A. J., Clark, A. L., Piepoli, M., Volterrani, M., & Poole-Wilson, P. A. (1994). Symptoms and quality of life in heart failure: the muscle hypothesis. *Br Heart J, 72*(2 Suppl), S36-39.

Cohen-Solal, A., Barnier, P., Pessione, F., Seknadji, P., Logeart, D., Laperche, T., & Gourgon, R. (1997). Comparison of the long-term prognostic value of peak exercise oxygen pulse and peak oxygen uptake in patients with chronic heart failure. *Heart, 78*(6), 572-576.

Cohen-Solal, A., Tabet, J. Y., Logeart, D., Bourgoin, P., Tokmakova, M., & Dahan, M. (2002). A non-invasively determined surrogate of cardiac power ('circulatory power') at peak exercise is a powerful prognostic factor in chronic heart failure. *Eur Heart J, 23*(10), 806-814. doi: 10.1053/euhj.2001.2966

Cohn, J. N., Johnson, G. R., Shabetai, R., Loeb, H., Tristani, F., Rector, T., . . . Fletcher, R. (1993). Ejection fraction, peak exercise oxygen consumption, cardiothoracic ratio, ventricular arrhythmias, and plasma norepinephrine as determinants of prognosis in heart failure. The V-HeFT VA Cooperative Studies Group. *Circulation, 87*(6 Suppl), VI5-16.

Cohn, J. N., & Rector, T. S. (1988). Prognosis of congestive heart failure and predictors of mortality. *Am J Cardiol, 62*(2), 25A-30A.

Coleridge, J. C., & Kidd, C. (1963). Reflex effects of stimulating baroreceptors in the pulmonary artery. *J Physiol, 166*(1), 197-210.

Collis, T., Devereux, R. B., Roman, M. J., de Simone, G., Yeh, J., Howard, B. V., . . . Welty, T. K. (2001). Relations of stroke volume and cardiac output to body composition: the strong heart study. *Circulation, 103*(6), 820-825.

Cooke, G. A., Marshall, P., al-Timman, J. K., Wright, D. J., Riley, R., Hainsworth, R., & Tan, L. B. (1998). Physiological cardiac reserve: development of a non-invasive method and first estimates in man. *Heart, 79*(3), 289-294.

Coote, J. H., Hilton, S. M., & Perez-Gonzalez, J. F. (1971). The reflex nature of the pressor response to muscular exercise. *J Physiol, 215*(3), 789-804.

Coote, J. H., & Perez-Gonzalez, J. F. (1970). The response of some sympathetic neurones to volleys in various afferent nerves. *J Physiol, 208*(2), 261-278.

Corra, U., Mezzani, A., Bosimini, E., Scapellato, F., Imparato, A., & Giannuzzi, P. (2002). Ventilatory response to exercise improves risk stratification in patients with chronic heart failure and intermediate functional capacity. *Am Heart J, 143*(3), 418-426.

Coutsos, M., Sala-Mercado, J. A., Ichinose, M., Li, Z., Dawe, E. J., & O'Leary, D. S. (2013). Muscle metaboreflex-induced coronary vasoconstriction limits ventricular contractility during dynamic exercise in heart failure. *Am J Physiol Heart Circ Physiol, 304*(7), H1029-1037. doi: 10.1152/ajpheart.00879.2012

Crisafulli, A., Piras, F., Chiappori, P., Vitelli, S., Caria, M. A., Lobina, A., . . . Melis, F. (2007). Estimating stroke volume from oxygen pulse during exercise. *Physiol Meas, 28*(10), 1201-1212. doi: 10.1088/0967-3334/28/10/006

Crisafulli, A., Piras, F., Filippi, M., Piredda, C., Chiappori, P., Melis, F., . . . Concu, A. (2011). Role of heart rate and stroke volume during muscle metaboreflex-induced cardiac output increase: differences between activation during and after exercise. *The Journal of Physiological Sciences, 61*(5), 385-394.

Crisafulli, A., Salis, E., Pittau, G., Lorrai, L., Tocco, F., Melis, F., . . . Concu, A. (2006). Modulation of cardiac contractility by muscle metaboreflex following efforts of different intensities in humans. *Am J Physiol Heart Circ Physiol, 291*(6), H3035-3042. doi: 10.1152/ajpheart.00221.2006

Crisafulli, A., Salis, E., Tocco, F., Melis, F., Milia, R., Pittau, G., . . . Concu, A. (2007). Impaired central hemodynamic response and exaggerated vasoconstriction during muscle metaboreflex activation in heart failure patients. *Am J Physiol Heart Circ Physiol, 292*(6), H2988-2996. doi: 10.1152/ajpheart.00008.2007

Crisafulli, A., Scott, A. C., Wensel, R., Davos, C. H., Francis, D. P., Pagliaro, P., . . . Piepoli, M. F. (2003). Muscle metaboreflex-induced increases in stroke volume.

Med Sci Sports Exerc, 35(2), 221-228; discussion 229. doi: 10.1249/01.MSS.0000048639.02548.24

Daley, J. C., 3rd, Khan, M. H., Hogeman, C. S., & Sinoway, L. I. (2003). Autonomic and vascular responses to reduced limb perfusion. *J Appl Physiol (1985), 95*(4), 1493-1498. doi: 10.1152/japplphysiol.00344.2002

Damato, A. N., Galante, J. G., & Smith, W. M. (1966). Hemodynamic response to treadmill exercise in normal subjects. *J Appl Physiol, 21*(3), 959-966.

de Simone, G., Devereux, R. B., Daniels, S. R., Mureddu, G., Roman, M. J., Kimball, T. R., . . . Contaldo, F. (1997). Stroke volume and cardiac output in normotensive children and adults. Assessment of relations with body size and impact of overweight. *Circulation, 95*(7), 1837-1843.

Dexter, L., Whittenberger, J. L., Haynes, F. W., Goodale, W. T., Gorlin, R., & Sawyer, C. G. (1951). Effect of exercise on circulatory dynamics of normal individuals. *J Appl Physiol, 3*(8), 439-453.

Dibner-Dunlap, M. E., & Thames, M. (1992). Control of sympathetic nerve activity by vagal mechanoreflexes is blunted in heart failure. *Circulation, 86*, 1929-1929.

Dickstein, K., Cohen-Solal, A., Filippatos, G., McMurray, J. J., Ponikowski, P., Poole-Wilson, P. A., . . . Guidelines, E. S. C. C. f. P. (2008). ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the diagnosis and treatment of acute and chronic heart failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur J Heart Fail, 10*(10), 933-989. doi: 10.1016/j.ejheart.2008.08.005

Dittmann, H., Voelker, W., Karsch, K. R., & Seipel, L. (1987). Influence of sampling site and flow area on cardiac output measurements by Doppler echocardiography. *J Am Coll Cardiol, 10*(4), 818-823.

Domanski, M. J., Mitchell, G. F., Norman, J. E., Exner, D. V., Pitt, B., & Pfeffer, M. A. (1999). Independent prognostic information provided by sphygmomanometrically determined pulse pressure and mean arterial pressure in patients with left ventricular dysfunction. *J Am Coll Cardiol, 33*(4), 951-958.

Donoghue, S., Felder, R. B., Jordan, D., & Spyer, K. M. (1984). The central projections of carotid baroreceptors and chemoreceptors in the cat: a neurophysiological study. *J Physiol, 347*, 397-409.

Edwards, P. D., Bull, R. K., & Coulden, R. (1998). CT measurement of main pulmonary artery diameter. *Br J Radiol, 71*(850), 1018-1020. doi: 10.1259/bjr.71.850.10211060

Eichhorn, E. J., Bedotto, J. B., Malloy, C. R., Hatfield, B. A., Deitchman, D., Brown, M., . . . Grayburn, P. A. (1990). Effect of beta-adrenergic blockade on myocardial function and energetics in congestive heart failure. Improvements in hemodynamic, contractile, and diastolic performance with bucindolol. *Circulation, 82*(2), 473-483.

Eisenhofer, G., Friberg, P., Rundqvist, B., Quyyumi, A. A., Lambert, G., Kaye, D. M., . . . Esler, M. D. (1996). Cardiac sympathetic nerve function in congestive heart failure. *Circulation, 93*(9), 1667-1676.

Eldridge, M. W., Braun, R. K., Yoneda, K. Y., & Walby, W. F. (2006). Effects of altitude and exercise on pulmonary capillary integrity: evidence for subclinical high-altitude pulmonary edema. *J Appl Physiol (1985), 100*(3), 972-980. doi: 10.1152/japplphysiol.01048.2005

Eldridge, M. W., Podolsky, A., Richardson, R. S., Johnson, D. H., Knight, D. R., Johnson, E. C., . . . Severinghaus, J. W. (1996). Pulmonary hemodynamic response to exercise in subjects with prior high-altitude pulmonary edema. *J Appl Physiol (1985), 81*(2), 911-921.

Epstein, S. E., Beiser, G. D., Stampfer, M., Robinson, B. F., & Braunwald, E. (1967). Characterization of the circulatory response to maximal upright exercise in normal subjects and patients with heart disease. *Circulation, 35*(6), 1049-1062.

Fick, A. (1870). Uber die Messung des Blutquantums in den Herzventrikel. *Phys Med Ges, 2*(XVI).

Fisher, J. P., Seifert, T., Hartwich, D., Young, C. N., Secher, N. H., & Fadel, P. J. (2010). Autonomic control of heart rate by metabolically sensitive skeletal muscle afferents in humans. *J Physiol, 588*(Pt 7), 1117-1127. doi: 10.1113/jphysiol.2009.185470

Fishman, A. P., Fritts, H. W., Jr., & Cournand, A. (1960). Effects of acute hypoxia and exercise on the pulmonary circulation. *Circulation, 22*, 204-215.

Floras, J. S. (1993). Clinical aspects of sympathetic activation and parasympathetic withdrawal in heart failure. *J Am Coll Cardiol, 22*(4 Suppl A), 72A-84A.

Forman, D. E., Butler, J., Wang, Y., Abraham, W. T., O'Connor, C. M., Gottlieb, S. S., . . . Stevenson, L. W. (2004). Incidence, predictors at admission, and impact of worsening renal function among patients hospitalized with heart failure. *J Am Coll Cardiol, 43*(1), 61-67.

Fox, K., Cowie, M., Wood, D., Coats, A., Gibbs, J., Underwood, S., . . . Sutton, G. (2001). Coronary artery disease as the cause of incident heart failure in the population. *Eur Heart J, 22*(3), 228-236.

Franciosa, J. A., Baker, B. J., & Seth, L. (1985). Pulmonary versus systemic hemodynamics in determining exercise capacity of patients with chronic left ventricular failure. *Am Heart J, 110*(4), 807-813.

Franciosa, J. A., Leddy, C. L., Wilen, M., & Schwartz, D. E. (1984). Relation between hemodynamic and ventilatory responses in determining exercise capacity in severe congestive heart failure. *Am J Cardiol, 53*(1), 127-134.

Franciosa, J. A., Park, M., & Barry Levine, T. (1981). Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. *The American journal of cardiology, 47*(1), 33-39.

Francis, D. P., Shamim, W., Davies, L. C., Piepoli, M. F., Ponikowski, P., Anker, S. D., & Coats, A. J. (2000). Cardiopulmonary exercise testing for prognosis in chronic heart failure: continuous and independent prognostic value from VE/VCO2slope and peak VO2. *Eur Heart J, 21*(2), 154-161.

Francis, G. S., Goldsmith, S. R., & Cohn, J. N. (1982). Relationship of exercise capacity to resting left ventricular performance and basal plasma norepinephrine levels in patients with congestive heart failure. *Am Heart J, 104*(4 Pt 1), 725-731.

Frank, O. (1895). *Zur Dynamik des Herzmuskels*: Druck v. R. Oldenbourg.

Freedson, P. S. (1981). The influence of hemoglobin concentration on exercise cardiac output. *Int J Sports Med, 2*(2), 81-86. doi: 10.1055/s-2008-1034587

Freund, P. R., Rowell, L. B., Murphy, T. M., Hobbs, S. F., & Butler, S. H. (1979). Blockade of the pressor response to muscle ischemia by sensory nerve block in man. *Am J Physiol, 237*(4), H433-439.

Friedberg, M. K., Feinstein, J. A., & Rosenthal, D. N. (2007). Noninvasive assessment of pulmonary arterial capacitance by echocardiography. *J Am Soc Echocardiogr, 20*(2), 186-190. doi: 10.1016/j.echo.2006.08.009

Friedman, M., Wilkins, S. A., Jr., Rothfeld, A. F., & Bromberg, P. A. (1984). Effect of ventilation and perfusion imbalance on inert gas rebreathing variables. *J Appl Physiol Respir Environ Exerc Physiol, 56*(2), 364-369.

Galie, N., Hoeper, M. M., Humbert, M., Torbicki, A., Vachiery, J. L., Barbera, J. A., . . . Guidelines, E. S. C. C. f. P. (2009). Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J, 30*(20), 2493-2537. doi: 10.1093/eurheartj/ehp297

Gelberg, H. J., Rubin, S. A., Ports, T. A., Brundage, B. H., Parmley, W. W., & Chatterjee, K. (1979). Detection of left ventricular functional reserve by supine exercise hemodynamics in patients with severe, chronic heart failure. *Am J Cardiol, 44*(6), 1062-1067.

Ghio, S., Gavazzi, A., Campana, C., Inserra, C., Klersy, C., Sebastiani, R., . . . Tavazzi, L. (2001). Independent and additive prognostic value of right ventricular systolic function and pulmonary artery pressure in patients with chronic heart failure. *J Am Coll Cardiol, 37*(1), 183-188.

Gladwell, V., & Coote, J. (2002). Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: a role for mechanoreceptors. *J Physiol, 540*(3), 1095-1102.

Gleason, W. L., & Braunwald, E. (1962). Studies on Starling's Law of the Heart VI. Relationships between Left Ventricular End-Diastolic Volume and Stroke Volume in Man with Observations on the Mechanism of Pulsus Alternans. *Circulation, 25*(5), 841-848.

Griffin, B. P., Shah, P. K., Ferguson, J., & Rubin, S. A. (1991). Incremental prognostic value of exercise hemodynamic variables in chronic congestive heart failure secondary to coronary artery disease or to dilated cardiomyopathy. *Am J Cardiol, 67*(9), 848-853.

Grollman, A. (1929). The determination of the cardiac output of man by the use of acetylene. *Am. J. Physiol, 88*, 432-445.

Grothues, F., Smith, G. C., Moon, J. C., Bellenger, N. G., Collins, P., Klein, H. U., & Pennell, D. J. (2002). Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *Am J Cardiol, 90*(1), 29-34.

Guazzi, M., Reina, G., Tumminello, G., & Guazzi, M. D. (2005). Exercise ventilation inefficiency and cardiovascular mortality in heart failure: the critical independent prognostic value of the arterial CO2 partial pressure. *Eur Heart J, 26*(5), 472-480. doi: 10.1093/eurheartj/ehi060

Guo, A., Vulchanova, L., Wang, J., Li, X., & Elde, R. (1999). Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. *Eur J Neurosci, 11*(3), 946-958.

Guyton, A. C., Armstrong, G. G., & Chipley, P. L. (1956). Pressure-volume curves of the arterial and venous systems in live dogs. *Am J Physiol, 184*(2), 253-258.

Guyton, A. C., Polizo, D., & Armstrong, G. G. (1954). Mean circulatory filling pressure measured immediately after cessation of heart pumping. *Am J Physiol, 179*(2), 261-267.

Hagberg, J. M., Allen, W. K., Seals, D. R., Hurley, B. F., Ehsani, A. A., & Holloszy, J. O. (1985). A hemodynamic comparison of young and older endurance athletes during exercise. *J Appl Physiol (1985), 58*(6), 2041-2046.

Haldeman, G. A., Croft, J. B., Giles, W. H., & Rashidee, A. (1999). Hospitalization of patients with heart failure: National Hospital Discharge Survey, 1985 to 1995. *Am Heart J, 137*(2), 352-360. doi: 10.1053/hj.1999.v137.95495

Hammond, R. L., Augustyniak, R. A., Rossi, N. F., Churchill, P. C., Lapanowski, K., & O'Leary, D. S. (2000). Heart failure alters the strength and mechanisms of the muscle metaboreflex. *Am J Physiol Heart Circ Physiol, 278*(3), H818-828.

Hammond, R. L., Augustyniak, R. A., Rossi, N. F., Lapanowski, K., Dunbar, J. C., & O'Leary, D. S. (2001). Alteration of humoral and peripheral vascular responses during graded exercise in heart failure. *J Appl Physiol (1985), 90*(1), 55-61.

Hanna, R. L., Hayes, S. G., & Kaufman, M. P. (2002). alpha,beta-Methylene ATP elicits a reflex pressor response arising from muscle in decerebrate cats. *J Appl Physiol (1985), 93*(3), 834-841. doi: 10.1152/japplphysiol.00237.2002

Hanna, R. L., & Kaufman, M. P. (2003). Role played by purinergic receptors on muscle afferents in evoking the exercise pressor reflex. *J Appl Physiol (1985), 94*(4), 1437-1445. doi: 10.1152/japplphysiol.01011.2002

Hanna, R. L., & Kaufman, M. P. (2004). Activation of thin-fiber muscle afferents by a P2X agonist in cats. *J Appl Physiol (1985), 96*(3), 1166-1169. doi: 10.1152/japplphysiol.01020.2003

Hansen, J., Thomas, G. D., Jacobsen, T. N., & Victor, R. G. (1994). Muscle metaboreflex triggers parallel sympathetic activation in exercising and resting human skeletal muscle. *Am J Physiol, 266*(6 Pt 2), H2508-2514.

Harrison, D. C., Pirages, S., Robison, S. C., & Wintroub, B. U. (1969). The pulmonary and systemic circulatory response to dopamine infusion. *Br J Pharmacol, 37*(3), 618-626.

Hayes, S. G., Kindig, A. E., & Kaufman, M. P. (2005). Comparison between the effect of static contraction and tendon stretch on the discharge of group III and IV muscle afferents. *Journal of Applied Physiology, 99*(5), 1891-1896.

Heart Failure Society of America (HFSA) practice guidelines. HFSA guidelines for management of patients with heart failure caused by left ventricular systolic dysfunction--pharmacological approaches. (1999). *J Card Fail, 5*(4), 357-382.

Hecht, H. S., Karahalios, S. E., Ormiston, J. A., Schnugg, S. J., Hopkins, J. M., & Singh, B. N. (1982). Patterns of exercise response in patients with severe left ventricular dysfunction: radionuclide ejection fraction and hemodynamic cardiac performance evaluations. *Am Heart J, 104*(4 Pt 1), 718-724.

Heerdt, P. M., Pond, C. G., Blessios, G. A., & Rosenbloom, M. (1992). Inaccuracy of cardiac output by thermodilution during acute tricuspid regurgitation. *Ann Thorac Surg, 53*(4), 706-708.

Heidenreich, P. A., Trogdon, J. G., Khavjou, O. A., Butler, J., Dracup, K., Ezekowitz, M. D., . . . Outcomes, R. (2011). Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation, 123*(8), 933-944. doi: 10.1161/CIR.0b013e31820a55f5

Hermansen, L., Ekblom, B., & Saltin, B. (1970). Cardiac output during submaximal and maximal treadmill and bicycle exercise. *J Appl Physiol, 29*(1), 82-86.

Herr, M. D., Imadojemu, V., Kunselman, A. R., & Sinoway, L. I. (1999). Characteristics of the muscle mechanoreflex during quadriceps contractions in humans. *J Appl Physiol (1985), 86*(2), 767-772.

Higginbotham, M. B., Morris, K. G., Conn, E. H., Coleman, R. E., & Cobb, F. R. (1983). Determinants of variable exercise performance among patients with severe left ventricular dysfunction. *Am J Cardiol, 51*(1), 52-60.

Higginbotham, M. B., Morris, K. G., Williams, R. S., McHale, P. A., Coleman, R. E., & Cobb, F. R. (1986). Regulation of stroke volume during submaximal and maximal upright exercise in normal man. *Circ Res, 58*(2), 281-291.

Hoeper, M. M., Maier, R., Tongers, J., Niedermeyer, J., Hohlfeld, J. M., Hamm, M., & Fabel, H. (1999). Determination of cardiac output by the Fick method, thermodilution, and acetylene rebreathing in pulmonary hypertension. *Am J Respir Crit Care Med, 160*(2), 535-541. doi: 10.1164/ajrccm.160.2.9811062

Holland, D. J., Prasad, S. B., & Marwick, T. H. (2010). Contribution of exercise echocardiography to the diagnosis of heart failure with preserved ejection fraction (HFpEF). *Heart, 96*(13), 1024-1028. doi: 10.1136/hrt.2009.183947

Holmgren, A. (1956). Circulatory changes during muscular work in man; with special reference to arterial and central venous pressures in the systemic circulation. *Scand J Clin Lab Invest, 8 Suppl 24*, 1-97.

Holverda, S., Gan, C. T., Marcus, J. T., Postmus, P. E., Boonstra, A., & Vonk-Noordegraaf, A. (2006). Impaired stroke volume response to exercise in pulmonary arterial hypertension. *J Am Coll Cardiol, 47*(8), 1732-1733. doi: 10.1016/j.jacc.2006.01.048

Horsfield, K. (1978). Morphometry of the small pulmonary arteries in man. *Circ Res, 42*(5), 593-597.

Horwitz, L. D., Atkins, J. M., & Leshin, S. J. (1972). Role of the Frank-Starling mechanism in exercise. *Circ Res, 31*(6), 868-875.

Hsia, C. C., Herazo, L. F., Ramanathan, M., & Johnson, R. L., Jr. (1995). Cardiac output during exercise measured by acetylene rebreathing, thermodilution, and Fick techniques. *J Appl Physiol (1985), 78*(4), 1612-1616.

Hughes, J. M., Glazier, J. B., Maloney, J. E., & West, J. B. (1968). Effect of lung volume on the distribution of pulmonary blood flow in man. *Respir Physiol, 4*(1), 58-72.

Hughes, J. M., Lockwood, D. N., Jones, H. A., & Clark, R. J. (1991). DLCO/Q and diffusion limitation at rest and on exercise in patients with interstitial fibrosis. *Respir Physiol, 83*(2), 155-166.

Ichinose, M., Sala-Mercado, J. A., O'Leary, D. S., Hammond, R. L., Coutsos, M., Ichinose, T., . . . Iellamo, F. (2008). Spontaneous baroreflex control of cardiac output during dynamic exercise, muscle metaboreflex activation, and heart failure. *Am J Physiol Heart Circ Physiol, 294*(3), H1310-1316. doi: 10.1152/ajpheart.01187.2007

Ichinose, M. J., Sala-Mercado, J. A., Coutsos, M., Li, Z., Ichinose, T. K., Dawe, E., & O'Leary, D. S. (2010). Modulation of cardiac output alters the mechanisms of the muscle metaboreflex pressor response. *Am J Physiol Heart Circ Physiol, 298*(1), H245-250. doi: 10.1152/ajpheart.00909.2009

Iellamo, F., Sala-Mercado, J. A., Ichinose, M., Hammond, R. L., Pallante, M., Ichinose, T., . . . O'Leary, D. S. (2007). Spontaneous baroreflex control of heart rate during exercise and muscle metaboreflex activation in heart failure. *Am J Physiol Heart Circ Physiol, 293*(3), H1929-1936. doi: 10.1152/ajpheart.00564.2007

Isserles, S. A., & Breen, P. H. (1991). Can changes in end-tidal PCO2 measure changes in cardiac output? *Anesthesia & Analgesia, 73*(6), 808-814.

Iwamoto, Parnavelas, J., Kaufman, M., Botterman, B., & Mitchell, J. (1984). Activation of caudal brainstem cell groups during the exercise pressor reflex in the cat as elucidated by 2-[< sup> 14</sup> C] deoxyglucose. *Brain research, 304*(1), 178-182.

Iwamoto, G. A., Kaufmann, M. P., Botterman, B. R., & Mitchell, J. H. (1982). Effects of lateral reticular nucleus lesions on the exercise pressor reflex in cats. *Circ Res, 51*(3), 400-403.

Iwamoto, G. A., Waldrop, T. G., Kaufman, M. P., Botterman, B. R., Rybicki, K. J., & Mitchell, J. H. (1985). Pressor reflex evoked by muscular contraction: contributions by neuraxis levels. *J Appl Physiol (1985), 59*(2), 459-467.

Jakovljevic, D. G., Popadic-Gacesa, J. Z., Barak, O. F., Nunan, D., Donovan, G., Trenell, M. I., . . . Brodie, D. A. (2012). Relationship between peak cardiac pumping capability and indices of cardio-respiratory fitness in healthy individuals. *Clin Physiol Funct Imaging, 32*(5), 388-393. doi: 10.1111/j.1475-097X.2012.01143.x

Jancso, G., Kiraly, E., & Jancso-Gabor, A. (1977). Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. *Nature, 270*(5639), 741-743.

Jessup Likoff, M., Chandler, S. L., & Kay, H. R. (1987). Clinical determinants of mortality in chronic congestive heart failure secondary to idiopathic dilated or to ischemic cardiomyopathy. *The American journal of cardiology, 59*(6), 634-638.

Johnson, B. D., Beck, K. C., Proctor, D. N., Miller, J., Dietz, N. M., & Joyner, M. J. (2000). Cardiac output during exercise by the open circuit acetylene washin method: comparison with direct Fick. *J Appl Physiol (1985), 88*(5), 1650-1658.

Johnson, B. D., Joyner, M. J., & Olson, T. P. (2014). Influence of the metaboreflex on arterial blood pressure in heart failure patients. *Am Heart J*.

Jondeau, G., Katz, S. D., Zohman, L., Goldberger, M., McCarthy, M., Bourdarias, J. P., & LeJemtel, T. H. (1992). Active skeletal muscle mass and cardiopulmonary

reserve. Failure to attain peak aerobic capacity during maximal bicycle exercise in patients with severe congestive heart failure. *Circulation, 86*(5), 1351-1356.
Kannengiesser, G. J., Opie, L. H., & van der Werff, T. J. (1979). Impaired cardiac work and oxygen uptake after reperfusion of regionally ischaemic myocardium. *J Mol Cell Cardiol, 11*(2), 197-207.
Kao, W., Winkel, E. M., Johnson, M. R., Piccione, W., Lichtenberg, R., & Costanzo, M. R. (1997). Role of maximal oxygen consumption in establishment of heart transplant candidacy for heart failure patients with intermediate exercise tolerance. *Am J Cardiol, 79*(8), 1124-1127.
Kasch, F. W., Phillips, W. H., Carter, J. E., & Boyer, J. L. (1973). Cardiovascular changes in middle-aged men during two years of training. *J Appl Physiol, 34*(1), 53-57.
Katz, S. D., Maskin, C., Jondeau, G., Cocke, T., Berkowitz, R., & LeJemtel, T. (2000). Near-maximal fractional oxygen extraction by active skeletal muscle in patients with chronic heart failure. *J Appl Physiol (1985), 88*(6), 2138-2142.
Kaufman, M. P., Iwamoto, G. A., Longhurst, J. C., & Mitchell, J. H. (1982). Effects of capsaicin and bradykinin on afferent fibers with ending in skeletal muscle. *Circ Res, 50*(1), 133-139.
Kaufman, M. P., Longhurst, J. C., Rybicki, K. J., Wallach, J. H., & Mitchell, J. H. (1983). Effects of static muscular contraction on impulse activity of groups III and IV afferents in cats. *J Appl Physiol Respir Environ Exerc Physiol, 55*(1 Pt 1), 105-112.
Kaufman, M. P., & Rybicki, K. J. (1987). Discharge properties of group III and IV muscle afferents: their responses to mechanical and metabolic stimuli. *Circ Res, 61*(4 Pt 2), I60-65.
Kaufman, M. P., Rybicki, K. J., Waldrop, T. G., & Ordway, G. A. (1984). Effect of ischemia on responses of group III and IV afferents to contraction. *J Appl Physiol Respir Environ Exerc Physiol, 57*(3), 644-650.
Kaufman, M. P., Waldrop, T. G., Rybicki, K. J., Ordway, G. A., & Mitchell, J. H. (1984). Effects of static and rhythmic twitch contractions on the discharge of group III and IV muscle afferents. *Cardiovasc Res, 18*(11), 663-668.
Keller, M. L., Johnson, B. D., Joyner, M., & Olson, T. P. (2014). Influence of the metaboreflex on arterial blood pressure in heart failure patients. *Am Heart J, 167*, 521-528.
Kitzman, D. W., Higginbotham, M. B., Cobb, F. R., Sheikh, K. H., & Sullivan, M. J. (1991). Exercise intolerance in patients with heart failure and preserved left ventricular systolic function: failure of the Frank-Starling mechanism. *J Am Coll Cardiol, 17*(5), 1065-1072.
Kleber, F. X., Vietzke, G., Wernecke, K. D., Bauer, U., Opitz, C., Wensel, R., . . . Glaser, S. (2000). Impairment of ventilatory efficiency in heart failure: prognostic impact. *Circulation, 101*(24), 2803-2809.
Kniffki, K. D., Mense, S., & Schmidt, R. F. (1978). Responses of group IV afferent units from skeletal muscle to stretch, contraction and chemical stimulation. *Exp Brain Res, 31*(4), 511-522.

Komajda, M., Carson, P. E., Hetzel, S., McKelvie, R., McMurray, J., Ptaszynska, A., . . . Massie, B. M. (2011). Factors associated with outcome in heart failure with preserved ejection fraction: findings from the Irbesartan in Heart Failure with Preserved Ejection Fraction Study (I-PRESERVE). *Circ Heart Fail, 4*(1), 27-35. doi: 10.1161/CIRCHEARTFAILURE.109.932996

Komamura, K., Shannon, R. P., Ihara, T., Shen, Y. T., Mirsky, I., Bishop, S. P., & Vatner, S. F. (1993). Exhaustion of Frank-Starling mechanism in conscious dogs with heart failure. *Am J Physiol, 265*(4 Pt 2), H1119-1131.

Kronenberg, M. W., Konstam, M. A., Edens, T. R., Howe, D. M., Dolan, N., Udelson, J. E., . . . Yusuf, S. (1998). Factors influencing exercise performance in patients with left ventricular dysfunction. SOLVD Investigators. Studies of Left Ventricular Dysfunction. *J Card Fail, 4*(3), 159-167.

Lang, C. C., Agostoni, P., & Mancini, D. M. (2007). Prognostic significance and measurement of exercise-derived hemodynamic variables in patients with heart failure. *J Card Fail, 13*(8), 672-679. doi: 10.1016/j.cardfail.2007.05.004

Lang, C. C., Karlin, P., Haythe, J., Lim, T. K., & Mancini, D. M. (2009). Peak cardiac power output, measured noninvasively, is a powerful predictor of outcome in chronic heart failure. *Circ Heart Fail, 2*(1), 33-38. doi: 10.1161/CIRCHEARTFAILURE.108.798611

Lang, C. C., Karlin, P., Haythe, J., Tsao, L., & Mancini, D. M. (2007). Ease of noninvasive measurement of cardiac output coupled with peak VO2 determination at rest and during exercise in patients with heart failure. *Am J Cardiol, 99*(3), 404-405. doi: 10.1016/j.amjcard.2006.08.047

Laukkanen, J. A., Kurl, S., Salonen, J. T., Lakka, T. A., & Rauramaa, R. (2006). Peak oxygen pulse during exercise as a predictor for coronary heart disease and all cause death. *Heart, 92*(9), 1219-1224.

Lavie, C. J., Milani, R. V., & Mehra, M. R. (2004). Peak exercise oxygen pulse and prognosis in chronic heart failure. *Am J Cardiol, 93*(5), 588-593. doi: 10.1016/j.amjcard.2003.11.023

Lee, D. S., Austin, P. C., Rouleau, J. L., Liu, P. P., Naimark, D., & Tu, J. V. (2003). Predicting mortality among patients hospitalized for heart failure. *JAMA: the journal of the American Medical Association, 290*(19), 2581-2587.

Leshnower, B. G., Potts, J. T., Garry, M. G., & Mitchell, J. H. (2001). Reflex cardiovascular responses evoked by selective activation of skeletal muscle ergoreceptors. *J Appl Physiol (1985), 90*(1), 308-316.

Lewis, B. M., Houssay, H. E., Haynes, F. W., & Dexter, L. (1953). The dynamics of both right and left ventricles at rest and during exercise in patients with heart failure. *Circulation research, 1*(4), 312-320.

Lewis, J. F., Kuo, L. C., Nelson, J. G., Limacher, M. C., & Quinones, M. A. (1984). Pulsed Doppler echocardiographic determination of stroke volume and cardiac output: clinical validation of two new methods using the apical window. *Circulation, 70*(3), 425-431.

Lewis, S. F., & Haller, R. G. (1989). Skeletal muscle disorders and associated factors that limit exercise performance. *Exerc Sport Sci Rev, 17*, 67-113.

Li, J., King, N. C., & Sinoway, L. I. (2003). ATP concentrations and muscle tension increase linearly with muscle contraction. *J Appl Physiol (1985), 95*(2), 577-583. doi: 10.1152/japplphysiol.00185.2003

Li, J., Sinoway, A. N., Gao, Z., Maile, M. D., Pu, M., & Sinoway, L. I. (2004). Muscle mechanoreflex and metaboreflex responses after myocardial infarction in rats. *Circulation, 110*(19), 3049-3054. doi: 10.1161/01.CIR.0000147188.46287.1B

Li, J., & Sinoway, L. I. (2002). ATP stimulates chemically sensitive and sensitizes mechanically sensitive afferents. *Am J Physiol Heart Circ Physiol, 283*(6), H2636-2643. doi: 10.1152/ajpheart.00395.2002

Lim, J. G., McAveney, T. J., Fleg, J. L., Shapiro, E. P., Turner, K. L., Bacher, A. C., . . . Stewart, K. J. (2005). Oxygen pulse during exercise is related to resting systolic and diastolic left ventricular function in older persons with mild hypertension. *American heart journal, 150*(5), 941-946.

Lipkin, D. P., Canepa-Anson, R., Stephens, M. R., & Poole-Wilson, P. A. (1986). Factors determining symptoms in heart failure: comparison of fast and slow exercise tests. *Br Heart J, 55*(5), 439-445.

Lipkin, D. P., & Poole-Wilson, P. A. (1985). Measurement of cardiac output during exercise by the thermodilution and direct Fick techniques in patients with chronic congestive heart failure. *Am J Cardiol, 56*(4), 321-324.

Lipkin, D. P., & Poole-Wilson, P. A. (1986). Symptoms limiting exercise in chronic heart failure. *Br Med J (Clin Res Ed), 292*(6527), 1030-1031.

Lipkin, D. P., Scriven, A. J., Crake, T., & Poole-Wilson, P. A. (1986). Six minute walking test for assessing exercise capacity in chronic heart failure. *Br Med J (Clin Res Ed), 292*(6521), 653-655.

Loeppky, J. A., Hoekenga, D. E., Greene, E. R., & Luft, U. C. (1984). Comparison of noninvasive pulsed Doppler and Fick measurements of stroke volume in cardiac patients. *Am Heart J, 107*(2), 339-346.

Lu, C., Nicolosi, G. L., Burelli, C., Cassin, M., Zardo, F., Brieda, M., . . . Zanuttini, D. (1992). Limitations in the assessment of changes of cardiac output by Doppler echocardiography under various hemodynamic conditions. *Am J Cardiol, 70*(15), 1370-1374.

Lykidis, C. K., Kumar, P., Vianna, L. C., White, M. J., & Balanos, G. M. (2010). A respiratory response to the activation of the muscle metaboreflex during concurrent hypercapnia in man. *Exp Physiol, 95*(1), 194-201. doi: 10.1113/expphysiol.2009.049999

Lykidis, C. K., White, M. J., & Balanos, G. M. (2008). The pulmonary vascular response to the sustained activation of the muscle metaboreflex in man. *Exp Physiol, 93*(2), 247-253. doi: 10.1113/expphysiol.2007.09487

MacLean, D. A., Imadojemu, V. A., & Sinoway, L. I. (2000). Interstitial pH, K(+), lactate, and phosphate determined with MSNA during exercise in humans. *Am J Physiol Regul Integr Comp Physiol, 278*(3), R563-571.

Mahapatra, S., Nishimura, R. A., Oh, J. K., & McGoon, M. D. (2006). The prognostic value of pulmonary vascular capacitance determined by Doppler echocardiography in patients with pulmonary arterial hypertension. *J Am Soc Echocardiogr, 19*(8), 1045-1050. doi: 10.1016/j.echo.2006.03.008

Mahapatra, S., Nishimura, R. A., Sorajja, P., Cha, S., & McGoon, M. D. (2006). Relationship of pulmonary arterial capacitance and mortality in idiopathic pulmonary arterial hypertension. *J Am Coll Cardiol, 47*(4), 799-803. doi: 10.1016/j.jacc.2005.09.054

Mahler, D. A., Parker, H. W., & Andresen, D. C. (1985). Physiologic changes in rowing performance associated with training in collegiate women rowers. *Int J Sports Med, 6*(4), 229-233. doi: 10.1055/s-2008-1025846

Mancini, Katz, S., Donchez, L., & Aaronson, K. (1996). Coupling of hemodynamic measurements with oxygen consumption during exercise does not improve risk stratification in patients with heart failure. *Circulation, 94*(10), 2492-2496.

Mancini, D. M., Eisen, H., Kussmaul, W., Mull, R., Edmunds, L. H., Jr., & Wilson, J. R. (1991). Value of peak exercise oxygen consumption for optimal timing of cardiac transplantation in ambulatory patients with heart failure. *Circulation, 83*(3), 778-786.

Mark, A. L., Victor, R. G., Nerhed, C., & Wallin, B. G. (1985). Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circ Res, 57*(3), 461-469.

Marshall, P., Al-Timman, J., Riley, R., Wright, J., Williams, S., Hainsworth, R., & Tan, L. B. (2001). Randomized controlled trial of home-based exercise training to evaluate cardiac functional gains. *Clin Sci (Lond), 101*(5), 477-483.

Marwick, T. H., Nemec, J. J., Pashkow, F. J., Stewart, W. J., & Salcedo, E. E. (1992). Accuracy and limitations of exercise echocardiography in a routine clinical setting. *J Am Coll Cardiol, 19*(1), 74-81.

Maskin, C. S., Forman, R., Sonnenblick, E. H., Frishman, W. H., & LeJemtel, T. H. (1983). Failure of dobutamine to increase exercise capacity despite hemodynamic improvement in severe chronic heart failure. *Am J Cardiol, 51*(1), 177-182.

Maslow, A., Stearns, G., Bert, A., Feng, W., Price, D., Schwartz, C., . . . Loring, S. (2001). Monitoring end-tidal carbon dioxide during weaning from cardiopulmonary bypass in patients without significant lung disease. *Anesth Analg, 92*(2), 306-313.

Mason, D. T., Spann, J., Zelis, R., & Amsterdam, E. A. (1971). Comparison of the contractile state of the normal, hypertrophied, and failing heart in man. *Cardiac Hypertrophy, 433*, 444.

McCloskey, D. I., & Mitchell, J. H. (1972). Reflex cardiovascular and respiratory responses originating in exercising muscle. *J Physiol, 224*(1), 173-186.

McCord, J. L., Tsuchimochi, H., & Kaufman, M. P. (2009). Acid-sensing ion channels contribute to the metaboreceptor component of the exercise pressor reflex. *Am J Physiol Heart Circ Physiol, 297*(1), H443-449. doi: 10.1152/ajpheart.00328.2009

McDaniel, J., Fjeldstad, A. S., Ives, S., Hayman, M., Kithas, P., & Richardson, R. S. (2010). Central and peripheral contributors to skeletal muscle hyperemia: response to passive limb movement. *J Appl Physiol (1985), 108*(1), 76-84. doi: 10.1152/japplphysiol.00895.2009

McDonagh, T. A., Morrison, C. E., Lawrence, A., Ford, I., Tunstall-Pedoe, H., McMurray, J. J., & Dargie, H. J. (1997). Symptomatic and asymptomatic left-

ventricular systolic dysfunction in an urban population. *Lancet, 350*(9081), 829-833. doi: 10.1016/S0140-6736(97)03033-X

McGoon, M., Gutterman, D., Steen, V., Barst, R., McCrory, D. C., Fortin, T. A., . . . American College of Chest, P. (2004). Screening, early detection, and diagnosis of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. *Chest, 126*(1 Suppl), 14S-34S. doi: 10.1378/chest.126.1_suppl.14S

McLaughlin, V. V., Archer, S. L., Badesch, D. B., Barst, R. J., Farber, H. W., Lindner, J. R., . . . Accf/Aha. (2009). ACCF/AHA 2009 expert consensus document on pulmonary hypertension: a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association: developed in collaboration with the American College of Chest Physicians, American Thoracic Society, Inc., and the Pulmonary Hypertension Association. *Circulation, 119*(16), 2250-2294. doi: 10.1161/CIRCULATIONAHA.109.192230

McLure, L. E., Brown, A., Lee, W. N., Church, A. C., Peacock, A. J., & Johnson, M. K. (2011). Non-invasive stroke volume measurement by cardiac magnetic resonance imaging and inert gas rebreathing in pulmonary hypertension. *Clin Physiol Funct Imaging, 31*(3), 221-226. doi: 10.1111/j.1475-097X.2010.01004.x

McMichael, J., & Sharpey-Schafer, E. P. (1944). CARDIAC OUTPUT IN MAN BY A DIRECT FICK METHOD: Effects of Posture, Venous Pressure Change, Atropine, And Adrenaline. *Br Heart J, 6*(1), 33-40.

Meiler, S. E., Ashton, J. J., Moeschberger, M. L., Unverferth, D. V., & Leier, C. V. (1987). An analysis of the determinants of exercise performance in congestive heart failure. *Am Heart J, 113*(5), 1207-1217.

Meintjes, A. F., Nobrega, A. C., Fuchs, I. E., Ally, A., & Wilson, L. B. (1995). Attenuation of the exercise pressor reflex. Effect of opioid agonist on substance P release in L-7 dorsal horn of cats. *Circ Res, 77*(2), 326-334.

Metra, M., Faggiano, P., D'Aloia, A., Nodari, S., Gualeni, A., Raccagni, D., & Dei Cas, L. (1999). Use of cardiopulmonary exercise testing with hemodynamic monitoring in the prognostic assessment of ambulatory patients with chronic heart failure. *J Am Coll Cardiol, 33*(4), 943-950.

Michael, G. J., & Priestley, J. V. (1999). Differential expression of the mRNA for the vanilloid receptor subtype 1 in cells of the adult rat dorsal root and nodose ganglia and its downregulation by axotomy. *J Neurosci, 19*(5), 1844-1854.

Middlekauff, H. R., Chiu, J., Hamilton, M. A., Fonarow, G. C., Maclellan, W. R., Hage, A., . . . Patel, J. (2004). Muscle mechanoreceptor sensitivity in heart failure. *Am J Physiol Heart Circ Physiol, 287*(5), H1937-1943. doi: 10.1152/ajpheart.00330.2004

Middlekauff, H. R., Nitzsche, E. U., Hoh, C. K., Hamilton, M. A., Fonarow, G. C., Hage, A., & Moriguchi, J. D. (2001). Exaggerated muscle mechanoreflex control of reflex renal vasoconstriction in heart failure. *J Appl Physiol (1985), 90*(5), 1714-1719.

Minotti, J. R., Christoph, I., Oka, R., Weiner, M. W., Wells, L., & Massie, B. M. (1991). Impaired skeletal muscle function in patients with congestive heart failure.

Relationship to systemic exercise performance. *J Clin Invest*, *88*(6), 2077-2082. doi: 10.1172/JCI115537
Mitchell, & Schmidt. (1983). Cardiovascular reflex control by afferent fibers from skeletal muscle receptors. *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow, 3*, 623.
Mitchell, & Schmidt. (2011). Cardiovascular Reflex Control by Afferent Fibers from Skeletal Muscle Receptors. *Comprehensive Physiology*.
Mitchell, G. F., Moyé, L. A., Braunwald, E., Rouleau, J.-L., Bernstein, V., Geltman, E. M., . . . Investigators, S. (1997). Sphygmomanometrically determined pulse pressure is a powerful independent predictor of recurrent events after myocardial infarction in patients with impaired left ventricular function. *Circulation, 96*(12), 4254-4260.
Mitchell, J. H., Kaufman, M. P., & Iwamoto, G. A. (1983). The exercise pressor reflex: its cardiovascular effects, afferent mechanisms, and central pathways. *Annu Rev Physiol, 45*(1), 229-242. doi: 10.1146/annurev.ph.45.030183.001305
Moraes, D. L., Colucci, W. S., & Givertz, M. M. (2000). Secondary pulmonary hypertension in chronic heart failure: the role of the endothelium in pathophysiology and management. *Circulation, 102*(14), 1718-1723.
Nagy, J. I., & van der Kooy, D. (1983). Effects of neonatal capsaicin treatment on nociceptive thresholds in the rat. *J Neurosci, 3*(6), 1145-1150.
Nattie, E. E., & Li, A. (2002). CO2 dialysis in nucleus tractus solitarius region of rat increases ventilation in sleep and wakefulness. *J Appl Physiol (1985), 92*(5), 2119-2130. doi: 10.1152/japplphysiol.01128.2001
Negrao, C. E., Rondon, M. U., Tinucci, T., Alves, M. J., Roveda, F., Braga, A. M., . . . Middlekauff, H. R. (2001). Abnormal neurovascular control during exercise is linked to heart failure severity. *Am J Physiol Heart Circ Physiol, 280*(3), H1286-1292.
O'Leary, D. S., Sala-Mercado, J. A., Augustyniak, R. A., Hammond, R. L., Rossi, N. F., & Ansorge, E. J. (2004). Impaired muscle metaboreflex-induced increases in ventricular function in heart failure. *Am J Physiol Heart Circ Physiol, 287*(6), H2612-2618. doi: 10.1152/ajpheart.00604.2004
O'Leary, D. S., Sala-Mercado, J. A., Hammond, R. L., Ansorge, E. J., Kim, J. K., Rodriguez, J., . . . Ichinose, M. (2007). Muscle metaboreflex-induced increases in cardiac sympathetic activity vasoconstrict the coronary vasculature. *J Appl Physiol (1985), 103*(1), 190-194. doi: 10.1152/japplphysiol.00139.2007
O'Leary, D. S., Augustyniak, R. A., Ansorge, E. J., & Collins, H. L. (1999). Muscle metaboreflex improves O2delivery to ischemic active skeletal muscle. *American Journal of Physiology-Heart and Circulatory Physiology, 276*(4), H1399-H1403.
Oelberg, D. A., Medoff, B. D., Markowitz, D. H., Pappagianopoulos, P. P., Ginns, L. C., & Systrom, D. M. (1998). Systemic oxygen extraction during incremental exercise in patients with severe chronic obstructive pulmonary disease. *Eur J Appl Physiol Occup Physiol, 78*(3), 201-207. doi: 10.1007/s004210050408
Ogawa, T., Spina, R. J., Martin, W. H., 3rd, Kohrt, W. M., Schechtman, K. B., Holloszy, J. O., & Ehsani, A. A. (1992). Effects of aging, sex, and physical training on cardiovascular responses to exercise. *Circulation, 86*(2), 494-503.

Olson, Miller, A. D., Hulsebus, M. L., Flood, K. S., Johnson, B. D., & Borlaug, B. A. (2008). Lung Diffusion and Alveolar-Capillary Recruitment during Exercise in Heart Failure with Preserved Ejection Fraction. *Journal of cardiac failure, 14*(6), S25.

Olson, L. J., Snyder, E. M., Beck, K. C., & Johnson, B. D. (2006). Reduced rate of alveolar-capillary recruitment and fall of pulmonary diffusing capacity during exercise in patients with heart failure. *J Card Fail, 12*(4), 299-306. doi: 10.1016/j.cardfail.2006.01.010

Olson, T. P., Denzer, D. L., Sinnett, W. L., Wilson, T., & Johnson, B. D. (2013). Prognostic value of resting pulmonary function in heart failure. *Clin Med Insights Circ Respir Pulm Med, 7*, 35-43. doi: 10.4137/CCRPM.S12525

Olson, T. P., Joyner, M. J., Dietz, N. M., Eisenach, J. H., Curry, T. B., & Johnson, B. D. (2010). Effects of respiratory muscle work on blood flow distribution during exercise in heart failure. *J Physiol, 588*(Pt 13), 2487-2501. doi: 10.1113/jphysiol.2009.186056

Olson, T. P., Joyner, M. J., Eisenach, J. H., Curry, T. B., & Johnson, B. D. (2014). Influence of locomotor muscle afferent inhibition on the ventilatory response to exercise in heart failure. *Exp Physiol, 99*(2), 414-426. doi: 10.1113/expphysiol.2013.075937

Olson, T. P., Joyner, M. J., & Johnson, B. D. (2010). Influence of locomotor muscle metaboreceptor stimulation on the ventilatory response to exercise in heart failure. *Circ Heart Fail, 3*(2), 212-219. doi: 10.1161/CIRCHEARTFAILURE.109.879684

Osada, N., Chaitman, B. R., Miller, L. W., Yip, D., Cishek, M. B., Wolford, T. L., & Donohue, T. J. (1998). Cardiopulmonary exercise testing identifies low risk patients with heart failure and severely impaired exercise capacity considered for heart transplantation. *J Am Coll Cardiol, 31*(3), 577-582.

Paton, J. F., Deuchars, J., Li, Y. W., & Kasparov, S. (2001). Properties of solitary tract neurones responding to peripheral arterial chemoreceptors. *Neuroscience, 105*(1), 231-248.

Patterson, S., & Starling, E. (1914). On the mechanical factors which determine the output of the ventricles. *The Journal of physiology, 48*(5), 357-379.

Paulus, W. J., Tschöpe, C., Sanderson, J. E., Rusconi, C., Flachskampf, F. A., Rademakers, F. E., . . . Leite-Moreira, A. F. (2007). How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. *European heart journal, 28*(20), 2539-2550.

Permutt, S., Bromberger-Barnea, B., & Bane, H. N. (1962). Alveolar pressure, pulmonary venous pressure, and the vascular waterfall. *Med Thorac, 19*(4), 239-260.

Piepoli, M. (1998). Central role of peripheral mechanisms in exercise intolerance in chronic heart failure: the muscle hypothesis. *Cardiologia, 43*(9), 909-917.

Piepoli, M., Clark, A. L., & Coats, A. J. (1995). Muscle metaboreceptors in hemodynamic, autonomic, and ventilatory responses to exercise in men. *Am J Physiol, 269*(4 Pt 2), H1428-1436.

Piepoli, M., Clark, A. L., Volterrani, M., Adamopoulos, S., Sleight, P., & Coats, A. J. (1996). Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training. *Circulation, 93*(5), 940-952.

Piepoli, M. F., Dimopoulos, K., Concu, A., & Crisafulli, A. (2008). Cardiovascular and ventilatory control during exercise in chronic heart failure: role of muscle reflexes. *Int J Cardiol, 130*(1), 3-10. doi: 10.1016/j.ijcard.2008.02.030

Piepoli, M. F., Kaczmarek, A., Francis, D. P., Davies, L. C., Rauchhaus, M., Jankowska, E. A., . . . Ponikowski, P. (2006). Reduced peripheral skeletal muscle mass and abnormal reflex physiology in chronic heart failure. *Circulation, 114*(2), 126-134. doi: 10.1161/CIRCULATIONAHA.105.605980

Piepoli, M. F., Ponikowski, P., Clarka, A. L., Banasiak, W., Capucci, A., & Coats, A. J. (1999). A neural link to explain the "muscle hypothesis" of exercise intolerance in chronic heart failure. *Am Heart J, 137*(6), 1050-1056.

Pietra, G. G., Capron, F., Stewart, S., Leone, O., Humbert, M., Robbins, I. M., . . . Tuder, R. M. (2004). Pathologic assessment of vasculopathies in pulmonary hypertension. *J Am Coll Cardiol, 43*(12 Suppl S), 25S-32S. doi: 10.1016/j.jacc.2004.02.033

Pilote, L., Silberberg, J., Lisbona, R., & Sniderman, A. (1989). Prognosis in patients with low left ventricular ejection fraction after myocardial infarction. Importance of exercise capacity. *Circulation, 80*(6), 1636-1641.

Pirofsky, B. (1953). The determination of blood viscosity in man by a method based on Poiseuille's law. *J Clin Invest, 32*(4), 292-298. doi: 10.1172/JCI102738

Poliner, L. R., Dehmer, G. J., Lewis, S. E., Parkey, R. W., Blomqvist, C. G., & Willerson, J. T. (1980). Left ventricular performance in normal subjects: a comparison of the responses to exercise in the upright and supine positions. *Circulation, 62*(3), 528-534.

Pomeroy, G., Ardell, J. L., & Wurster, R. D. (1986). Spinal opiate modulation of cardiovascular reflexes in the exercising dog. *Brain Res, 381*(2), 385-389.

Ponikowski, P. P., Chua, T. P., Francis, D. P., Capucci, A., Coats, A. J., & Piepoli, M. F. (2001). Muscle ergoreceptor overactivity reflects deterioration in clinical status and cardiorespiratory reflex control in chronic heart failure. *Circulation, 104*(19), 2324-2330.

Potts, J. T., Fuchs, I. E., Li, J., Leshnower, B., & Mitchell, J. H. (1999). Skeletal muscle afferent fibres release substance P in the nucleus tractus solitarii of anaesthetized cats. *J Physiol, 514 (Pt 3)*, 829-841.

Randle Adair, J., Hamilton, B. L., Scappaticci, K. A., Helke, C. J., & Gillis, R. A. (1977). Cardiovascular responses to electrical stimulation of the medullary raphe area of the cat. *Brain research, 128*(1), 141-145.

Reeves, J. T., Groves, B. M., Cymerman, A., Sutton, J. R., Wagner, P. D., Turkevich, D., & Houston, C. S. (1990). Operation Everest II: cardiac filling pressures during cycle exercise at sea level. *Respiration physiology, 80*(2), 147-154.

Reeves, R. B., & Park, H. K. (1992). CO uptake kinetics of red cells and CO diffusing capacity. *Respir Physiol, 88*(1-2), 1-21.

Reindl, I., Wernecke, K. D., Opitz, C., Wensel, R., Konig, D., Dengler, T., . . . Kleber, F. X. (1998). Impaired ventilatory efficiency in chronic heart failure: possible role of pulmonary vasoconstriction. *Am Heart J, 136*(5), 778-785.

Remes, J., MlEttinen, H., Reunanen, A., & Pyörälä, K. (1991). Validity of clinical diagnosis of heart failure in primary health care. *European heart journal, 12*(3), 315-321.

Remme, W. J., Swedberg, K., Task Force for the, D., & Treatment of Chronic Heart Failure, E. S. o. C. (2001). Guidelines for the diagnosis and treatment of chronic heart failure. *Eur Heart J, 22*(17), 1527-1560. doi: 10.1053/euhj.2001.2783

Rerych, S. K., Scholz, P. M., Newman, G. E., Sabiston Jr, D. C., & Jones, R. H. (1978). Cardiac function at rest and during exercise in normals and in patients with coronary heart disease: evaluation by radionuclide angiocardiography. *Annals of surgery, 187*(5), 449.

Rich, S., & Rabinovitch, M. (2008). Diagnosis and treatment of secondary (non-category 1) pulmonary hypertension. *Circulation, 118*(21), 2190-2199. doi: 10.1161/CIRCULATIONAHA.107.723007

Riley, R. L., Himmelstein, A., & et al. (1948). Studies of the pulmonary circulation at rest and during exercise in normal individuals and in patients with chronic pulmonary disease. *Am J Physiol, 152*(2), 372-382.

Rodeheffer, R. J., Gerstenblith, G., Becker, L., Fleg, J., Weisfeldt, M., & Lakatta, E. (1984). Exercise cardiac output is maintained with advancing age in healthy human subjects: cardiac dilatation and increased stroke volume compensate for a diminished heart rate. *Circulation, 69*(2), 203-213.

Roger, V. L., Go, A. S., Lloyd-Jones, D. M., Benjamin, E. J., Berry, J. D., Borden, W. B., . . . Fox, C. S. (2012). Heart Disease and Stroke Statistics—2012 Update A Report From the American Heart Association. *Circulation, 125*(1), e2-e220.

Rose, C. P., Burgess, J. H., & Cousineau, D. (1983). Reduced aortocoronary sinus extraction of epinephrine in patients with left ventricular failure secondary to long-term pressure or volume overload. *Circulation, 68*(2), 241-244.

Ross, J., Gault, J. H., Mason, D. T., Linhard, J. W., & Braunwald, E. (1966). Left ventricular performance during muscular exercise in patients with and without cardiac dysfunction. *Circulation, 34*(4), 597-608.

Ross, J., Jr., & Braunwald, E. (1964). Studies on Starling's Law of the Heart. Ix. The Effects of Impeding Venous Return on Performance of the Normal and Failing Human Left Ventricle. *Circulation, 30*(5), 719-727.

Ross, J., Jr., Morrow, A. G., Mason, D. T., & Braunwald, E. (1966). Left ventricular function following replacement of the aortic valve. Hemodynamic responses to muscular exercise. *Circulation, 33*(4), 507-516.

Rotto, D. M., & Kaufman, M. P. (1988). Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. *J Appl Physiol (1985), 64*(6), 2306-2313.

Rotto, D. M., Stebbins, C. L., & Kaufman, M. P. (1989). Reflex cardiovascular and ventilatory responses to increasing H+ activity in cat hindlimb muscle. *J Appl Physiol (1985), 67*(1), 256-263.

Roughton, F. J., & Forster, R. E. (1957). Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J Appl Physiol, 11*(2), 290-302.

Roul, G., Germain, P., & Bareiss, P. (1998). Does the 6-minute walk test predict the prognosis in patients with NYHA class II or III chronic heart failure? *Am Heart J, 136*(3), 449-457.

Roul, G., Moulichon, M. E., Bareiss, P., Gries, P., Koegler, A., Sacrez, J., . . . Sacrez, A. (1995). Prognostic factors of chronic heart failure in NYHA class II or III: value of invasive exercise haemodynamic data. *Eur Heart J, 16*(10), 1387-1398.

Rowland, T. W., Melanson, E. L., Popowski, B. E., & Ferrone, L. C. (1998). Test-retest reproducibility of maximum cardiac output by Doppler echocardiography. *Am J Cardiol, 81*(10), 1228-1230.

Rubin, S. A., & Brown, H. V. (1984). Ventilation and gas exchange during exercise in severe chronic heart failure. *Am Rev Respir Dis, 129*(2 Pt 2), S63-64.

Rybicki, K. J., Kaufman, M. P., Kenyon, J. L., & Mitchell, J. H. (1984). Arterial pressure responses to increasing interstitial potassium in hindlimb muscle of dogs. *Am J Physiol, 247*(4 Pt 2), R717-721.

Sala-Mercado, J. A., Hammond, R. L., Kim, J. K., McDonald, P. J., Stephenson, L. W., & O'Leary, D. S. (2007). Heart failure attenuates muscle metaboreflex control of ventricular contractility during dynamic exercise. *Am J Physiol Heart Circ Physiol, 292*(5), H2159-2166. doi: 10.1152/ajpheart.01240.2006

Sala-Mercado, J. A., Hammond, R. L., Kim, J. K., Rossi, N. F., Stephenson, L. W., & O'Leary, D. S. (2006). Muscle metaboreflex control of ventricular contractility during dynamic exercise. *Am J Physiol Heart Circ Physiol, 290*(2), H751-757. doi: 10.1152/ajpheart.00869.2005

Saltin, B. (1964). Cardiac output during submaximal and maximal work1.

Saltin, B., & Astrand, P. O. (1967). Maximal oxygen uptake in athletes. *J Appl Physiol, 23*(3), 353-358.

Salton, C. J., Chuang, M. L., O'Donnell, C. J., Kupka, M. J., Larson, M. G., Kissinger, K. V., . . . Manning, W. J. (2002). Gender differences and normal left ventricular anatomy in an adult population free of hypertension. A cardiovascular magnetic resonance study of the Framingham Heart Study Offspring cohort. *J Am Coll Cardiol, 39*(6), 1055-1060.

Sandstede, J., Lipke, C., Beer, M., Hofmann, S., Pabst, T., Kenn, W., . . . Hahn, D. (2000). Age- and gender-specific differences in left and right ventricular cardiac function and mass determined by cine magnetic resonance imaging. *Eur Radiol, 10*(3), 438-442. doi: 10.1007/s003300050072

Sarnoff, S. J., & Berglund, E. (1952). Pressure-volume characteristics and stress relaxation in the pulmonary vascular bed of the dog. *Am J Physiol, 171*(1), 238-244.

Sato, A. (1973). Spinal and medullary reflex components of the somatosympathetic reflex discharges evoked by stimulation of the group IV somatic afferents. *Brain Res, 51*, 307-318.

Saur, J., Trinkmann, F., Doesch, C., Scherhag, A., Brade, J., Schoenberg, S. O., . . . Papavassiliu, T. (2010). The impact of pulmonary disease on noninvasive measurement of cardiac output by the inert gas rebreathing method. *Lung, 188*(5), 433-440. doi: 10.1007/s00408-010-9257-0

Schadt, J. C., McKown, M. D., McKown, D. P., & Franklin, D. (1984). Hemodynamic effects of hemorrhage and subsequent naloxone treatment in conscious rabbits. *Am J Physiol, 247*(3 Pt 2), R497-505.

Scharf, C., Merz, T., Kiowski, W., Oechslin, E., Schalcher, C., & Brunner-La Rocca, H. P. (2002). Noninvasive assessment of cardiac pumping capacity during exercise predicts prognosis in patients with congestive heart failure. *Chest, 122*(4), 1333-1339.

Schlader, Z. J., Mundel, T., Barnes, M. J., & Hodges, L. D. (2010). Peak cardiac power output in healthy, trained men. *Clin Physiol Funct Imaging, 30*(6), 480-484. doi: 10.1111/j.1475-097X.2010.00959.x

Schnabel Jr, T., Sacker, M. A., Allen, M., & Lewis, D. (1961). Some Observations on the Relationship of Venous Return and Cardiac Function in Humans. *Transactions of the American Clinical and Climatological Association, 72*, 150.

Schwinger, R., Böhm, M., Koch, A., Schmidt, U., Morano, I., Eissner, H.-J., . . . Erdmann, E. (1994). The failing human heart is unable to use the Frank-Starling mechanism. *Circulation research, 74*(5), 959-969.

Scott, A. C., Davies, L. C., Coats, A. J., & Piepoli, M. (2002). Relationship of skeletal muscle metaboreceptors in the upper and lower limbs with the respiratory control in patients with heart failure. *Clin Sci (Lond), 102*(1), 23-30.

Scott, A. C., Francis, D. P., Coats, A. J., & Piepoli, M. F. (2003). Reproducibility of the measurement of the muscle ergoreflex activity in chronic heart failure. *Eur J Heart Fail, 5*(4), 453-461.

Scott, A. C., Francis, D. P., Davies, L. C., Ponikowski, P., Coats, A. J., & Piepoli, M. F. (2000). Contribution of skeletal muscle 'ergoreceptors' in the human leg to respiratory control in chronic heart failure. *J Physiol, 529*(3), 863-870.

Scott, A. C., Wensel, R., Davos, C. H., Georgiadou, P., Ceri Davies, L., Coats, A. J., . . . Piepoli, M. F. (2004). Putative contribution of prostaglandin and bradykinin to muscle reflex hyperactivity in patients on Ace-inhibitor therapy for chronic heart failure. *Eur Heart J, 25*(20), 1806-1813. doi: 10.1016/j.ehj.2004.07.018

Scott, A. C., Wensel, R., Davos, C. H., Georgiadou, P., Kemp, M., Hooper, J., . . . Piepoli, M. F. (2003). Skeletal muscle reflex in heart failure patients: role of hydrogen. *Circulation, 107*(2), 300-306.

Scott, A. C., Wensel, R., Davos, C. H., Kemp, M., Kaczmarek, A., Hooper, J., . . . Piepoli, M. F. (2002). Chemical mediators of the muscle ergoreflex in chronic heart failure: a putative role for prostaglandins in reflex ventilatory control. *Circulation, 106*(2), 214-220.

Scrutenid, Lagioia, R., Ricci, A., Clemente, M., Boni, L., & Rizzon, P. (1994). Prediction of mortality in mild to moderately symptomatic patients with left ventricular dysfunction The role of the New York Heart Association classification, cardiopulmonary exercise testing, two-dimensional echocardiography and Holter Monitoring. *European heart journal, 15*(8), 1089-1095.

Sheriff, D. D., Augustyniak, R. A., & O'Leary, D. S. (1998). Muscle chemoreflex-induced increases in right atrial pressure. *Am J Physiol, 275*(3 Pt 2), H767-775.
Shibutani, K., Muraoka, M., Shirasaki, S., Kubal, K., Sanchala, V. T., & Gupte, P. (1994). Do changes in end-tidal PCO2 quantitatively reflect changes in cardiac output? *Anesth Analg, 79*(5), 829-833.
Shipley, R. E., & Wilson, C. (1951). An improved recording rotameter. *Proc Soc Exp Biol Med, 78*(3), 724-728.
Shoemaker, J. K., Kunselman, A. R., Silber, D. H., & Sinoway, L. I. (1998). Maintained exercise pressor response in heart failure. *J Appl Physiol (1985), 85*(5), 1793-1799.
Silber, D. H., Sutliff, G., Yang, Q. X., Smith, M. B., Sinoway, L. I., & Leuenberger, U. A. (1998). Altered mechanisms of sympathetic activation during rhythmic forearm exercise in heart failure. *J Appl Physiol (1985), 84*(5), 1551-1559.
Simonneau, G., Robbins, I. M., Beghetti, M., Channick, R. N., Delcroix, M., Denton, C. P., . . . Souza, R. (2009). Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol, 54*(1 Suppl), S43-54. doi: 10.1016/j.jacc.2009.04.012
Sinoway, L., & Prophet, S. (1990). Skeletal muscle metaboreceptor stimulation opposes peak metabolic vasodilation in humans. *Circ Res, 66*(6), 1576-1584.
Smith, A. A., Cowburn, P. J., Parker, M. E., Denvir, M., Puri, S., Patel, K. R., & Cleland, J. G. (1999). Impaired pulmonary diffusion during exercise in patients with chronic heart failure. *Circulation, 100*(13), 1406-1410.
Smith, S. A., Mammen, P. P., Mitchell, J. H., & Garry, M. G. (2003). Role of the exercise pressor reflex in rats with dilated cardiomyopathy. *Circulation, 108*(9), 1126-1132. doi: 10.1161/01.CIR.0000084538.40542.56
Smith, S. A., Mitchell, J. H., Naseem, R. H., & Garry, M. G. (2005). Mechanoreflex mediates the exaggerated exercise pressor reflex in heart failure. *Circulation, 112*(15), 2293-2300. doi: 10.1161/CIRCULATIONAHA.105.566745
Smith, S. A., Williams, M. A., Mitchell, J. H., Mammen, P. P., & Garry, M. G. (2005). The capsaicin-sensitive afferent neuron in skeletal muscle is abnormal in heart failure. *Circulation, 111*(16), 2056-2065.
Snyder, E. M., Johnson, B. D., & Beck, K. C. (2005). An open-circuit method for determining lung diffusing capacity during exercise: comparison to rebreathe. *J Appl Physiol (1985), 99*(5), 1985-1991. doi: 10.1152/japplphysiol.00348.2005
Spiteri, M. A., Cook, D. G., & Clarke, S. W. (1988). Reliability of eliciting physical signs in examination of the chest. *Lancet, 1*(8590), 873-875.
Spranger, M. D., Sala-Mercado, J. A., Coutsos, M., Kaur, J., Stayer, D., Augustyniak, R. A., & O'Leary, D. S. (2013). Role of cardiac output versus peripheral vasoconstriction in mediating muscle metaboreflex pressor responses: dynamic exercise versus postexercise muscle ischemia. *Am J Physiol Regul Integr Comp Physiol, 304*(8), R657-663. doi: 10.1152/ajpregu.00601.2012
Starling, E. H. (1918). *The Linacre lecture on the law of the heart*: Longmans, Green, & Company.

Stebbins, C. L., Maruoka, Y., & Longhurst, J. C. (1986). Prostaglandins contribute to cardiovascular reflexes evoked by static muscular contraction. *Circ Res, 59*(6), 645-654.

Sterns, Ettinger, S., Gray, K., Whisler, S., Mosher, T., Smith, M., & Sinoway, L. (1991). Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation, 84*(5), 2034-2039.

Stevenson, L. W., & Perloff, J. K. (1989). The limited reliability of physical signs for estimating hemodynamics in chronic heart failure. *JAMA, 261*(6), 884-888.

Strange, S., Secher, N. H., Pawelczyk, J. A., Karpakka, J., Christensen, N. J., Mitchell, J. H., & Saltin, B. (1993). Neural control of cardiovascular responses and of ventilation during dynamic exercise in man. *J Physiol, 470*(1), 693-704.

Stratton, J. R., Levy, W. C., Cerqueira, M. D., Schwartz, R. S., & Abrass, I. B. (1994). Cardiovascular responses to exercise. Effects of aging and exercise training in healthy men. *Circulation, 89*(4), 1648-1655.

Stringer, W. W., Hansen, J. E., & Wasserman, K. (1997). Cardiac output estimated noninvasively from oxygen uptake during exercise. *J Appl Physiol (1985), 82*(3), 908-912.

Sullivan, Knight, Higginbotham, & Cobb. (1989a). Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure. Muscle blood flow is reduced with maintenance of arterial perfusion pressure. *Circulation, 80*(4), 769-781.

Sullivan, M. J., Cobb, F. R., & Higginbotham, M. B. (1991). Stroke volume increases by similar mechanisms during upright exercise in normal men and women. *Am J Cardiol, 67*(16), 1405-1412.

Sullivan, M. J., Higginbotham, M. B., & Cobb, F. R. (1988). Increased exercise ventilation in patients with chronic heart failure: intact ventilatory control despite hemodynamic and pulmonary abnormalities. *Circulation, 77*(3), 552-559.

Sullivan, M. J., Knight, J. D., Higginbotham, M. B., & Cobb, F. R. (1989b). Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure. Muscle blood flow is reduced with maintenance of arterial perfusion pressure. *Circulation, 80*(4), 769-781.

Szlachcic, J., Masse, B. M., Kramer, B. L., & Tubau, J. (1985). Correlates and prognostic implication of exercise capacity in chronic congestive heart failure. *Am J Cardiol, 55*(8), 1037-1042.

Szlachcic, J., Massie, B. M., Kramer, B. L., Topic, N., & Tubau, J. (1985). Correlates and prognostic implication of exercise capacity in chronic congestive heart failure. *Am J Cardiol, 55*(8), 1037-1042.

Tallarida, R. J., Rusy, B. F., & Loughnane, M. H. (1970). Left ventricular wall acceleration and the law of Laplace. *Cardiovasc Res, 4*(2), 217-223.

Tamhane, R. M., Johnson, R. L., Jr., & Hsia, C. C. (2001). Pulmonary membrane diffusing capacity and capillary blood volume measured during exercise from nitric oxide uptake. *Chest, 120*(6), 1850-1856.

Tan, L. B. (1986). Cardiac pumping capability and prognosis in heart failure. *Lancet, 2*(8520), 1360-1363.

Taylor, B. J., Mojica, C. R., Olson, T. P., Woods, P. R., Frantz, R. P., & Johnson, B. D. (2013). A possible role for systemic hypoxia in the reactive component of pulmonary hypertension in heart failure. *J Card Fail, 19*(1), 50-59. doi: 10.1016/j.cardfail.2012.11.005

Taylor, B. J., Olson, T. P., Chul Ho, K., Maccarter, D., & Johnson, B. D. (2013). Use of noninvasive gas exchange to track pulmonary vascular responses to exercise in heart failure. *Clin Med Insights Circ Respir Pulm Med, 7*, 53-60. doi: 10.4137/CCRPM.S12178

Tolle, J. J., Waxman, A. B., Van Horn, T. L., Pappagianopoulos, P. P., & Systrom, D. M. (2008). Exercise-induced pulmonary arterial hypertension. *Circulation, 118*(21), 2183-2189. doi: 10.1161/CIRCULATIONAHA.108.787101

Trinkmann, F., Papavassiliu, T., Kraus, F., Leweling, H., Schoenberg, S. O., Borggrefe, M., . . . Saur, J. (2009). Inert gas rebreathing: the effect of haemoglobin based pulmonary shunt flow correction on the accuracy of cardiac output measurements in clinical practice. *Clin Physiol Funct Imaging, 29*(4), 255-262. doi: 10.1111/j.1475-097X.2009.00861.x

Tsuchimochi, H., Matsukawa, K., Komine, H., & Murata, J. (2002). Direct measurement of cardiac sympathetic efferent nerve activity during dynamic exercise. *Am J Physiol Heart Circ Physiol, 283*(5), H1896-1906. doi: 10.1152/ajpheart.00112.2002

van der Lee, I., Zanen, P., Stigter, N., van den Bosch, J. M., & Lammers, J. W. (2007). Diffusing capacity for nitric oxide: reference values and dependence on alveolar volume. *Respir Med, 101*(7), 1579-1584. doi: 10.1016/j.rmed.2006.12.001

VanIterson, E. H., Snyder, E. M., Joyner, M. J., Johnson, B. D., & Olson, T. P. (2014). Effect of Locomotor Muscle Afferent Feedback Inhibition on Cardiac Power During Submaximal Exercise in Heart Failure. *Circulation, 130*(Suppl 2), A16770-A16770.

Vasan, R. S., Larson, M. G., Benjamin, E. J., Evans, J. C., Reiss, C. K., & Levy, D. (1999). Congestive heart failure in subjects with normal versus reduced left ventricular ejection fraction: prevalence and mortality in a population-based cohort. *J Am Coll Cardiol, 33*(7), 1948-1955.

Victor, R. G., Bertocci, L. A., Pryor, S. L., & Nunnally, R. L. (1988). Sympathetic nerve discharge is coupled to muscle cell pH during exercise in humans. *Journal of Clinical Investigation, 82*(4), 1301.

Victor, R. G., Seals, D. R., & Mark, A. L. (1987). Differential control of heart rate and sympathetic nerve activity during dynamic exercise. Insight from intraneural recordings in humans. *J Clin Invest, 79*(2), 508-516. doi: 10.1172/JCI112841

Villars, P. S., Hamlin, S. K., Shaw, A. D., & Kanusky, J. T. (2004). Role of diastole in left ventricular function, I: Biochemical and biomechanical events. *Am J Crit Care, 13*(5), 394-403; quiz 404-395.

Vorluni, L., & Volianitis, S. (2008). Baroreflex control of sinus node during dynamic exercise in humans: effect of muscle mechanoreflex. *Acta Physiol (Oxf), 192*(3), 351-357. doi: 10.1111/j.1748-1716.2007.01766.x

Vorluni, L., & Volianitis, S. (2010). Interaction of cardiac and muscle mechanical afferents on baroreflex control of the sinus node during dynamic exercise. *Scand J Med Sci Sports, 20*(3), 434-440. doi: 10.1111/j.1600-0838.2009.00902.x

Wang, H. J., Li, Y. L., Gao, L., Zucker, I. H., & Wang, W. (2010). Alteration in skeletal muscle afferents in rats with chronic heart failure. *J Physiol, 588*(Pt 24), 5033-5047. doi: 10.1113/jphysiol.2010.199562

Warburton, D. E., Haykowsky, M. J., Quinney, H. A., Blackmore, D., Teo, K. K., & Humen, D. P. (2002). Myocardial response to incremental exercise in endurance-trained athletes: influence of heart rate, contractility and the Frank-Starling effect. *Exp Physiol, 87*(5), 613-622.

Washburn, E. W. (1921). The dynamics of capillary flow. *Physical review, 17*(3), 273.

Weber, K. T., & Janicki, J. S. (1985). Cardiopulmonary exercise testing for evaluation of chronic cardiac failure. *Am J Cardiol, 55*(2), 22A-31A.

Weber, K. T., Kinasewitz, G. T., Janicki, J. S., & Fishman, A. P. (1982). Oxygen utilization and ventilation during exercise in patients with chronic cardiac failure. *Circulation, 65*(6), 1213-1223.

Werko, L., Berseus, S., & Lagerlof, H. (1949). A comparison of the direct Fick and the Grollman methods for determination of the cardiac output in man. *J Clin Invest, 28*(3), 516-520.

West, J. B., Dollery, C. T., & Naimark, A. (1964). Distribution of Blood Flow in Isolated Lung; Relation to Vascular and Alveolar Pressures. *J Appl Physiol, 19*(4), 713-724.

Wheatley, C. M., Foxx-Lupo, W. T., Cassuto, N. A., Wong, E. C., Daines, C. L., Morgan, W. J., & Snyder, E. M. (2011). Impaired lung diffusing capacity for nitric oxide and alveolar-capillary membrane conductance results in oxygen desaturation during exercise in patients with cystic fibrosis. *J Cyst Fibros, 10*(1), 45-53. doi: 10.1016/j.jcf.2010.09.006

Whipp, B. J., Higgenbotham, M. B., & Cobb, F. C. (1996). Estimating exercise stroke volume from asymptotic oxygen pulse in humans. *J Appl Physiol (1985), 81*(6), 2674-2679.

White, M. J., Lykidis, C. K., & Balanos, G. M. (2013). The pulmonary vascular response to combined activation of the muscle metaboreflex and mechanoreflex. *Exp Physiol, 98*(3), 758-767. doi: 10.1113/expphysiol.2012.068528

Willens, H. J., Blevins, R. D., Wrisley, D., Antonishen, D., Reinstein, D., & Rubenfire, M. (1987). The prognostic value of functional capacity in patients with mild to moderate heart failure. *Am Heart J, 114*(2), 377-382.

Williams, Jackson, M., Cooke, G. A., Barker, D., Patwala, A., Wright, D. J., . . . Tan, L.-B. (2005). How do different indicators of cardiac pump function impact upon the long-term prognosis of patients with chronic heart failure? *American heart journal, 150*(5), 983. e981-983. e986.

Williams, S. G., Cooke, G. A., Wright, D. J., Parsons, W. J., Riley, R. L., Marshall, P., & Tan, L. B. (2001). Peak exercise cardiac power output; a direct indicator of cardiac function strongly predictive of prognosis in chronic heart failure. *Eur Heart J, 22*(16), 1496-1503. doi: 10.1053/euhj.2000.2547

Williams, S. G., Tzeng, B. H., Barker, D., & Tan, L. B. (2005). Comparison and relation of indirect and direct dynamic indexes of cardiac pumping capacity in chronic heart failure. *Am J Cardiol, 96*(8), 1149-1150. doi: 10.1016/j.amjcard.2005.06.051

Wilson, Martin, J. L., & Ferraro, N. (1984). Impaired skeletal muscle nutritive flow during exercise in patients with congestive heart failure: role of cardiac pump dysfunction as determined by the effect of dobutamine. *Am J Cardiol, 53*(9), 1308-1315.

Wilson, Rayos, G. H., Yeoh, T. K., & Gothard, P. (1995). Dissociation between peak exercise oxygen consumption and hemodynamic dysfunction in potential heart transplant candidates. *Journal of the American College of Cardiology, 26*(2), 429-435.

Wilson, J. R., Martin, J. L., Schwartz, D., & Ferraro, N. (1984). Exercise intolerance in patients with chronic heart failure: role of impaired nutritive flow to skeletal muscle. *Circulation, 69*(6), 1079-1087.

Zelis, R., Mason, D. T., & Braunwald, E. (1969). Partition of blood flow to the cutaneous and muscular beds of the forearm at rest and during leg exercise in normal subjects and in patients with heart failure. *Circ Res, 24*(6), 799-806.

Zhou, B., Conlee, R. K., Jensen, R., Fellingham, G. W., George, J. D., & Fisher, A. G. (2001). Stroke volume does not plateau during graded exercise in elite male distance runners. *Medicine and science in sports and exercise, 33*(11), 1849-1854.