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Causes And Potential Treatment For Altered Muscle Metaboreflex Control Of Ventricular Vascular Interactions In Heart Failure

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CAUSES AND POTENTIAL TREATMENT FOR ALTERED MUSCLE METABOREFLEX CONTROL OF VENTRICULAR VASCULAR INTERACTIONS IN HEART FAILURE

by

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Advisor Date

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DEDICATION

This dissertation is dedicated to my parents, who gave unconditional love and support in my pursuit of higher education and research.

To my siblings who encouraged me to strive for a greater future.

To my dear friends whose company and understanding of the requirements of a PhD has been paramount to my success.

To Kristin Richardson, my greatest supporter, my rock during highs and lows, the love of my life, without you this would have been an impossible feat.

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CHAPTER 1: INTRODUCTION

Cardiovascular adaptations during exercise are dependent on the effects of three major neural control mechanisms. The first is central command, which is associated with alterations in parasympathetic activity and minimally impacts sympathetic activity (62, 175, 178, 184). The second is the arterial baroreflex which is responsible for autonomic control of heart rate, and systemic vascular resistance for the maintenance of blood pressure (4, 31, 37, 60, 73, 77, 82, 87, 93, 107, 116, 119, 149, 152). The last is known as the exercise pressor response which is due to activation of skeletal muscle afferents. These afferents can be generally subdivided into mechanosensitive type III afferents, and metabosensitive type IV afferents, which together culminate in robust increases mean arterial pressure driven by increased cardiac output via enhanced ventricular function, tachycardia, and central blood volume mobilization (19, 58, 69, 70, 80, 100, 115, 120, 121, 127, 131, 145, 163) in combination with peripheral vasoconstriction. The relative roles of cardiac output vs. the peripheral vasculature varies with workload as well as patho-physiological state.

A profound modulator of central and peripheral cardiovascular function during exercise is activation of metabolite-sensitive skeletal muscle afferents caused mismatch between oxygen demand and oxygen delivery (5, 47, 49, 143, 150, 153). Ischemia results in an enhanced metabolite load that leads to the activation of multiple receptors located on type III polymodal and type IV skeletal muscle afferents (2, 91, 92, 117, 142, 159, 160, 162, 165, 169). These afferents in turn elicit a sympathetic mediated response causing increased ventricular function, tachycardia, peripheral vasoconstriction and enhanced central blood volume mobilization to increase cardiac output and mean arterial pressure and thereby increase active skeletal muscle perfusion (13, 19, 24, 42, 43, 45, 49, 115,

127, 128, 131, 140, 145, 151, 153). Although the muscle metaboreflex does enhance ventricular performance coronary vasoconstriction occurs that functionally limits these increases (14, 40). In addition to the stifling of ventricular performance there is also opposition to reflex induced vasoconstriction via adrenal release of epinephrine and subsequent β_2 mediated vasodilation within the active skeletal muscle from which the reflex originates (94).

In heart failure, ventricular function is compromised and the mechanisms by which muscle metaboreflex activation increases mean arterial pressure are altered (64, 65, 78). The traditional mechanisms of increasing cardiac output is stifled by reductions in ventricular contractility brought on by the pathology of heart failure and enhanced coronary vasoconstriction (13, 41, 129, 144). This in turn shifts the reflex to raise mean arterial pressure by inducing profound systemic vasoconstriction that even effects the active skeletal muscle from which the reflex originates (13, 64, 95, 98, 99). Thus, a positive feedback loop can arise in which enhanced vasoconstriction leads to further limitations in skeletal muscle perfusion that then further exaggerates the sympathetic activity of the muscle metaboreflex.

Inasmuch as changes in ventricular and vascular performance during muscle metaboreflex activation have been observed before and during heart failure, no study has yet assessed the efficiency of the interaction between the left ventricle and the systemic vasculature; termed ventricular-vascular coupling with regard to muscle metaboreflex activation. This interaction is assessed by the ratio of effective arterial elastance to ventricular maximal elastance (32-34, 39, 54, 57, 89, 102). Effective arterial elastance is an index of vascular load that integrates vascular impedance and conductance, systolic and diastolic time decay, and resistance (16, 17, 29, 33, 34, 57, 97, 110, 111, 113, 133,

134, 138, 139, 171). The muscle metaboreflex in healthy subjects has been shown to produce vasodilation and vasoconstriction dependent on the vascular bed and both impact aspects of effective arterial elastance. Ventricular maximal elastance is an assessment of how well the left ventricle contracts. Muscle metaboreflex activation enhances ventricular maximal elastance and these increases are severely attenuated in heart failure (13, 41, 144). Whether or not effective arterial elastance in healthy or heart failure subjects matches the changes in ventricular elastance to maintain ventricular-vascular coupling and optimal energy transfer and thereby increase stroke work during muscle metaboreflex activation is unknown.

Ventricular contractile dynamics during muscle metaboreflex activation have been evaluated extensively showing that ventricular maximal elastance, pre-load recruitable stroke work, and dP/dt_{MAX} are all increased (13, 14, 40, 41, 127, 144, 145). However, assessment of relaxation dynamics and their relationship to contractile dynamics has only been evaluated in vitro. In vitro studies have shown in multiple genetic models of cardiovascular disease that the ratio of force generation per unit time (dF/dt_{MAX} and $_{MIN}$) of the left ventricle sarcomeres varies minimally regardless of changes in frequency of contraction, length of muscle stretch, and β adrenergic stimulation (85, 86). No study has evaluated if these effects are maintained in a whole organ *in vivo*

The muscle metaboreflex is elicited by metabolites interacting with metabosensitive afferents. Multiple receptors exist on metabosensitive afferents such as purinergic (P2X), acid sensing ion channels (ASICs), μ opioid receptors (μ), and transient receptor potential vanilloid 1 cation channels (TRPV1) (2, 6-10, 20, 56, 59, 66, 70, 75, 91, 92, 136, 142, 156, 161, 169, 176, 177, 179, 181). Multiple studies have investigated short term treatments aimed to attenuate signaling through these receptors and thus attenuate

pressor responses during exercise in various cardiovascular pathologies and alleviate the sensation of fatigue (8-10, 20, 136, 169, 177, 181). However, these treatments are dependent on systemically administered therapeutic compounds that may have off target effects. Thus, the need for a selective long-term method of attenuation of metabosensitive afferents is needed. TRPV1 channels are activated by changes in H+ concentration but are also capsaicin sensitive. Resiniferatoxin (RTX) is an ultra-potent dominant negative agonist of TRPV1 channels that achieve chronic ablation through calcium cytotoxicity that has been used to attenuate metabosensitive cardiac sympathetic afferents in heart failure, as well as the relief of nociceptive pain (1, 26-28, 46, 61, 108, 146, 162, 173, 181). Whether or not administration of intrathecal RTX is capable of effectively attenuating metabosensitive afferents responsible for the muscle metaboreflex has yet to be determined. If RTX can effectively attenuate muscle metaboreflex responses in healthy subjects it poses a potential therapy to alleviate over sympatho-excitation during exercise in heart failure.

These studies investigate muscle metaboreflex control of ventricular-vascular coupling, ventricular dynamics, and whether these responses can be attenuated via chronic ablation of skeletal muscle afferents. These studies utilized a chronically instrumented canine model in which each animal serves as its own control in a longitudinal design.

SPECIFIC AIM 1: Evaluating the effect of muscle metaboreflex activation on effective arterial elastance and ventricular-vascular coupling before and after induction of heart failure.

I hypothesize that muscle metaboreflex activation will increase effective arterial elastance to match ventricular maximal elastance, maintaining ventricular-vascular coupling, such that optimal energy transfer from the left ventricle to the systemic

circulation is maintained. In heart failure, metaboreflex-induced increases in arterial elastance are increased and with impaired ability to raise ventricular elastance the ventricular-vascular relationship becomes further uncoupled and thus worsens the already sub optimal energy transfer in heart failure.

SPECIFIC AIM 2: Assessment of ventricular contraction and relaxation dynamics during muscle metaboreflex activation before and after induction of heart failure.

I hypothesize that muscle metaboreflex activation will alter the ventricular contraction-relaxation ratio such that relaxation and contraction will become coupled at similar rates. Heart failure will attenuate increases in relaxation and contraction such that minimal changes will occur across all workloads. Furthermore, similar to previous observation on the impact of coronary vasoconstriction in heart failure on ventricular contractility, I hypothesize heart failure will significantly attenuate relaxation function such that it becomes a likely contributor to reductions in the ability to raise cardiac output.

SPECIFIC AIM 3: Evaluate the efficacy of chronic ablation of metabosensitive skeletal muscle afferents during muscle metaboreflex activation using intrathecal RTX

I hypothesize that intrathecal RTX will significantly attenuate muscle metaboreflex responses. The effects of RTX will last weeks thereby indicating a possible mechanism to reduce exaggerated sympathetic activity during exercise in heart failure.

CHAPTER 2: MUSCLE METABOREFLEX-INDUCED INCREASES IN EFFECTIVE ARTERIAL ELASTANCE: EFFECT OF HEART FAILURE

(This Chapter contains previously published material. See Appendix B.)

ABSTRACT

Dynamic exercise elicits robust increases in sympathetic activity in part due to muscle metaboreflex activation (MMA) – a pressor response triggered by activation of skeletal muscle afferents. MMA during dynamic exercise increases arterial pressure by increasing cardiac output via increases in heart rate, ventricular contractility, and central blood volume mobilization. In heart failure ventricular function is compromised and MMA elicits peripheral vasoconstriction. Ventricular-vascular coupling reflects the efficiency of energy transfer from the left ventricle to the systemic circulation and is calculated as the ratio of effective arterial elastance (E_a) to left ventricular maximal elastance (E_{max}). The effect of MMA on E_a in normal subjects is unknown. Furthermore, whether muscle metaboreflex control of E_a is altered in heart failure has not been investigated. We utilized two previously published methods of evaluating E_a: (end systolic pressure ÷ stroke volume (E_{aPV})) and (heart rate x vascular resistance (E_{aZ})) during rest, mild treadmill exercise and MMA (induced via partial reductions in hindlimb blood flow imposed during exercise) in chronically instrumented conscious canines before and after induction of heart failure via rapid ventricular pacing. In healthy animals MMA elicits significant increases in effective arterial elastance and stroke work that likely maintains ventricular-vascular coupling. In heart failure E_a is high and MMA-induced increases are exaggerated which further exacerbates the already uncoupled ventricular-vascular relationship which likely contributes to the impaired ability to raise stroke work and cardiac output during exercise in heart failure.

INTRODUCTION

During exercise, the ability to maintain adequate systemic perfusion is dependent on optimal ventricular-vascular coupling (89, 90, 101). This relationship is an index of the efficiency to transfer energy from the left ventricle to the arterial circulation, and is calculated as the ratio of left-ventricular end-systolic elastance (E_{ES}) and effective arterial elastance (E_a) (29, 97, 111, 112, 148, 168, 170-172). E_a can be assessed by end-systolic pressure divided by stroke volume, as well as heart rate times systemic vascular resistance (29, 35, 97, 133, 171), and both of these relationships have been used as minimally invasive measures of E_a in humans (11, 21, 32-34, 36, 57, 97, 125, 139). E_a is an index of ventricular afterload and integrates the effects of pressure, resistance, impedance, and cardiac cycle time decay (36, 97, 170, 172). E_{ES} accounts for changes in end-systolic volume and end-systolic pressure, and thereby integrates left-ventricular dynamics into the ventricular-vascular coupling ratio in a manner relatable to the effects on ventricular afterload (21, 22, 29, 36, 97, 102, 111, 139, 170). Both E_a and E_{ES} can be altered by a variety of physiological and pharmacological interventions which affect ventricular performance and vasomotor tone which thereby will alter the ventricular-vascular the coupling ratio which, under optimal conditions, ranges between 0.6 and 1.2 in humans (3, 22, 71, 111). Recently, E_a has been used as a diagnostic assessment measure of ventricular-vascular coupling and ventricular afterload (16, 33, 34, 88, 133, 138). In healthy individuals and canines, E_a increases in response to exercise in parallel with the rise in ventricular performance which maintains an optimal stroke work (34, 125, 133, 134). In pathophysiological conditions such as heart failure, E_a increases whereas E_{ES} decreases, and ventricular-vascular coupling is reduced which compromises the ability to maintain or increase stroke work (102, 110, 111, 147).

When oxygen demand in skeletal muscle exceeds oxygen delivery, metabolites accumulate in the muscle interstitium and stimulate group III and IV afferents which elicit activation of a powerful pressor reflex termed the muscle metaboreflex. Activation of this reflex in healthy individuals during submaximal dynamic exercise induces a pressor response predominantly caused by increases in cardiac output which occurs via increases in heart rate, ventricular contractility and central blood volume mobilization (2, 12, 21-23, 26, 31, 34, 48, 58-60, 62, 68-71, 77). Little net change in systemic vascular conductance is observed as sympathetically-driven vasoconstriction is offset by β_2-mediated vasodilation in response to epinephrine release (6, 8, 19, 36, 38, 40, 51, 58). The rise in total systemic blood flow partially corrects the mismatch between O_2 delivery and O_2 demand which likely acts to maintain performance for a given workload (11, 24, 25, 49, 55, 64). As workload rises above relatively mild levels, skeletal muscle afferents become active, and once workloads reach moderate levels, the muscle metaboreflex is likely tonically active (6, 7, 47, 65, 185).

Muscle metaboreflex activation can elicit marked increases in left-ventricular maximal elastance (40, 145). In heart failure, the muscle metaboreflex can be over active due to poor skeletal muscle perfusion and can induce profound peripheral vasoconstriction (44, 65, 94, 95) which may increase E_a. Increased E_a, coupled with the inability to raise ventricular performance in heart failure (40, 144), could lead to ventricular-vascular uncoupling which could further reduce the capacity to raise stroke work. To what extent MMA influences arterial elastance and affects ventricular-vascular coupling in normal subjects is unknown. Furthermore, how these relationships are altered in subjects with marked systolic dysfunction has not been investigated. We hypothesized that, in normal subjects, MMA will increase E_a in parallel with increases in E_{ES} thereby

maintaining optimal ventricular-vascular coupling and stroke work. However, in heart failure, metaboreflex-induced increases in E_a, without concomitant increases in ventricular function, will result in ventricular-vascular uncoupling and reduced stroke work.

METHODS

Experimental Subjects

Five adult mongrel canines (1 male, 4 females) of approximately 19-25 kg were selected based on willingness to walk on a motor-driven treadmill and then acclimatized to a workload of 3.2 km/hr with 0% incline. We have shown that gender does not affect the strength or mechanisms of the muscle metaboreflex in normal canines (104). No experiments were performed during the estrus phase. All experimental and surgical procedures utilized for this study comply with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and were approved by the Wayne State University Institutional Animal Care and Use Committee (IACUC). Animals utilized in this study underwent a seven-day acclimation period with laboratory surroundings and personnel and exercised on their own volition during all experiments.

Surgical Instrumentation

All animals were instrumented in a series of surgical procedures spanning over a period of at least six weeks using standard aseptic techniques. Prior to each surgery, animals were sedated with an intramuscular injection acepromazine (0.4-0.5 mg/kg) 30 minutes prior to induction of anesthesia with intravenous administration of ketamine (5 mg/kg) and diazepam (0.2-0.3 mg/kg). Anesthesia was then maintained pre-operatively and during surgery with (1-3%) isoflurane gas. Preoperative analgesics included [Fentanyl 75-125 μg/h (72) transdermal delivery] buprenorphine (0.01-0.03 mg/kg IM) and carprofen (4.4 mg/kg IV). Postoperatively acepromazine (0.2-0.3 mg/kg IV) and

buprenorphine (0.01-0.03) were administered. Acute proactive antibiotics (cephalexin 30 mg/kg IV) were administered pre- and post-operatively, and prophylactic antibiotics (cephalexin 30 mg/kg PO BID) were administered to prevent microbial infection.

The first surgical procedure performed was a left thoracotomy exposing the heart through the 3rd/4th intercostal space. The pericardium was incised to allow placement of a telemetric pressure transducer tip (TA11 PA-D70, DSI) into the apex of the left ventricle with the implant body tethered subcutaneously. The upper section of dissected pericardium was then retracted for placement of a blood flow transducer (20PAU, Transonic Systems) around the ascending aortic trunk to measure cardiac output. Four stainless steel pacing leads (0-FLexon, Ethicon) were attached to the right ventricle free wall for subsequent induction of heart failure via rapid ventricular pacing. For studies unrelated to these observations, a blood flow probe (3PSB, Transonic Systems) was placed on the circumflex artery. All leads and wires were tunneled subcutaneously and exteriorized between the scapulae. The pericardium and ribs were reapproximated and the chest was closed in layers.

The second procedure occurred no earlier than two weeks post-thoracotomy. Through a left retroperitoneal approach, all arterial blood vessels caudal to the renal artery and cranial to incision point near the iliac crest were ligated. To measure systemic arterial pressure, a 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) was inserted into the most accessible cranial ligated branch of the terminal aorta. In order, cranial to caudal, a 10 PAU blood flow transducer (Transonic Systems) and two 8-10mm hydraulic occluders (DocXS Biomedical Products) were placed caudal to the systemic arterial pressure catheter to measure and manipulate terminal aortic blood flow. Cables, catheters and occluder lines were then tunneled subcutaneously and exteriorized

between the scapulae. A 4PSB flow probe (Transonic Systems) was placed on the left renal artery and, when accessible, a second 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) was placed in the inferior mesenteric artery (N=2) for purposes unrelated to this study. In the event the mesenteric artery was inaccessible during a separate procedure, no earlier than two weeks after recovery, a 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) was placed in the femoral artery branch through a vertical incision in the right hindlimb. Cables and catheters were exteriorized subcutaneously from their point of origin to the scapulae. The retroperitoneal space was then closed in successive layers.

A third procedure, unrelated to this study, was performed after a two-week recovery period. Via a midline incision in the neck both carotid arteries were accessed and one 4-6 mm hydraulic occluder (DocXS Biomedical Products) was placed on each side. Occluder lines were tunneled subcutaneously to the scapulae and exteriorized. The neck was closed in successive layers and animals recovered for 10-14 days prior to any experimental procedures.

Data Acquisition

All animals were acclimated in the laboratory 10-20 minutes prior to being led to the motor-driven treadmill. Arterial pressure was measured through a connection to a pressure transducer (Transpac IV, ICU Medical). Heart rate and left-ventricular pressure were derived from a left-ventricular pressure sensor (DSI). All flows were measured by connecting flow probes to benchtop flowmeters (Transonic Systems). Hemodynamic variables were monitored and recorded in real time through an A-D converter (iWorx) output into Labscribe2 acquisition and analysis software (iWorx).

Experimental Procedures

Hemodynamic parameters were recorded under steady-state conditions of rest, mild free-flow exercise (3.2 km/h 0% grade), and during sustained exercise with successive reductions in hindlimb blood flow (via partial inflation of the terminal aortic hydraulic occluders to 50-60% of free-flow conditions). After completion of control experiments, heart failure with reduced ejection fraction was induced via rapid ventricular pacing as described in previous studies (13, 18, 41, 64, 65, 78, 79, 83, 95, 98, 129, 131, 144). Briefly, the ventricular pacing wires were connected to a pacemaker, and over several days the rate was increased to ~230 bpm. Rapid ventricular pacing was continued for ~30 days until clear clinical indices of moderate ventricular dysfunction were observed, which included tachycardia in the absence of pacing, low cardiac output, low stroke volume and compromised ventricular contractility shown by dP/dt_{MAX} and $_{MIN}$. The experiments were then repeated as in control. The pacemaker was disconnected ~30 minutes prior to every experiment and then reconnected after the experiment was completed. Thus, each animal served as its own control for these longitudinally designed studies.

Analysis

All *in vivo* hemodynamics (mean arterial pressure, heart rate, cardiac output, left-ventricular pressure, hindlimb blood flow) were continuously recorded for each experiment. Steady state values at rest, during mild exercise, and with MMA were averaged over minute. All other hemodynamic variables were derived mathematically from *in vivo* measurements. For measures of E_a, we utilized two equations initially developed by Kelly et al. (97), one of which has been modified to account for the hydraulic effects of vascular occluder inflation used to activate the muscle metaboreflex. Kelly et al.

(97) denoted the first equation as E_{aPV} and the second as E_{aZ}.

Equation 1:

E_{aPV} = ESP ÷ SV

Equation 2:

E_{aZ} = NIVR x HR

* NIVR = MAP ÷ (CO - HLBF)

- NIVR is non-ischemic vascular resistance and is resistance of all vascular beds except the ischemic hindlimb
- HLBF is hindlimb blood flow and is sequentially partially reduced to elicit muscle metaboreflex activation via hindlimb vascular occluders

Since during metaboreflex activation arterial pressure can increase somewhat simply due to the passive, mechanical effects of the occluder, we adjusted the latter vascular based equation (E_{aZ}) by using only vascular resistance of all non-ischemic beds which thereby only accounts for increases in pressure due to reflex activation (140).

For this study, multiple experiments were conducted on each animal and steady-state values taken at rest, exercise, and maximal muscle metaboreflex activation were averaged across experiments for each individual animal. Averaged steady-state values for each animal were then averaged across all animals, thus each animal contributed to the mean values only once.

Statistical Analysis

All statistics were performed with Systat Software (Systat 11.0) and data are reported as mean ± SE and statistical significance was determined by an α level of $P < 0.05$. The data were analyzed via Two-way ANOVA for Repeated Measures and when a significant interaction was observed, individual means were compared using C Matrix

Test for Simple Effects. The effect of heart failure on the changes induced by MMA (changes from steady state exercise) were compared via Student's Paired T-Tests. Additionally, a Bland-Altman plot was constructed to evaluate the agreement between our two measures of E_a similar to Kelly et al. (97).

RESULTS

Figure 2.1 shows the average hemodynamic parameters during control and after the induction of heart failure at rest, during mild exercise and during MMA. Figure 2.2 shows the resultant calculated levels of E_a: E_{aPV} and E_{aZ} and stroke work in each setting and the changes which occurred between exercise and exercise with MMA. In control experiments during the transition from rest to exercise cardiac output significantly increases with no significant change in mean arterial pressure. During MMA cardiac output increased which occurred via large significant increases in heart rate and

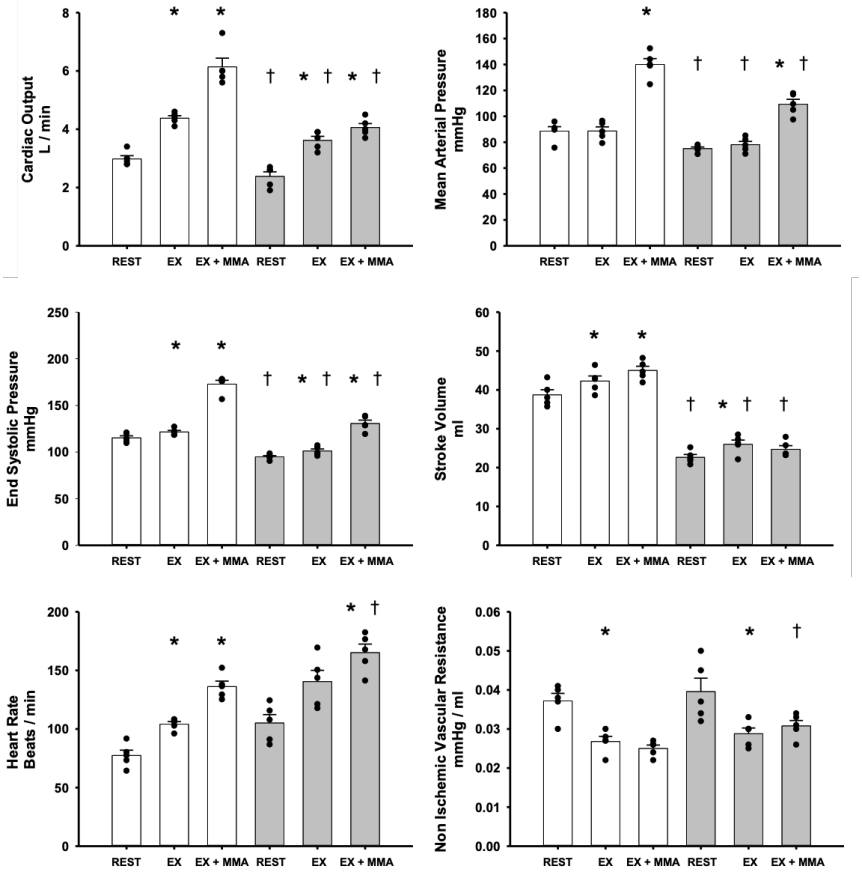

Figure 2.1 *Average one minute steady state values of cardiac output, mean arterial pressure, end systolic pressure, stroke volume, non-ischemic vascular resistance, heart rate, at rest (REST), during free-flow mild exercise (EX), and EX with muscle metaboreflex activation (EX + MMA) induced by graded reductions in hindlimb blood flow of 50-60% before (white bars) and after induction of heart failure (grey bars). Standard error is shown on bar graphs with individual data points overlaid. Statistical significance compared to the previous setting shown as * $P < 0.05$. Comparisons between control and heart failure within each setting shown as † $P < 0.05$. (N=5).*

small but significant increases in stroke volume. Mean arterial pressure during MMA increased significantly due to increased cardiac output with minimal changes in non-ischemic vascular resistance. In control experiments, neither index of E_a changed from rest to mild exercise: E_{aPV} was unaffected as little change in either end-systolic pressure or stroke volume occurred and E_{aZ} was unaffected as decreases in resistance were balanced by increases in heart rate. In response to MMA, both indices of E_a increased: E_{aPV} increased due to large, significant increases in end-systolic pressure with small, significant increases in stroke volume, and E_{aZ} increased due to large increases in heart rate offsetting small reductions in resistance. Stroke work was used as an additional index of ventricular-vascular coupling. Stroke work increased significantly from rest to exercise and then again from exercise to MMA. Figure 2.3 shows left-ventricular dP/dt as an index

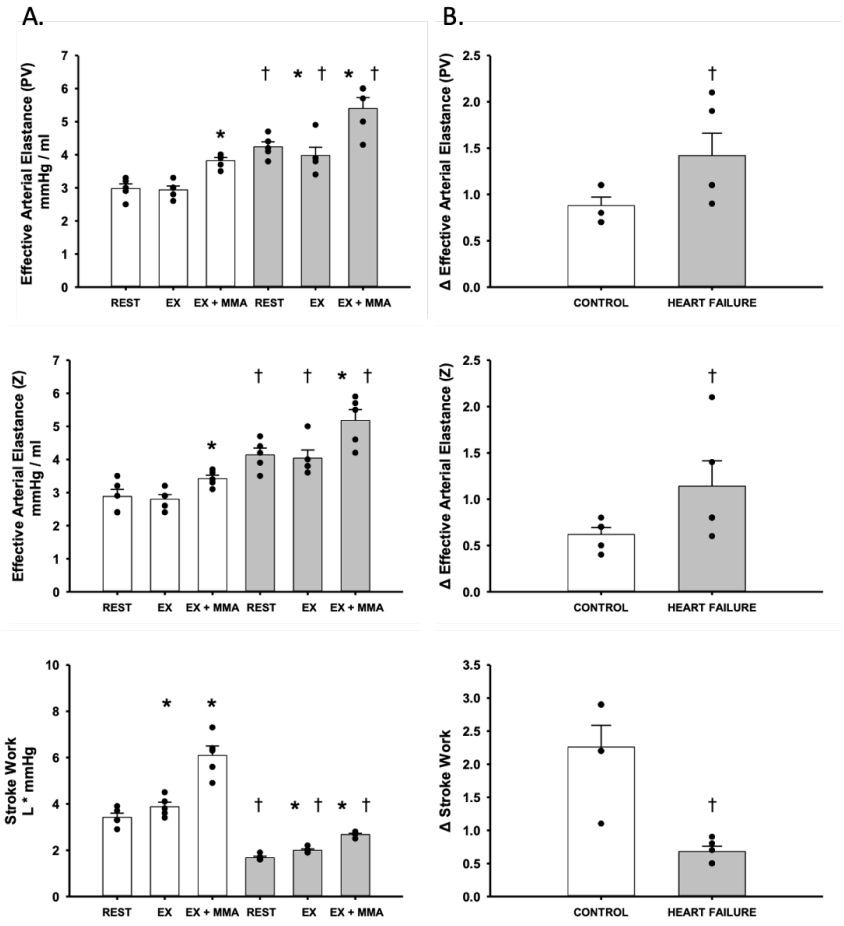

Figure 2.2. ***A)*** *Average one-minute steady state indices and of effective arterial elastance derived from traditional pressure volume loop calculation [(E_{aPV}) end systolic pressure (ESP) divided by stroke volume (SV)] and from vascular components [(E_{aZ}) heart rate (HR) multiplied by non-ischemic vascular resistance (NIVR)]. Mean values shown at rest (REST), during free-flow mild exercise (EX), and EX with muscle metaboreflex activation (EX + MMA) induced by graded reductions in hindlimb blood flow of 50-60% before (white bars) and after induction of heart failure (grey bars).* ***B)*** *Average changes between one-minute steady state values during exercise and exercise with muscle metaboreflex activation. Standard error is shown on bar graphs and individual data points are plotted. Statistical significance between successive settings shown as * P < 0.05. Comparisons between control and heart failure shown as † P < 0.05. (N=5).*

of the ventricular contribution to the ventricular-vascular coupling relationship. Ventricular contractility (dP/dt_{max}) and lusitropy (dP/dt_{min}), significantly increased from rest to mild exercise. During MMA, there were large significant increases in ventricular performance.

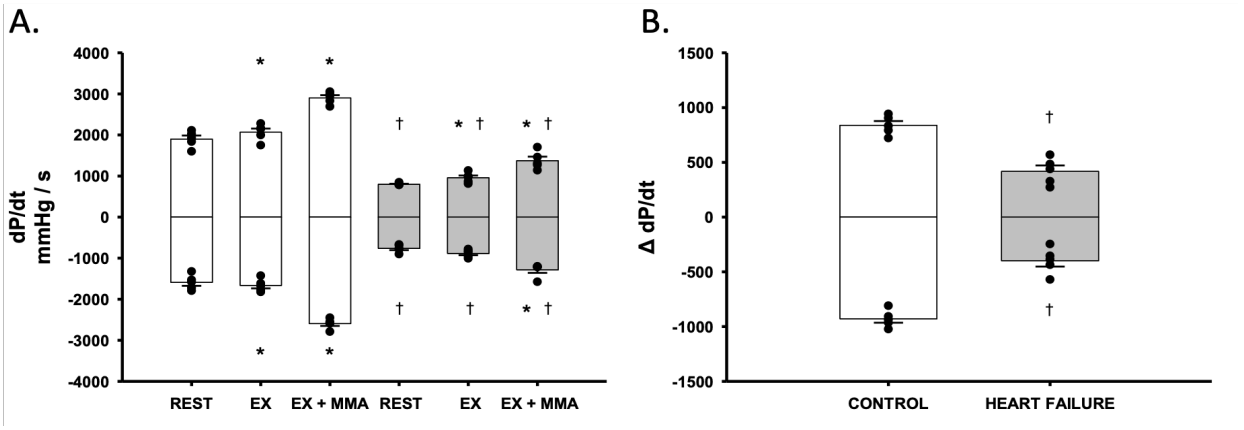

Figure 2.3. A)*Average one-minute steady state indices of ventricular function dP/dt_{MAX} and dP/dt_{MIN} taken from the first derivative of the left ventricular pressure wave. Mean values shown at rest (REST), during free-flow mild exercise (EX), and EX with muscle metaboreflex activation (EX + MMA) induced by graded reductions in hindlimb blood flow of 50-60% before (white bars) and after induction of heart failure (grey bars).* ***B)*** *Average changes between one-minute steady state values during exercise and exercise with muscle metaboreflex activation. Standard error is shown on bar graphs and individual data points are plotted. Statistical significance between successive settings shown as * P < 0.05. Comparisons between control and heart failure shown as † P < 0.05. (N=5).*

In heart failure, end-systolic pressure and stroke volume were significantly lower and heart rate was significantly higher when compared to control (Figure 2.1). Significant reductions in stroke volume with limited significant increases in heart rate in heart failure lead to significant reductions in cardiac output and mean arterial pressure across all workloads. In heart failure increases in cardiac output with MMA were attenuated. After the induction of heart failure, both indices showed that E_a was significantly increased across all settings vs. control. In heart failure, no significant changes were observed from rest to exercise in either index of E_a: for E_{aPV}, similar to control experiments, minimal changes in end-systolic pressure and stroke volume were observed and, again for E_{aZ}, reductions in resistance were offset by increases in heart rate. In response to MMA in heart failure, E_{aPV} significantly increased as a result of significant increases in end-systolic pressure with no significant changes in stroke volume and E_{aZ} significantly increased due

to large, significant increases in heart rate with minimal changes in nonischemic vascular resistance. These muscle metaboreflex-induced increases in E_a were enhanced by heart failure by approximately 35% vs. control. In heart failure, stroke work is significantly increased from rest to exercise, but did not further increase in response to MMA, likely as a result of ventricular-vascular uncoupling. Ventricular function is significantly reduced in heart failure (Figure 2.3). In heart failure, ventricular contractility (dP/dt_{max}) and lusitropy (dP/dt_{min}), significantly increased from rest to mild exercise. Muscle metaboreflex activation in heart failure elicits significant increases in ventricular contractility compared to exercise conditions. However, the capacity to increase ventricular function during MMA is significantly attenuated in heart failure when compared to healthy animals.

Figure 2.4 illustrates a Bland-Altman plot constructed to assess the effects of bias

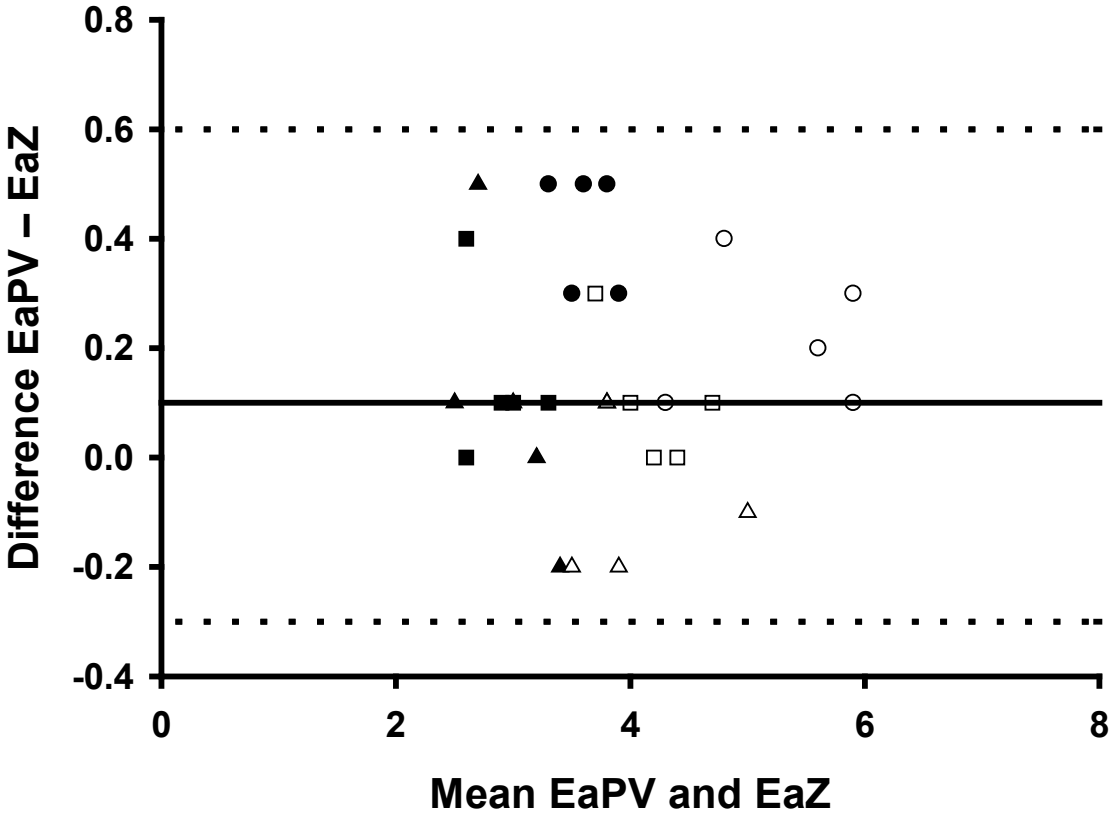

Figure 2.4 *Bland Altman plot evaluating the agreement between E_{aPV} and E_{aZ} before (filled) and after induction of heart failure (open) during rest (squares), free flow exercise (triangles), and exercise with peak muscle metaboreflex activation (circles). Dash lines indicate upper and lower limits of agreement, and the solid line indicates bias. (N=5).*

and agreement between our measures of E_a (E_{aPV} and E_{aZ}) in the 5 animals before and after the induction of heart failure. This plot was constructed by taking the averages of E_{aPV} and E_{aZ} from each animal during each steady state as a means to remove any single animal's bias in the analysis that may impact the 95% confidence interval. For comparisons of measurements, we utilized all collected E_a measures across each workload before and after induction of heart failure and found that the bias between the two measures was only 0.1 and all data points fall within the limits of agreement.

DISCUSSION

This is the first study to demonstrate that muscle metaboreflex activation increases E_a. Furthermore, we showed that muscle metaboreflex-induced increases in E_a are exaggerated after induction of heart failure. In control experiments, metaboreflex-induced increases in E_a occur in parallel with increases in ventricular performance as shown by increases in dP/dt_{MAX} and $_{MIN}$ (13, 40, 41, 144, 145), which thereby serves to sustain the ability to increase stroke work via efficient energy transfer from the left ventricle to the systemic circulation. In contrast, after induction of heart failure, ventricular performance is compromised (40, 41, 144, 145) and E_a is significantly elevated even at rest. In this setting MMA elicits substantial further increases in E_a, yet without concomitant increases in ventricular function, the ventricular – vascular interaction becomes further uncoupled which likely contributes to the impaired ability to raise stroke work and cardiac output.

Arterial Elastance

E_a has been used to estimate the arterial load imposed on ventricular function to gain insight into the regulation of systemic blood flow in normal subjects and in patients with declining ventricular function and increased vascular stiffing with aging and disease (11, 33-35, 57, 97, 103, 125, 138, 139, 148).

E_a was first utilized to estimate stroke volume from vascular parameters and ventricular pressure, but later evolved as a methodology to assess ventricular efficiency as it relates to stroke work (29, 171). Optimal stroke work reflects efficient ventricular-vascular coupling and ensures that for a given cardiac cycle, blood flow mechanics are maintained, thereby preserving adequate systemic blood flow. When the ventricular-vascular relationship becomes uncoupled, the ability of the heart to perform work is impaired. Thus, changes in stroke work indirectly assess changes in ventricular-vascular coupling. We observed that MMA during submaximal dynamic exercise evokes significant increases in E_a as well as stroke work, suggesting that optimal ventricular energetics are maintained. However, after induction of heart failure, E_a is significantly increased and stroke work is significantly reduced, indicating ventricular-vascular uncoupling. With MMA there are further increases in E_a and significant decreases in stroke work. We have previously shown that during MMA in animals after the induction of heart failure there is no increase in E_{ES} (13, 40, 144). Thus, with our present findings demonstrating that E_a rises from an already elevated level and our previous findings that E_{ES} does not change from an already depressed level, we conclude that during MMA in heart failure the already compromised ventricular-vascular relationship becomes even more uncoupled. This likely contributes to the impaired stroke work and cardiac output. With diminished ability to raise cardiac output, the ability of the muscle metaboreflex to raise blood flow to active skeletal muscle is reduced which likely contributes to exercise intolerance and enhanced sympatho-activation observed in heart failure (13, 41, 64, 65, 78, 95, 129, 131, 144).

Maintenance of ventricular-vascular coupling is paramount to cardiovascular efficiency. Instances of altered ventricular-vascular coupling have been observed in a myriad of conditions in which cardiovascular performance is altered or reduced (32, 33,

53, 57, 102, 125, 134). During exercise in healthy subjects, exercise performance is highly dependent on the efficiency of the cardiovascular system to maintain adequate perfusion of active tissues. Traditionally, during submaximal dynamic exercise, the muscle metaboreflex has been considered as a heart-centric reflex that drives the pressor response through increased cardiac output achieved via tachycardia, enhanced ventricular performance, and increased central blood volume mobilization (131, 145). If the ventricular component of ventricular-vascular coupling is not matched by similar changes in arterial properties, ventricular-vascular efficiency would be depressed which could negatively impact systemic blood flow and perfusion pressure. Conversely, if arterial properties were to greatly exceed the contribution of ventricular function, such as with enhanced sympatho-excitation in heart failure and hypertension (64, 95, 166), then energy generated by the heart would be stifled by the increased afterload which could thereby limit perfusion during exercise.

The impact of vascular modulation plays a key role in determining the impact of E_a on ventricular-vascular dynamics. We previously observed that MMA can modulate vascular resistance thru β_2-mediated dilation, vasoconstriction of ischemic skeletal muscle and coronary vascular beds, as well as shifts in reflex characteristics during high-intensity workloads and pathophysiological states (19, 40, 65, 94-96, 140, 166). Furthermore, the baroreflex modulates vascular tone and has been proposed as a buffer to muscle metaboreflex control of vascular function (13, 79, 98-100). To what extent the arterial baroreflex and other vascular-centric sympatho-excitatory stimuli may assist or oppose optimal ventricular-vascular coupling during MMA is unknown.

Methodological Considerations

Our two methods to assess E_a are adaptations of previous derivations and agree

with very small bias as shown by the Bland-Altman plot (Figure 4) (97). We modified one of the methods to calculate E_a to take into account the passive hydraulic effects of the vascular occluder used to activate the muscle metaboreflex. We feel the small observed bias occurs in part due to the location of the arterial pressure catheter. This catheter is located downstream within the aorta at a point in which we feel the impact of systemic impedance and resistance are more influential on the calculation of E_{aZ}. Inasmuch as the ventricle is also subject to these factors, we believe variability in the lumped effect of impedance and resistance at the aortic valve are reduced as this point is the culmination of wave reflection, resistance and impedance. We used NIVR in place of TPR to eliminate the effects of the vascular occluder on measured resistance and this substantially reduced the bias between the two indices of E_a. Inflation of the vascular occluder also raises pressure due to the passive hydraulic effect (19). We can subtract the difference between observed mean arterial pressure and the calculated effect of the rise in mean arterial pressure due to inflation of the occluder (19, 150) and this in turn normalizes the difference in the two measures accounting for bias observed across individual conditions such that the absolute value of the bias calculated a Bland-Altman plot remains the same. In regard to values near or just above our limits of agreement, we find that applying this estimate brings them closer to the mean bias and well within the 95% confidence interval. Therefore, we believe the data presented in this study coincide with Kelly et al. (97), and that E_{aPV} and E_{aZ} may act as interchangeable measures of E_a for future studies so long as accommodations are made to account for the impact of the hydraulic effects of the occluder. Both indices of E_a increased with MMA in parallel with increases in ventricular contractility (40, 41, 144, 145).

Muscle Metaboreflex in Heart Failure

Our previous studies have shown that the strength and mechanisms of the muscle metaboreflex are altered in heart failure (13, 41, 64, 83, 95, 99, 129, 131, 144) which are supported by subsequent studies in humans (15, 42-45, 50, 58, 59, 67, 76, 77, 122, 141, 154, 155, 158-161). Whether the strength (gain) of this reflex is increased or decreased is of some debate (10, 19, 23, 41, 44, 51, 78, 95, 99, 114, 117, 118, 120, 121, 129, 131, 154, 157, 163) and our studies support both sides. Much depends on how the reflex strength is quantified. Clearly, with the impaired ventricular function in heart failure, the ability of the reflex to raise cardiac output is attenuated (13, 40, 64, 129). However, we have also shown that metaboreflex induced-peripheral vasoconstriction (64, 65) as well as constriction of the coronary circulation (41) and even the ischemic active skeletal muscle from which the reflex arises are enhanced in heart failure (95). Metaboreflex-induced increases in the release of renin and vasopressin are also substantially increased in heart failure as are the plasma levels of circulating norepinephrine indicating increased sympatho-activation (65). These latter observations would support an accentuated reflex. Further complicating the issue is that the metaboreflex is buffered by the arterial baroreflex (4, 93, 99, 100) which in normal animals reduces the pressor response by ~ 50% (152). The greater metaboreflex-induced pressor response observed after baroreceptor denervation occurs by substantial peripheral vasoconstriction now accompanying the rise in cardiac output (98). Thus, the baroreflex buffers the metaboreflex by preventing metaboreflex-induced peripheral vasoconstriction (98-100). In heart failure, baroreflex buffering is likely attenuated and baroreceptor denervation induces little change in the mechanisms of the muscle metaboreflex (98, 99). In heart failure, the metaboreflex switches from the normal response of increased cardiac output

and little vasoconstriction to limited increases in cardiac output and substantial vasoconstriction (41, 44, 64, 65, 95, 129, 131, 144). After baroreceptor denervation in heart failure this same pattern remains, but modestly exaggerated (99). These results would support the hypothesis that in heart failure, depressed baroreflex buffering may allow greater metaboreflex-induced peripheral vasoconstriction.

During mild exercise, a threshold level of hindlimb blood flow exists before the metaboreflex is engaged. In heart failure, the normal level of blood flow is lower, but a clear threshold is still seen during mild exercise. However, at moderate workloads the threshold of the muscle metaboreflex is much closer to the normal level of blood flow even in normal animals and minimal if any reduction in hindlimb blood flow is capable of eliciting, or further activating the muscle metaboreflex (64). Studies in humans also concluded that skeletal muscle afferents are tonically active and contribute to reflex cardiovascular responses to dynamic exercise at relatively low workloads (10). In heart failure, during moderate exercise the level of hindlimb blood flow is already below the threshold as ascribed in the control experiments prior to heart failure induction and any further reduction in blood flow causes further increases in sympathetic activity indicating that the reflex is over-active in heart failure due to impaired skeletal muscle blood flow (13, 19, 65, 140, 145)

Perspectives and Significance

This is the first study to evaluate muscle metaboreflex control of effective arterial elastance and thereby ventricular-vascular coupling and stroke work. Our results show that activation of the muscle metaboreflex increases effective arterial elastance to a similar extent as we have previously shown for maximal ventricular elastance (144, 145) which thus maintains ventricular-vascular coupling at optimal levels. Heart failure

enhances E_a at rest and reduces stroke work and ventricular maximal elastance (144, 145) causing ventricular-vascular uncoupling. With metaboreflex activation substantial further increases in E_a occur which are not paralleled by increases in maximal ventricular elastance (144) thereby further uncoupling the ventricular-vascular relationship which attenuates increases in stroke work, cardiac output and ultimately skeletal muscle perfusion. Impaired skeletal muscle blood flow during exercise likely over-activates the muscle metaboreflex contributing to the often profound sympatho-activation (6, 8, 10, 13, 65, 67, 126, 157, 169). Thus, limitations in skeletal muscle perfusion as a result of inadequate energy transfer from the left ventricle to the system circulation may be the cause of exaggerated sympathetic activation during exercise in heart failure which may contribute to exercise intolerance.

CHAPTER 3: VENTRICULAR CONTRACTION AND RELAXATION RATES DURING MUSCLE METABOREFLEX ACTIVATION IN HEART FAILURE: ARE THEY COUPLED?

ABSTRACT

The relationship between contraction and relaxation rates of the left ventricle varies with exercise and is paramount to the maintenance of cardiovascular performance during exercise. In *in vitro* models, this ratio was shown to be relatively unaltered by changes in sarcomere length, frequency of stimulation and β adrenergic stimulation. In this study we investigated whether the ratio of contraction to relaxation rate is maintained in the whole heart during exercise and sympathetic activation elicited by the muscle metaboreflex and whether heart failure alters these relationships. We observed that in healthy subjects the ratio of contraction to relaxation increases from rest to exercise as a result of a higher increase in contraction relative to relaxation. During muscle metaboreflex activation the ratio of contraction to relaxation is significantly reduced towards 1.0 due to a large increase in relaxation rate matching contraction rate. After induction of heart failure, contraction and relaxation rates are significantly reduced and increases during exercise are attenuated. A significant increase in the ratio was observed from rest to exercise although baseline ratio values were significantly reduced close to 1.0 when compared to healthy subjects. There was no significant change observed between exercise and muscle metaboreflex activation nor was the ratio during muscle metaboreflex activation significantly different between heart failure and control. We conclude that heart failure reduces the muscle metaboreflex gain and absolute values of contraction and relaxation rates. Furthermore, we observed that the ratio of contraction and relaxation rates is not significantly different, however, significant changes in the ratio in healthy subjects as a result of increased relaxation rate are abolished in heart failure.

INTRODUCTION

Ventricular performance is a key determinant of the cardiac response to dynamic exercise and the maintenance of exercise workload. A key reflex mediating increases in cardiac output in response to exercise is the muscle metaboreflex - initiated via increases in activity of skeletal muscle afferents sensitive to increases in metabolite concentration (2, 13, 40, 42, 43, 45, 64, 91, 92, 96, 121, 123, 127, 128, 131, 142, 151, 158-161). When activated this reflex causes significant increases in heart rate, stroke volume, ventricular performance, and central blood volume mobilization which increases cardiac output and thereby perfusion of the active skeletal muscle (42, 43, 45, 64, 96, 127, 128, 131, 140, 145). After induction of heart failure, the ability of this reflex to raise ventricular performance and thus cardiac output is attenuated, and the reflex now elicits profound peripheral vasoconstriction including within the ventricular myocardium and even the active skeletal muscle, the tissue from which the reflex originates (13, 41, 44, 64, 95, 99, 129, 144, 154, 157). Inasmuch as muscle metaboreflex control of central blood volume mobilization is maintained in heart failure (131), the mechanism for the reduction in the ability to raise cardiac output during muscle metaboreflex activation is likely impaired ventricular dynamics due to both the inherent dysfunction of the myocardium coupled with enhanced coronary vasoconstriction (13, 40, 41). Previously we have reported that left ventricular maximal elastance, preload recruitable stroke work, and dP/dt_{MAX} and $_{MIN}$ are all reduced and exhibit attenuated responses during muscle metaboreflex activation (MMA) in heart failure (13, 41, 129, 144). Previous in-vitro studies of sarcomere dynamics determined that the ratio of contraction to relaxation rates are maintained in cardiac muscle in response to changes in frequency, length, and beta adrenergic stimulation (85, 86). Whether or not aspects of single sarcomere dynamics will be reflected in the whole

heart in-vivo has yet to be determined. We assessed whether ventricular contraction and relaxation rates as determined by dP/dt_{MAX} and $_{MIN}$ in the canine model are coupled between rest and mild exercise and with pronounced further sympathetic activation induced by MMA. We also assessed whether heart failure affects these relationships. Secondly, we wished to determine the strength or gain of the muscle metaboreflex in the control of ventricular contraction and relaxation rates before and after the induction of heart failure. We hypothesized that muscle metaboreflex activation before or after heart failure will not impede contraction-relaxation coupling and heart failure will induce a significant reduction in the gain (strength) of ventricular contraction and relaxation rates.

METHODS

Experimental subjects. The experiments were performed on 14 adult mongrel canines (1 male, 13 females) of approximately 18-25 kg were selected on their willingness to walk on a motor driven treadmill prior to acclimatization to a workload of 3.2 km/h 0% grade. The gender mix was determined by the availability of animals from the vendor. Previously we have shown that sex does not significantly impact the strength or mechanisms of the muscle metaboreflex (104). No experiments during frank estrus were included in this study. All procedures, surgical and experimental used in this study comply with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and were approved by the Wayne State University Institutional Animal Care and Use Committee (IACUC). All animals in this study underwent a 7-14-day acclimation period to the laboratory surroundings and personnel prior to volitional treadmill exercise. All animals exercised voluntarily during all experiments.

Surgical Procedures

Animals underwent a series of surgical procedures spanning 4-6 weeks utilizing

standard aseptic techniques. All surgical procedures and medications used in this study have been described previously (13, 40, 41, 93, 95, 96, 115, 144, 145). Briefly the first procedure was a left thoracotomy thru the 3rd/4th intercostal space to access the heart. The pericardium was incised cranially to caudally to access the apex of the heart for insertion of a telemetric pressure transducer tip (TA11 PA-D70, DSI) to measure LV pressure, and to access the ascending aorta for placement of a blood flow transducer (20PAU, Transonic Systems) for measures of cardiac output. Four stainless steel pacing leads (0-Flexon, Ethicon) were attached to the right ventricle free wall for subsequent induction of heart failure via rapid ventricular pacing. 7 of 14 animals were surgically prepared with sonometric crystals in the long and short axis of the left ventricle, a blood flow transducer (3PSB, Transonic Systems) on the circumflex artery as an index of coronary blood flow, and vascular occluders (DocXS Biomedical Products) were placed on the superior and inferior vena cava for experimental questions unrelated to the current study. All cables, wires, and occluder lines were tunneled subcutaneously between the scapulae and animals recovered for 14 days prior to further experiments or surgical procedures.

In the second procedure a left retroperitoneal approach was performed to access the terminal aorta for placement of a 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) in a lumbar artery caudal to the left renal artery for measures of systemic arterial pressure. A blood flow transducer (10PAU Transonic Systems) was placed on the terminal aorta caudal to the catheter for measures of hindlimb blood flow, followed by two hydraulic vascular occulders (DocXS Biomedical Products) used to manipulate hindlimb blood flow during experiments. On the left renal artery, a blood flow transducer (4PSB Transonic Systems) was placed for studies unrelated to the present investigation. All cables,

catheters, and occluders were tunneled subcutaneously to the scapula and animals then recovered for a minimum of 14 days prior to any experiments or additional surgeries.

Six out of fourteen animals in this study underwent a third procedure for placement of vascular occluders (DocXS Biomedical Products) bilaterally on the carotid arteries for experimental questions unrelated to the current study. These animals were allowed to recover for 10-14 days prior to experiments of surgeries. Pre and postoperative care are described in previous studies (19, 40, 41, 94, 95, 115, 144, 145, 166).

Data Acquisition

Prior to the experiments the animals were acclimated to the laboratory setting for 15-20 minutes. Animals were then led to the motor driven treadmill for connection of monitoring equipment. Arterial pressure was measured through a direct connection from the fluid filled 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) to a pressure transducer (Transpac IV, ICU Medical). The telemetric pressure tip placed in the left ventricle was connected and left ventricular pressure and heart rate were derived. All flow probes were connected to bench top flow meters (Transonic Sysytems). All *in vivo* hemodynamic measurements were continuously monitored in real time through passage of signals through an A-D converter (iWorx) and output into Labscribe Acquisition software (iWorx).

Experimental Procedures

All in-vivo hemodynamics were measured under steady state conditions taken at rest, exercise (3.2km/h 0% grade), and during exercise. In 7 animals, responses were observed with successive reductions in hindlimb blood flow to approximately 40-60% of free flow conditions. Reductions in hindlimb blood flow were achieved through inflation of vascular occluders (DocXS Biomedical Products). In 7 animals, the muscle metaboreflex

was activated via a one-step reduction in hindlimb blood flow to values similar to the maximal occlusion used in the graded reduction protocol. After control experiments were performed in all 14 animals, the ventricular pacing leads were connected to a pacemaker for rapid ventricular pacing (200 to 240 bpm, ~ 30 days) to achieve heart failure with reduced ejection fraction as described in previous studies (13, 41, 64, 95, 115, 129, 131, 144). Heart failure was indicated by frank reduction in ventricular performance was apparent when the pacemaker was disconnected; e.g., substantial reductions in dP/dt_{MAX} and $_{MIN}$, tachycardia in the absence of pacing, reduced cardiac output, and hypotension at rest. After heart failure was induced the experiments were repeated. The pacemaker was disconnected approximately 30 minutes prior to each experiment and reconnected afterwards for maintenance of heart failure. Thus, each animal served as its own control in this longitudinally designed study.

Analysis

In vivo hemodynamics (cardiac output, hindlimb blood flow, renal blood flow, mean arterial pressure, left ventricular pressure) were measured and recorded continuously for each experiment. Average one-minute steady state values were taken at rest, exercise (3.2km/h) 0 % grade), and each successive reduction in hindlimb blood flow during exercise. Peak reductions in hindlimb blood flow (HLBF) were used to assess peak muscle metaboreflex activation. All other variables were derived from in-vivo steady state hemodynamic measures. The gain of the muscle metaboreflex was determined as the slope of the relationship between the reduction in hindlimb blood flow and the given reflex response once hindlimb blood flow had been reduced beyond metaboreflex threshold (13, 41, 65, 94, 95, 140, 166, 185). Multiple experiments were conducted on each animal in this study. Therefore, steady state values for each animal were averaged across all

experiments from that individual animal. Average values for each animal were then averaged across all animals such that each animal contributed only once to the mean values.

Statistical Analysis

All statistics were performed with Systat Software (Systat 13). Data is reported as standard error of the mean with statistical significance determined by an α level of $P < 0.05$. A Two-Way ANOVA for repeated measures was used for analysis and when significant interactions were observed means were compared using a C-Matrix Test for Simple Effects. Observations of muscle metaboreflex gain and changes observed from exercise to muscle metaboreflex activation between control and heart failure were compared using a Students Paired T-Test.

RESULTS

Figure 3.1 shows the average steady-state hemodynamic responses in 14 animals during rest, exercise (3.2 km/h 0% incline), and exercise with peak muscle metaboreflex activation before and after induction of heart failure. In normal subjects, significant increases in heart rate (HR), stroke volume (SV), cardiac output (CO), stroke work (SW, calculated as (MAP x SV)), and non-ischemic vascular conductance (NIVC) (conductance to all areas except the hindlimb calculated as (CO-HLBF)/MAP)) were observed from rest to exercise. With subsequent muscle metaboreflex activation, heart rate, stroke volume, cardiac output, mean arterial pressure, and effective arterial elastance (HR x (1/NIVC) all increased significantly. Non-ischemic vascular conductance approached significance ($p=0.053$) between exercise and muscle metaboreflex activation. After induction of heart failure, resting values of heart rate and effective arterial elastance were significantly increased and, stroke volume, cardiac output, stroke work, mean arterial pressure and

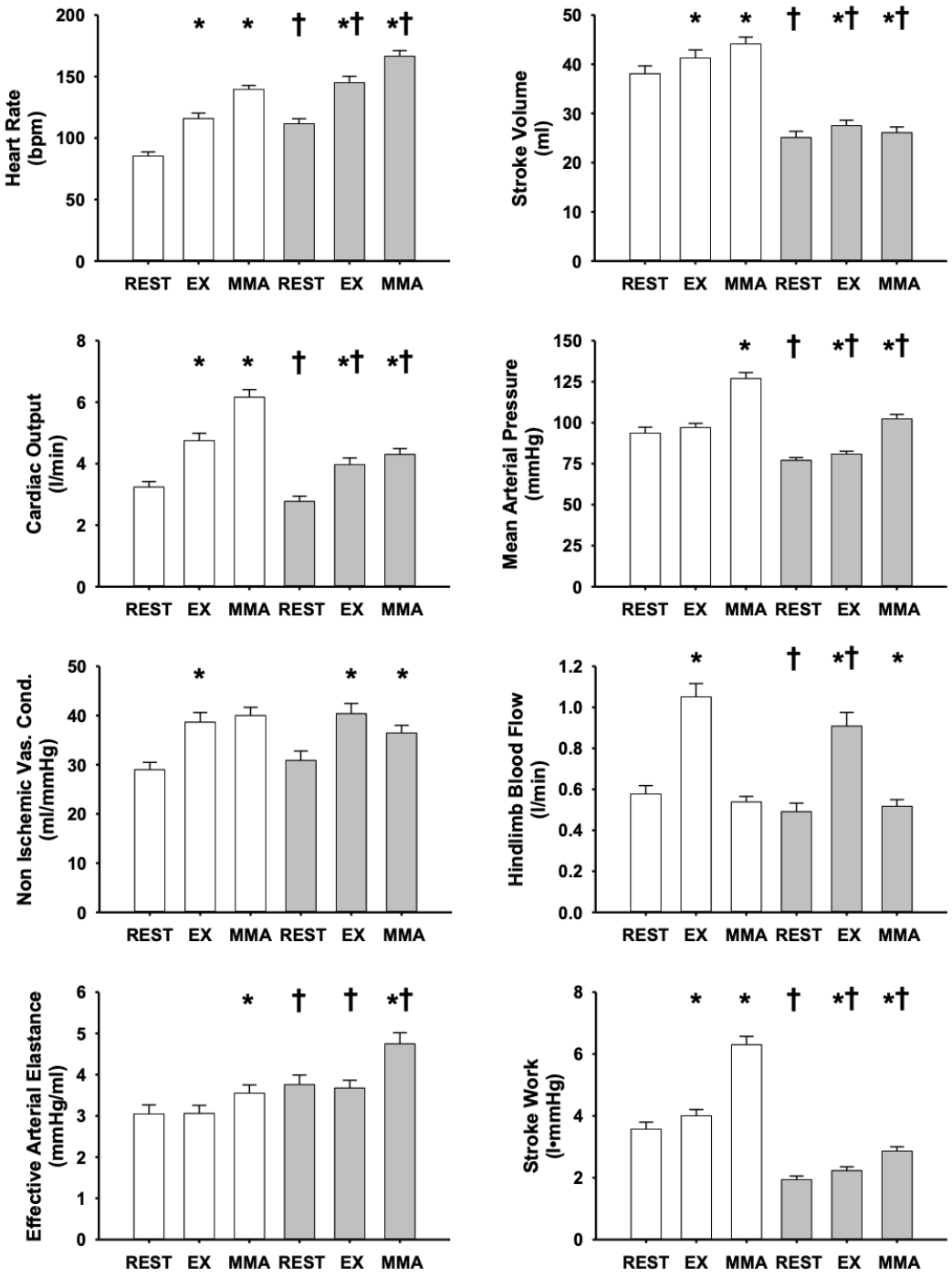

Figure 3.1 *Average one-minute steady state hemodynamic values taken at rest (REST), exercise (3.2 km/h 0% grade) (EX), and exercise with muscle metaboreflex activation (MMA) before (white) and after induction of heart failure via rapid ventricular pacing (gray). Standard error of the mean is shown on the bar graphs. Statistical significance against previous workload is shown as * P < 0.05. Comparisons between control and heart failure for a given workload shown as † P < 0.05. (N=14).*

hindlimb blood flow were significantly reduced and no difference was observed in non-ischemic vascular conductance. From rest to exercise stroke volume, cardiac output,

mean arterial pressure, non-ischemic vascular conductance and stroke work significantly increased and all except non-ischemic vascular conductance were significantly attenuated compared to control. Heart rate significantly increased from rest to exercise but was significantly higher vs. control. In heart failure from exercise to muscle metaboreflex activation all variables significantly increased. However, all variables were significantly attenuated when compared to control with the exceptions of heart rate and effective arterial elastance which were significantly increased. Hindlimb blood flow was significantly reduced in heart failure at rest. Significant increases were observed in hindlimb blood flow from rest to exercise however the steady state level during exercise was significantly attenuated compared to control. Hindlimb blood flow was mechanically reduced to comparable levels to elicit the muscle metaboreflex after induction of heart failure.

Figure 3.2 shows the average steady-state values in 14 animals during rest, exercise, and exercise with peak muscle metaboreflex activation before and after induction of heart failure. In control experiments significant increases were observed in

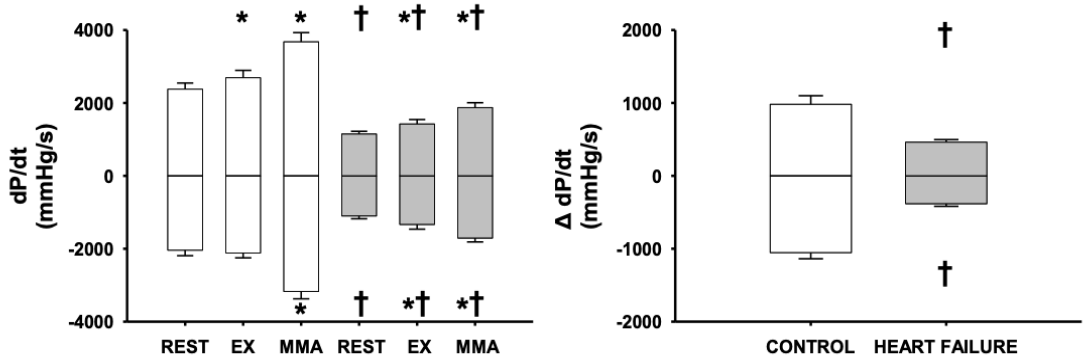

Figure 3.2 *Left average 1-minute steady state values of dP/dt $_{MAX}$ and $_{MIN}$ taken at rest (REST), exercise (3.2 km/h 0% grade) (EX), and exercise with muscle metaboreflex activation (MMA) before (white) and after induction of heart failure via rapid ventricular pacing (gray). Right shows the average change from EX to MMA in control and heart failure. Standard error of the mean is shown on the bar graphs. Statistical significance against previous workload is shown as * P < 0.05. Comparisons between control and heart failure for a given workload or change from previous workload shown as † P < 0.05. (N=14).*

dP/dt $_{MAX}$ from rest to exercise and from exercise to peak muscle metaboreflex activation. dP/dt $_{MIN}$ was not significantly changed from rest to exercise but was significantly increased from exercise to muscle metaboreflex activation. After induction of heart failure, dP/dt $_{MAX}$ and $_{MIN}$ were significantly reduced at rest and increased between rest and exercise and between exercise and muscle metaboreflex activation, however after induction of heart failure these responses were all attenuated.

Figure 3.3 shows the relationship between dP/dt $_{MAX}$ and $_{MIN}$ and hindlimb blood flow (left columns) and the slope (gain) of these relationships (right column) in 7 animals

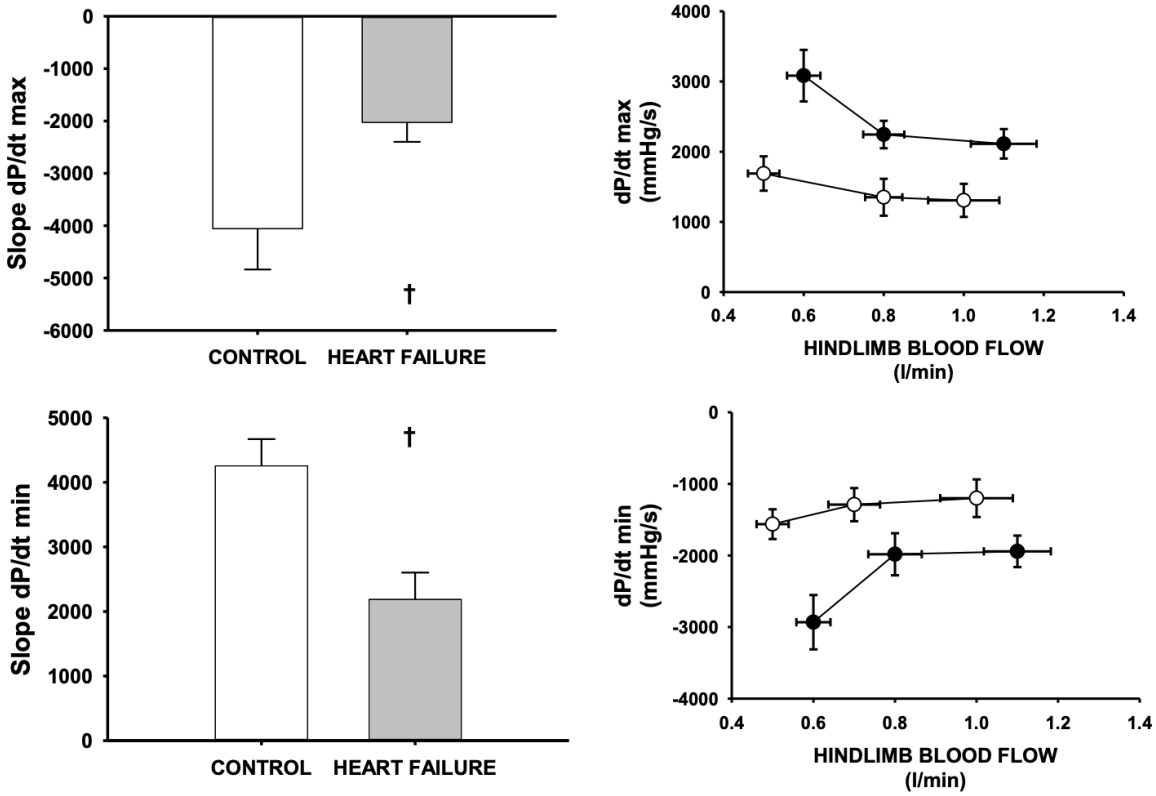

Figure 3.3 *Assessment of muscle metaboreflex gain as judged by the slope of the line from threshold to maximal muscle metaboreflex activation before (solid circles, white bars) and after induction of heart failure via rapid ventricular pacing (open circles, grey bars). Line graphs should be observed from the right point (exercise) to the middle point (reflex threshold) to the final point left (peak muscle metaboreflex activation) as hindlimb blood flow is reduced. Statistical significance between control and heart failure shown as † P < 0.05. (N=7).*

before and after induction of heart failure (data analyzed as described previously,(40, 41,

64, 93-96, 144, 145). The graphs on the left show the average values during steady-state exercise (right-hand points), the average threshold of the reflex (middle points) and the values observed at peak metaboreflex activation (left hand points) before and after induction of heart failure. In heart failure, the baseline levels of hindlimb blood flow during mild exercise were lower and ventricular contractility was decreased. There was no change in the threshold level of hindlimb blood flow in heart failure and upon muscle metaboreflex activation the slope of the relationships between hindlimb blood flow and dP/dt $_{MAX}$ and $_{MIN}$ were significantly lower.

Figure 3.4 shows the contraction/relaxation rate ratios at rest, exercise and metaboreflex activation (left) and the changes seen with metaboreflex activation (right) in 14 animals before and after induction of heart failure. In normal subjects, the

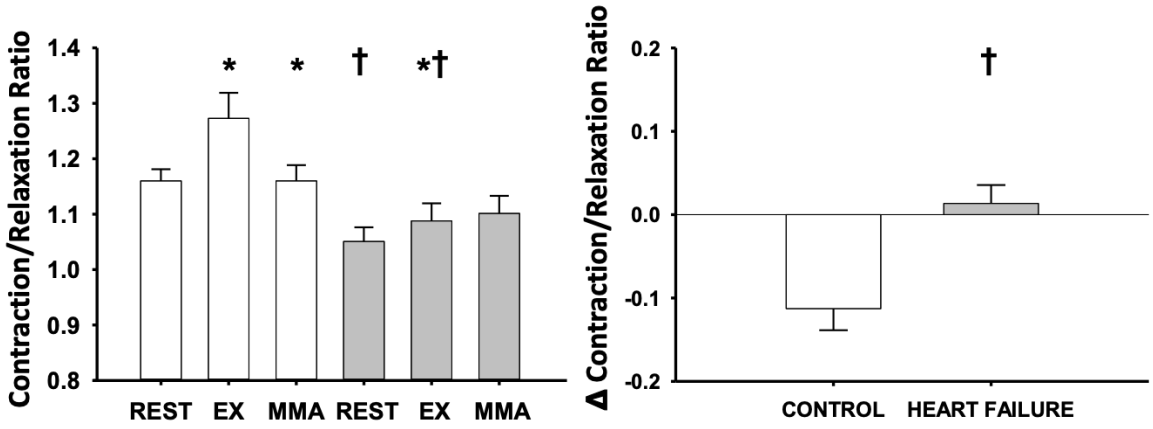

Figure 3.4 *Average one-minute steady state values of the ratio of dP/dt $_{MAX}$ divided by dP/dt $_{MIN}$ taken at rest (REST), exercise (3.2 km/h 0% grade) (EX), and exercise with muscle metaboreflex activation (EX + MMA) before (white) and after induction of heart failure via rapid ventricular pacing (gray). Right shows the average change from EX to EX + MMA in control and heart failure. Standard error of the mean is shown on the bar graphs. Statistical significance against previous workload is shown as * P < 0.05. Comparisons between control and heart failure for a given workload or change from previous workload shown as † P < 0.05. (N=14).*

contraction/relaxation ratio at rest is greater than 1.0 and significantly increases from rest to exercise which favors contraction. With muscle metaboreflex activation the ratio significantly reduces back towards 1.0 indicating a larger improvement in the relaxation

rate relative to the contraction rate during muscle metaboreflex activation. After induction of heart failure, the ratio is significantly lower at rest, there is a small but statistically significant increase from rest to exercise but no further significant changes in the ratio occurred during muscle metaboreflex activation. Thus, in normal animals metaboreflex activation results in a large reduction in the ratio whereas in heart failure there is little change.

DISCUSSION

This is the first study to demonstrate that muscle metaboreflex activation enhances ventricular relaxation rate relative to the contraction rate and that these increases are stifled in heart failure. Additionally, we observed that ventricular contraction and relaxation rates are maintained near a ratio of 1:1 in healthy and heart failure similar to what is observed in genetic models of rats (85, 86) however in-vivo this ratio does vary with exercise and metaboreflex activation. Significant differences in contraction-relaxation ratios were observed in healthy subjects between rest, exercise and with muscle metaboreflex activation. We observed that from rest to mild exercise there was a significant increase in the ratio favoring contraction rate. However, with subsequent muscle metaboreflex activation there was a reduction in the ratio to return closer to 1.0 implying significant greater increases in relaxation rate relative to contraction rate. This improved relaxation rate would favor improved ventricular filling time and thereby may aid in the ability to raise cardiac output. In heart failure the effect of metaboreflex activation on the contraction-relaxation ratio was abolished. This failure to substantially improve relaxation rate in heart failure may contribute to the impaired ability to raise cardiac output. In addition to the effects observed regarding contraction/relaxation ratios, this is the first study to quantify the gain of the muscle metaboreflex control of ventricular dP/dt_{MAX} and

MIN and the effects of heart failure on metaboreflex gain of dP/dt MAX and MIN. We observed that gain of the muscle metaboreflex control of both the ability to improve contraction and relaxation are substantially impaired in heart failure.

Previously, we have observed that during metaboreflex activation in normal subjects, the increased sympathetic activity to the heart limits coronary vasodilation due to activation of vascular alpha-adrenergic receptors and that this functional vasoconstriction restrains the ability to raise ventricular function (40). In heart failure, frank coronary vasoconstriction occurs and further restrains already compromised ventricular performance (13, 41). After blockade of alpha adrenergic receptors, this coronary vasoconstriction is reversed to coronary vasodilation during metaboreflex activation and coronary blood flow increases substantially and there are significant improvements in ventricular function (40, 41). Furthermore, we have shown that ventricular-vascular coupling is also markedly altered in heart failure as much greater increases in arterial elastance occurs which are unaccompanied by increases in ventricular elastance thereby further uncoupling an already impaired ventricular-vascular relationship (115). The higher the arterial elastance the less effective energy transfer becomes and this in turn reduces the ability to improve stroke work. Interestingly, even with reduced energy transfer and poor vascular accommodation, muscle metaboreflex-induced central blood volume mobilization in heart failure is sustained. (131). Thus, the inability to improve cardiac output in heart failure is likely not only a result of reduced ventricular contraction (13, 41, 129, 144) but also poor relaxation dynamics that may be affected by changes in coronary artery blood flow similar to contractile function as seen in our previous studies (13, 40, 41, 130, 166). In-depth analyses of diastolic function during exercise and metaboreflex activation in heart failure are not well understood, however in the present study we have

shown that relaxation rate is altered during muscle metaboreflex activation and this likely significantly impacts diastolic performance.

IMPLICATIONS

The muscle metaboreflex is a negative feedback reflex which acts to lessen metabolite accumulation in the skeletal muscle by increasing total systemic blood flow (e.g. cardiac output) as well as arterial O_2 content via red blood cell mobilization (40, 42, 45, 49, 76, 80, 127, 140, 145, 153). In healthy subjects during relative ischemia when the levels of metabolites rise within the skeletal muscle, they activate metabosensitive afferents. These afferents activate cardiovascular centers within the brain to increase sympathetic outflow. This culminates in tachycardia and increased ventricular function thereby raising cardiac output and β_2 mediated vasodilation via epinephrine release. Combined these enhance skeletal muscle blood flow and oxygen delivery which reduces the interstitial metabolite concentration (13, 47, 49, 128, 150, 151, 153). Muscle metaboreflex activation also enhances central blood volume mobilization thereby maintaining ventricular filling pressure which thereby supports the enhanced ventricular performance in order to sustain the reflex increase in cardiac output. In heart failure, during exercise skeletal muscle blood flow is low and is already near, at, or beyond the threshold required to elicit activation of skeletal muscle afferents depending on the workload (64, 65). Muscle metaboreflex activation causes vasoconstriction in inactive vascular beds and even within the active skeletal muscle - the vascular bed from which the reflex originates thereby exaggerating the original response developing a limiting positive feedback loop (94, 95). Although the muscle metaboreflex elicits tachycardia in heart failure, ventricular function is compromised and exaggerated coronary vasoconstriction (41) further limits reflex increases in cardiac output. Thus, the ability of

the reflex to improve perfusion to the ischemic muscle is compromised. Although central blood volume mobilization is sustained in heart failure (131), we observed in the present study that ventricular relaxation rate is depressed at rest and during mild exercise and the metaboreflex-induced reflex increases in relaxation rate are attenuated To what extent these impairments in diastolic function are related to exaggerated metaboreflex-induced coronary vasoconstriction are unknown.

CHAPTER 4: CHRONIC ABLATION OF TRPV1 SENSITIVE SKELETAL MUSCLE AFFERENTS ATTENUATES THE MUSCLE METABOREFLEX

ABSTRACT

Exercise intolerance is a hallmark feature seen in a variety of cardiovascular pathologies. One of the mechanisms by which these symptoms likely occur is enhanced activation of the muscle metaboreflex. The muscle metaboreflex vasoconstrictor response occurs as a result of activation of metabolite-sensitive afferent fibers located in ischemic active skeletal muscle some of which express the Transient Receptor Potential Vanilloid 1 (TRPV1) cation channel. Local cardiac and intrathecal administration of an ultra-potent noncompetitive, dominant negative agonist resiniferatoxin (RTX) can attenuate cardiac sympathetic afferent activation and sensations of nociceptive pain by ablating these TRPV1 sensitive afferents. This study investigated if intrathecal administration (L4-L6) of RTX (2 μg/kg) was capable of chronically attenuating the muscle metaboreflex elicited by partial reductions in hindlimb blood flow during mild exercise (3.2 km/h) in chronically instrumented conscious canines. In this study we observed that administration of intrathecal RTX significantly attenuated muscle metaboreflex induced increases in mean arterial pressure (27 ± 5.0 mmHg to 6 ± 8.2 mmHg), cardiac output (1.40 ± 0.2 L/min to 0.28 ± 0.1 L/min) and stroke work (2.27 ± 0.2 L*mmHg to 1.01 ± 0.2 L*mmHg). These effects were maintained until 78 ± 10 days post RTX at which point the efficacy of RTX injection was tested in a terminal experiment by intra-arterial administration of capsaicin (20 μg/kg). A significant reduction in mean arterial pressure response (+45.7±6.5 mmHg pre RTX vs +19.7±3.1mmHg post RTX) was observed. We conclude that intrathecal administration of RTX can chronically attenuate the muscle metaboreflex and could potentially alleviate enhanced sympatho-activation observed in

cardiovascular disease states such as heart failure and hypertension.

INTRODUCTION

Whole body dynamic exercise presents one of the greatest challenges to cardiovascular control as heart rate and cardiac output rise from resting values to maximal levels during peak workloads (19, 24, 38, 42, 43, 45, 49, 78, 93, 127, 128, 145, 151, 155). Blood flow to inactive vascular beds is reduced in many species and vasodilation in the active skeletal muscle and even the coronary circulations are restrained by the substantial increases in sympathetic activity (40, 41, 94, 95). These marked changes in autonomic activity occur through the action and likely interaction between activation of central command, resetting of the arterial baroreflex, and activation of skeletal muscle afferents. In cardiovascular dysfunction, these challenges often become exacerbated as systemic perfusion and cardiac function may be compromised resulting in massive sympathetic activation. One potential mechanism mediating the enhanced sympathetic activity during exercise in cardiovascular disease is over-activation skeletal muscle afferents, in particular those activated via accumulation of metabolites due to suboptimal oxygen delivery – termed the muscle metaboreflex (1, 2, 47, 66, 70, 91, 92, 123, 135, 142, 150, 153, 158, 160-162). Even in normal subjects this reflex is thought to become tonically active at relatively modest workloads and activation of this reflex can cause substantial increases in sympathetic activity which elicits increases in cardiac output via tachycardia, increased ventricular contractility and central blood volume mobilization (13, 43, 45, 49, 58, 59, 67, 93, 96, 127, 128, 131, 140, 145, 151, 154, 155). Arterial elastance rises in parallel with ventricular maximal elastance thereby optimizing ventricular-vascular coupling and energy transfer from the left ventricle to the systemic circulation (115, 144, 145). Peripheral vasoconstriction can also occur which is countered by adrenal

epinephrine release and β_2 mediated vasodilation (13, 94, 96, 140). Normally, these responses act to correct about 50% of any blood flow deficit to the active muscle. Splanchnic constriction mobilizing red blood cells increases arterial O_2 content and O_2 deficit is increased even further than blood flow as the reflex increase in blood flow now contains more O_2 (49, 153, 183) In pathophysiological states, even during moderate workloads this reflex becomes markedly over-activated and often profound vasoconstriction occurs in inactive vascular beds and even the ischemic active muscle and heart are vasoconstricted (41, 44, 64, 95, 129, 154, 166, 167). This exacerbates an already precarious situation leading to positive feedback amplification of sympathetic activity (94, 95). The potentially extreme levels of sympathetic activity may pose increased risks for adverse cardiovascular events such as stroke, myocardial infarction, ventricular arrhythmia and sudden cardiac death. Thus, mechanisms to ameliorate over-activation of skeletal muscle afferents during exercise in patients with cardiovascular disease may be beneficial.

Skeletal muscle metabo-sensitive afferents respond to a number of substances via stimulation of a variety of receptors. These receptors include TRPV1 channels which are non-selective cation channels known to be stimulated by a variety of stimuli such as temperature, and H+ ion concentration (1, 48, 55, 61, 174). Recent studies have investigated the impact of stimulating or antagonizing TRPV1 expressing afferents utilizing capsaicin, capsaizapine, their analogs, and other various pharmaceutical compounds (72, 74, 84, 124, 162, 165, 181, 186, 187). These studies found that activation of TRPV1 receptors has been linked not only to initiation of blood pressure responses but also the sensation of nociceptive pain (15, 26, 28, 84, 106, 108, 124, 146, 177). Short term attenuation of type III and IV afferents including those expressing TRPV1, during

exercise, decreases the sensation of muscle fatigue as well as the pressor responses during exercise via activation of Mu opioid channels. (8, 20, 56). Whether these afferents can be chronically ablated is unclear. In this study we hypothesized that chronic ablation of TRPV1 expressing afferent neurons in the hindlimbs could be used to chronically reduce the strength or gain of the muscle metaboreflex. In a longitudinally designed study, we first established the strength of the muscle metaboreflex in chronically instrumented canines during mild exercise. We then used an ultra-potent dominant negative agonist of capsaicin, resiniferatoxin (RTX) injected into the intra-thecal space to partially destroy afferents expressing TRPV1 afferents. We repeated the experiments from 7 to 78 $\pm$ 10 days after injection and found that the strength of the muscle metaboreflex was chronically attenuated by over 50%. We conclude that this approach could be useful to attenuate exaggerated sympathetic activation during exercise in patients with cardiovascular disease.

METHODS

Experimental subjects

Six adult mongrel canines (1 male, 5 female) of approximately 19-25 kg were selected based on willingness to walk on a motor driven treadmill. We have previously shown that sex does not affect the strength or mechanisms of muscle metaboreflex activation (104). Canines were acclimatized to exercise at a workload of 3.2 km/hr with a 0% grade. All experimental and surgical procedures utilized for this study comply with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and were approved by the Wayne State University Institutional Animal Care and Use Committee (IACUC). Animals utilized in this study underwent a fourteen-day acclimation period with the laboratory surroundings and personnel and exercised on their own volition during all

experiments.

Surgical Instrumentation

All animals underwent a series of surgical procedures over a period of four weeks using standard aseptic surgical techniques. Prior to each surgery, animals were sedated with an intramuscular injection of acepromazine (0.4-0.5 mg/kg) and administered a sub cutaneous injection of slow release analgesic buprenorphine SR (0.03 mg/kg) 30 minutes prior to induction of anesthesia. Induction was achieved by intravenous administration of ketamine (5 mg/kg) and diazepam (0.2-0.3 mg/kg). Anesthesia was maintained pre and intra operatively with (1-3%) isoflurane gas. Additional preoperative analgesic included intravenous administration of carprofen (4.4 mg/kg). Postoperatively acepromazine (0.2-0.3 mg/kg IV) and buprenorphine (0.01-0.03 mg/kg IM) were administered as needed. Acute proactive antibiotic (cephalexin 30 mg/kg IV) was administered pre and post operatively. Prophylactic antibiotic (cephalexin 30 mg/kg PO BID) were administered to prevent microbial infection.

The first surgical procedure was a left thoracotomy through the left $3^{rd}/4^{th}$ intercostal space to expose the heart. The pericardium was incised and parted for placement of a telemetric pressure transducer tip (TA11 PA-D70, DSI) into the apex of the left ventricle that was tunneled from the telemeter body tethered subcutaneously caudal to the thoracotomy incision site. The upper section of pericardium was then retracted dorsally to expose the aorta and pulmonary vein. A section of the aortic trunk was used for placement of a blood flow transducer (20 PAU, Transonic Systems) for measure of cardiac output. Unrelated to the investigations of this study, four stainless steel pacing leads (0-FLexon, Ethicon) were attached to the right ventricle free wall. All leads, and cables were tunneled subcutaneously and exteriorized between the scapulae.

The pericardium and ribs were reapproximated and the chest was closed in layers.

The final surgical procedure occurred at a minimum of 14 days post thoracotomy. A left retroperitoneal approach was used to access the terminal aorta. All arterial blood vessels caudal to the renal artery and cranial to the edge of the incision above the iliac crest were ligated. The most accessible cranial arterial branch caudal to the renal artery was catheterized with a 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) for measure of arterial pressure. In order, cranial to caudal, placed caudal to the arterial catheter, a blood flow transducer (10 PAU, Transonic Systems) and two 8-10mm hydraulic occluders (DocXS Biomedical Products) were placed to measure and manipulate hindlimb blood flow. All cables and occluder lines were tunneled subcutaneously and exteriorized between the scapulae.

Intrathecal RTX Administration and Terminal Capsaicin Response

Administration of RTX was performed after completion of all control experiments. Prior to this procedure animals were sedated, induced, anesthetically maintained, and treated with the same analgesics and aseptic techniques as described above in the surgical procedures. Flow probes and pressure transducers were connected. Heart rate and mean arterial pressure responses were taken during a one-minute steady-state prior to administration of each pharmaceutical agent, as well as, peak responses post administration for analysis. Prior to placement of the spinal needle, intra-arterial capsaicin (20 μg/kg) was administered through the catheter placed in the terminal aorta. After recovery from capsaicin administration a 20-22-gauge spinal needle was placed into the intrathecal space between the L4-L5 or L5-L6 vertebrae. Placement of the spinal needle was done based on animal positioning, alignment, and successful observation of cerebrospinal fluid through the needle. Spinal fluid equal to the volume amount of

intrathecal RTX administered to reach the desired dose volume (2 μg/kg) plus 0.5 ml to use as a flush was removed. RTX was then administered through the spinal needle at a concentration of 2 μg/kg followed by flush of 0.5 ml spinal fluid and removal of the spinal needle. After approximately 30 minutes and recovery from responses to RTX, a second intra-arterial injection of capsaicin (20 μg/kg) was performed. Animals were monitored post operatively and administered acepromazine (0.2-0.3 mg/kg IV) and buprenorphine (0.01-0.03 mg/kg IM) as needed. Metaboreflex experiments were repeated after a 7-10 day recovery period. After completion of all experiments a final terminal anesthetic procedure was performed to assess the long-term efficacy of the RTX injection. Animals were sedated, induced, treated with analgesics and anesthetically maintained as described above in the surgical procedures. All equipment was connected as described above and steady-state values of *in vivo* hemodynamics were taken during a one-minute steady-state prior to intra-arterial administration of capsaicin (20 μg/kg).

Data Acquisition

Animals were acclimated 10-20 minutes in the laboratory setting and then led to the treadmill and equipment. Arterial pressure was measured by connection of the 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton to a pressure transducer (Transpac IV, ICU Medical). Left ventricular pressure telemeter (DSI) and blood flow transducers were connected to their appropriate instrumentation. All flows and pressures were monitored and recorded in real time through an A-D converter (IWorx). A-D output was run through Labscribe4 acquisition and analysis software (IWorx).

Experimental Procedures

All hemodynamic parameters were measured under steady-state conditions during rest, mild free flow exercise (3.2 km/h 0% grade), and during sustained mild free flow

exercise with successive reductions in hindlimb blood flow used to activate the muscle metaboreflex. Hydraulic vascular occluders (DocXS) were used to achieve reductions in hindlimb blood flow to approximately 30% of free flow conditions for both control and RTX treated animals. Experiments were repeated after RTX thus each animal served as its own control in this longitudinally designed study.

Analysis

All *in vivo* hemodynamic parameters were continuously recorded during each experiment. One-minute averaged steady-state values were taken after a three-minute acclimation period at rest, free flow exercise, and during steady-state at each reduction of hindlimb blood flow (HLBF). Measures of mean arterial pressure, cardiac output, and hindlimb blood flow were measured *in vivo*. Heart rate was derived from the cardiac output waveform. All other hemodynamic parameters were mathematically derived from *in vivo* measurements.

Statistical Analysis

Systat Software (Systat 13.0) was used for statistical analysis. Data are reported as the mean $\pm$ SE and statistical significance was determined by an α level of $P < 0.05$. Data were analyzed using a Two-way ANOVA for repeated measures and when a significant interaction was observed, individual means were compared using C Matrix Test for Simple Effects. Direct observations on changes in muscle metaboreflex gain, and comparison in changes observed during surgical procedures were assessed by a Students Paired T-test.

RESULTS

Figure 4.1A shows the pressor responses to i.a. capsaicin (20 μg/kg) before and approximately 30 minutes post RTX injection. RTX markedly attenuated the pressor and

tachycardic responses to capsaicin. Figure 4.1B shows the responses to i.a. capsaicin performed in a terminal procedure 78 ± 10 days after intrathecal administration of RTX. Compared to the responses observed prior to RTX, the pressor and tachycardic responses to capsaicin were significantly attenuated by ~ 50%.

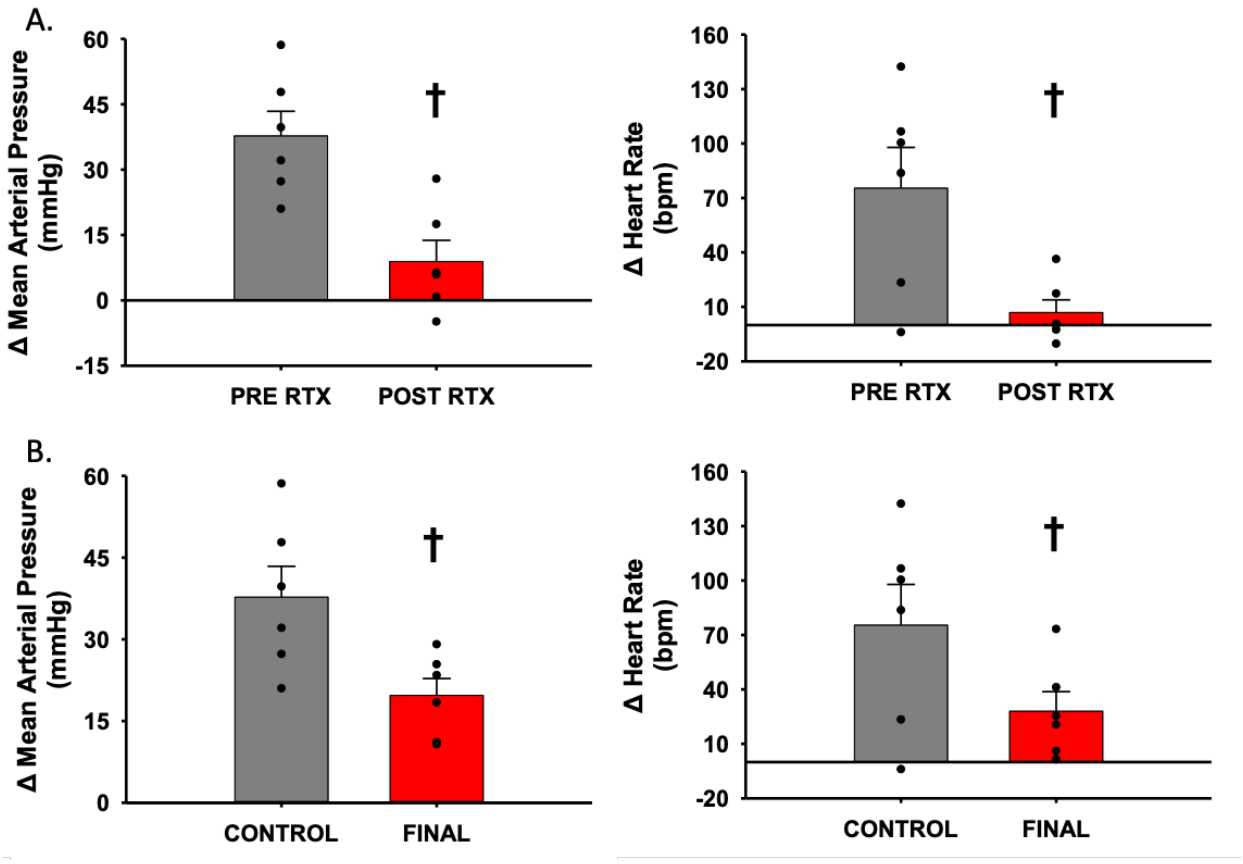

Figure 4.1A *Intraoperative responses to intra-arterial administration of capsaicin (20 μg/kg) while under isoflurane anesthesia. Pre RTX (Grey) and Post RTX (Red) are the delta responses comparing a one-minute steady-state prior to capsaicin administration to the maximal response for each variable after administration.* ***4.1B*** *intraoperative responses to intra- arterial capsaicin (20 μg/kg) prior too administration of RTX and during a terminal experiment performed 78 ± 14 days post injection of RTX. Bar graphs show standard error of the mean with individual data points overlain. Standard error shown on bar graphs with actual data points overlain. Statistical significance is shown compared to previous condition and shown as* ***†*** *(P < 0.05). (N=6)*

Figure 4.2 shows that in control experiments heart rate (HR), stroke volume (SV) cardiac output (CO) and stroke work (SW) increased significantly from rest to exercise whereas there were no significant changes in mean arterial pressure (MAP) or effective arterial elastance (E_{aZ}, an index of vascular load (97, 115)) likely as a result of the

significant increases in non-ischemic vascular conductance (NIVC, vascular conductance to all vascular beds except the hindlimbs calculated as (CO-HLBF) / MAP). During muscle metaboreflex activation HR, SV, CO, SW, E_{aZ} and MAP all increased significantly with no significant increases in NIVC when compared to the free flow exercise condition. Intrathecal RTX injection had no effect on MAP, HR, SV, CO, NIVC SW, or E_{aZ} at rest and during steady-state exercise. However, after RTX muscle metaboreflex responses were virtually abolished: only SW showed a small but statistically significant increases in response to similar reductions in HLBF.

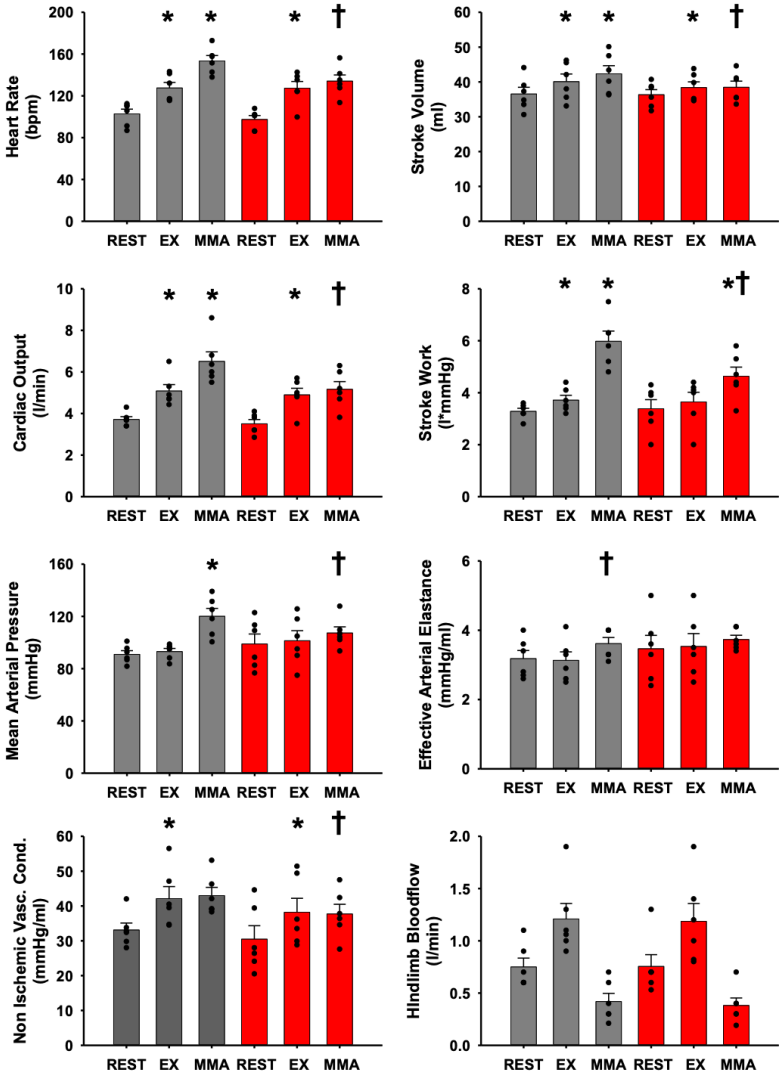

Figure 4.2 *One-minute averaged hemodynamic responses taken during steady-states at rest, exercise (3.2 km/h 0% grade) and during exercise with muscle metaboreflex activation before (Grey) and after administration of intrathecal RTX (2 μg/kg) (L4-L6) (Red). Bar graphs show standard error of the mean with individual data points overlain. Standard error shown on bar graphs with actual data points overlain. Statistical significance is shown as* * *(P < 0.05) compared to previous workload and significance to previous condition and shown as* † *(P < 0.05). (N=6).*

Figure 4.3 shows a comparison of changes observed in the hemodynamic values between steady-state mild exercise (3.2 km/h 0% grade) and steady-state maximal muscle metaboreflex activation. Increases in HR and SV were significantly attenuated which thereby lowered CO after intrathecal administration of RTX. The significant

reduction in the increase of MAP after intrathecal RTX is primarily a result of the lack of CO response, the normal increases in SW and E_{aZ} were also reduced.

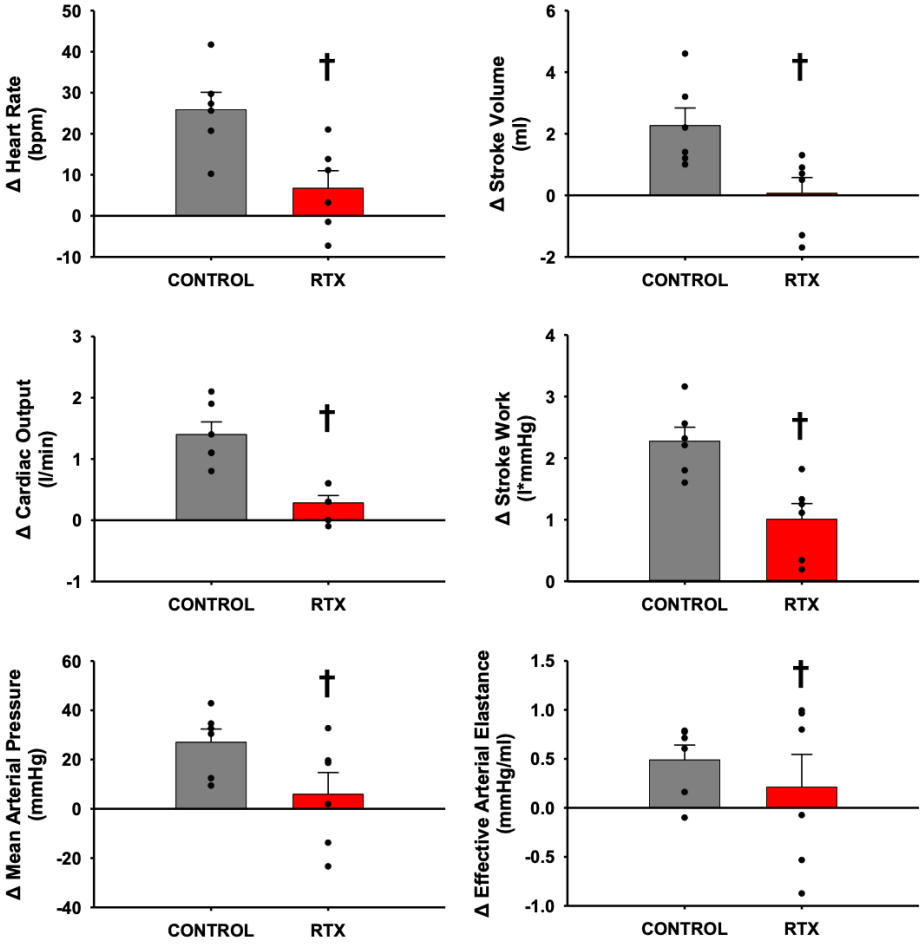

Figure 4.3. *Average change between one-minute steady-state values taken during exercise (3.2 km/h 0% grade) and during exercise with muscle metaboreflex activation before (Grey) and after administration of intrathecal RTX (2 μg/kg) (L4-L6) (Red). Standard error shown on bar graps with actual data points overlain. Statistical significance shown as* ***†*** *(P < 0.05) compare to previous condition. (N=6)*

Figure 4.4 is a comparison of the reflex gain defined as the slope of the regression line from the threshold of reflex to peak muscle metaboreflex activation of between MAP, CO, and HR vs. hindlimb blood flow before and after RTX. The slopes of MAP, CO, and HR were significantly attenuated after treatment with RTX. The slope of CO relationship showed the largest reduction by approximately 70 percent compared to reductions of MAP and HR of approximately 50%.

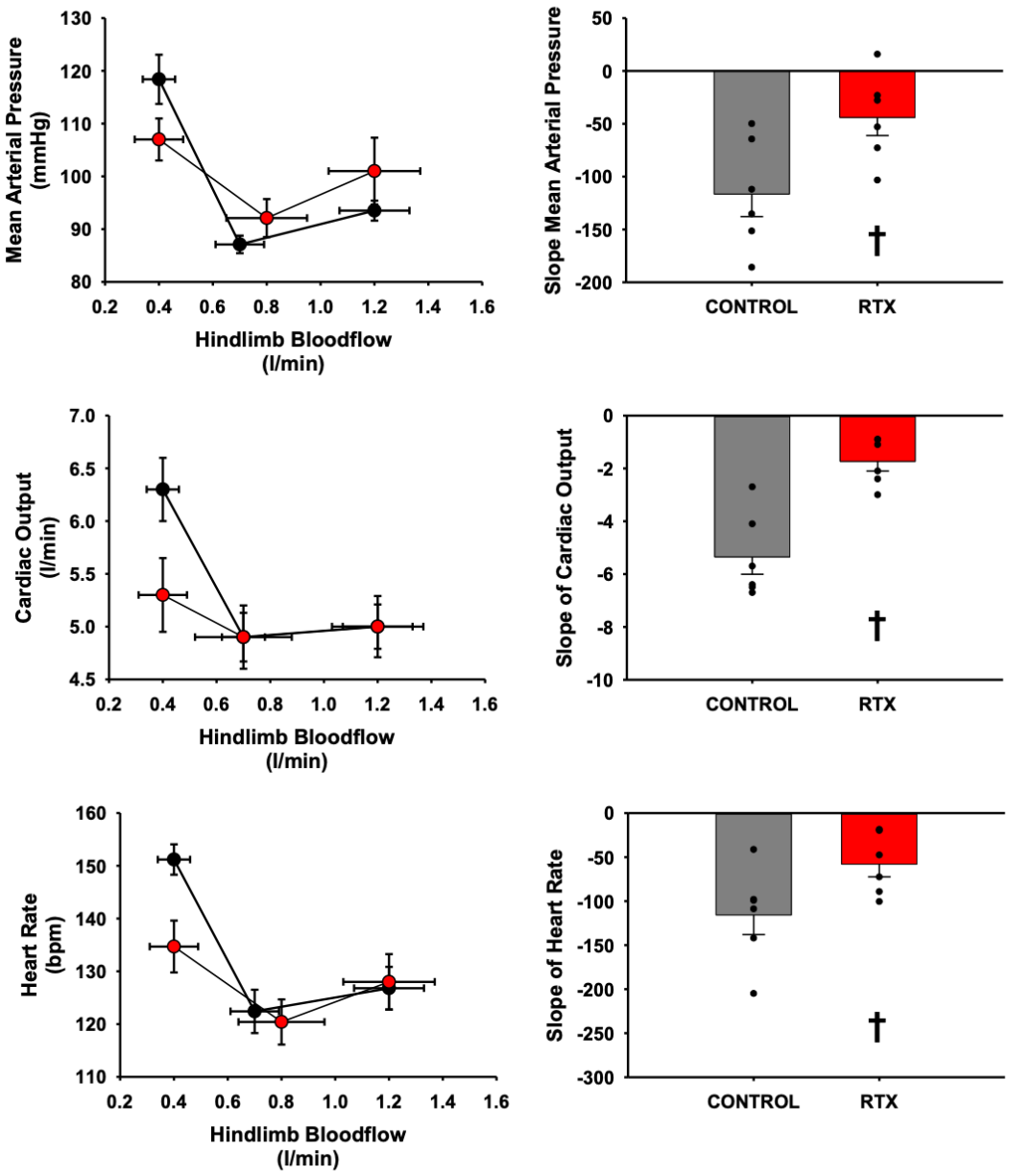

Figure 4.4 *Assessment of changes in the gain of the muscle metaboreflex determined by the slope of the linear regression taken from one-minute averaged steady-states at each successive reduction in hindlimb blood flow from threshold to peak reflex activation before (black circles, grey bars) and after administration of RTX (red circles, red bars). Line graphs should be observed from the right point (exercise) to the middle point (reflex threshold) to the final point left (peak muscle metaboreflex activation) as hindlimb blood flow is reduced. Standard error shown on bar graps with actual data points overlain. Statistical significance between control (Grey) and after administration of intrathecal RTX (2 μg/kg) (L4-L6) (Red) shown as* ***†*** *(P < 0.05). (N=6)*

DISCUSSION

This is the first study to demonstrate that chronic attenuation of muscle metaboreflex activation can be achieved by ablation of TRPV1 sensitive afferents via

intrathecal administration of RTX. We determined that intrathecal RTX attenuates the muscle metaboreflex primarily through significant reductions in heart rate and stroke volume and attenuation of ventricular performance indexed by stroke work. These reduced responses lower the reflex increase in cardiac output. Inasmuch as in normal subjects during submaximal dynamic exercise, the increase in cardiac output is the major mechanism mediating the rise in arterial pressure, with the reduced rise in cardiac output the metaboreflex pressor response was significantly attenuated.

Traditionally, the muscle metaboreflex increases cardiac output via the combined effects of tachycardia, increased ventricular contractility, and enhanced central blood volume mobilization (13, 127, 131, 145). This reflex arises due to activation of metabosensitive skeletal muscle afferents (2, 91, 92, 135, 142, 156, 161). These afferents are primarily type IV but some type III afferents, which primarily respond to mechanical stimulation, are also metabo-sensitive (91, 92, 162, 164, 165). These metabo-sensitive afferents respond to TRPV1 agonists (48, 84, 109, 162). In the present study we utilized a non-competitive agonist to ablate TRPV1 sensitive afferent fibers in the hindlimb skeletal muscle. Intrathecal RTX itself usually caused a pressor response likely due to irreversible activation of the TRPV1 receptors. Thirty minutes to one hour later, the reflex responses to i.a. capsaicin were significantly attenuated. Metaboreflex experiments were performed 7-78 $\pm$ 10 days after RTX and the responses were consistently reduced. In the terminal experiment the responses to i.a. capsaicin were reduced by approximately 50% indicating sustained significant ablation of afferents containing TRPV1 receptors after 78 $\pm$ 10 days. The lack of complete ablation is likely a result of multiple factors such as toxin volume delivered, intrathecal flow, and the levels of TRPV1 receptors available per neuron to accept RTX and achieve the level of cation

flow required for cytotoxicity. Furthermore, expression patterns of TRPV1 neurons likely vary per neuron and their upregulation and downregulation from various stimuli may play a role in injection efficacy. Wang et al. showed that TRPV1 protein levels can vary based on pathological state (179) possibly acting as a compensatory mechanism in response to enhanced levels of metabolites in muscle with reduced blood supply.

Peripheral vascular responses were altered post RTX. We observed a significant reduction in non-ischemic vascular resistance during muscle metaboreflex activation likely as a result of reduced β_2 mediated vasodilation observed in previous studies (94-96). We have shown that muscle metaboreflex activation enhances ventricular maximal elastance and effective arterial elastance such that ventricular-vascular coupling is maintained and stroke work is optimal (115, 145). In this study intrathecal RTX significantly attenuated increases in stroke work with no significant changes in effective arterial elastance. Reductions in stroke work is probable evidence of some degree of ventricular-vascular uncoupling, likely a result of reductions in ventricular maximal elastance during muscle metaboreflex activation post RTX. In addition to overall reductions in hemodynamic responses to graded reductions in hindlimb blood flow, we also observed significant reductions in the gain of the muscle metaboreflex. Calculation of muscle metaboreflex gain (strength) is quantified via the slope of the regression line between a given hemodynamic parameter vs. the reduction in hindlimb blood flow once beyond metaboreflex threshold. We observed that not only are maximum hemodynamic values attenuated but also that the slope of mean arterial pressure, heart rate, and cardiac output relationships vs. hindlimb blood flow are significantly reduced with no change in threshold indicating that reflex gain is reduced for all observed hemodynamic parameters. These experiments were performed only during mild exercise and to what extent

metaboreflex responses after RTX are different at higher workloads remains to be investigated.

IMPLICATIONS

Several cardiovascular diseases (e.g. heart failure, hypertension, peripheral vascular disease) alter the strength and mechanisms of the muscle metaboreflex (13, 41, 64, 129, 144, 166, 167). Impaired ventricular function can occur not only from the direct effect of the disease, but also from enhanced reflex coronary vasoconstriction (41, 166) which thereby contributes to the attenuated ability to raise cardiac output and thus correct metabolic mismatches in ischemic active skeletal muscle. Not only the heart, but also the ischemic muscle becomes a target for metaboreflex – induced vasoconstriction (95). Inability to improve perfusion of ischemic active skeletal muscle in heart failure causes enhanced sympathetic activation due to over-activation of the muscle metaboreflex (41, 95). In heart failure ventricular-vascular interaction is already uncoupled and enhanced sympathetic outflow further decouples this relationship which thereby lessens the ability to sustain or improve skeletal muscle blood flow (115). Impaired ventricular function and ventricular- vascular interactions leads to an enhanced vasoconstriction even within the ischemic muscle (95). Limitations in raising skeletal muscle blood flow are thus both the cause and effect of an enhanced metabolic mismatch that causes the enhanced sympathetic activation in heart failure and likely other cardiovascular pathologies such as hypertension (20, 30, 63, 166, 167) To date no study has assessed the effect of chronic chemical ablation of skeletal muscle afferents in cardiovascular disease during exercise. A common symptom of these pathologies is exercise intolerance and fatigue, such that quality of life and the will to exercise are reduced. Studies have shown that exercise training regimens are capable of improving and in some cases correcting abnormal

cardiovascular responses during exercise (3, 12, 15, 52, 68, 81, 105, 137, 180). Furthermore, multiple studies have shown that attenuation of skeletal muscle afferents by way of activation of mu opioid receptors located on lower body afferents improves cardiovascular responses during exercise and reduces fatigue (6-8, 20, 156). In the present study we observed that a reduction of skeletal muscle afferents via intrathecal RTX was capable of significantly attenuating muscle metaboreflex responses during exercise. In addition, we observed animals treated with RTX were capable of maintaining workload performance with greater reduction in hindlimb blood flow, therefore, RTX may also lessen the sensation of fatigue during exercise as observed in previous studies utilizing opioids (7-10, 20, 124, 136, 156, 169, 177). Thus, chronic ablation of skeletal muscle afferents may allow greater exercise-induced increases in cardiovascular fitness, less exercise intolerance and improved overall quality of life.

CHAPTER 5: CONCLUSIONS

The major findings from these studies are:

1) The muscle metaboreflex increases effective arterial elastance and maintains ventricular-vascular coupling to increase stroke work. In heart failure baseline levels of effective arterial elastance are higher and the ventricular-vascular relationship is uncoupled. Metaboreflex induced increases in effective elastance worsen the ventricular-vascular relationship and limit stroke work likely contributing to the impaired ability to raise cardiac output and therefore improve skeletal muscle perfusion.

2) The contraction and relaxation rate ratio *in vivo* favors contraction during exercise and muscle metaboreflex activation elicits robust increases in relaxation to bring the ratio towards 1:1. In heart failure the ventricular contraction-relaxation ratio again favors contraction albeit significantly attenuated compared to control. During muscle metaboreflex activation increases in relaxation rate are significantly stifled preventing any reflex induced change in the ratio. Additionally, muscle metaboreflex control of contraction and relaxation gain is attenuated. It is possible that the enhanced coronary vasoconstriction previously shown to effect contraction also effects relaxation function in heart failure.

3) Intrathecal RTX significantly attenuates muscle metaboreflex activation. We observed that reflex increases in heart rate, cardiac output, mean arterial pressure, stroke volume, stroke work, effective arterial elastance are all attenuated after administration of RTX. Furthermore, it is likely that β_2 mediated vasodilation is also inhibited based on the attenuation of various hemodynamic parameters and significantly lower non-ischemic vascular conductance observed after administration of RTX. Intrathecal administration was shown to be effective in attenuating muscle metaboreflex responses for 78 ± 10 days.

FUTURE DIRECTIONS

Future directions of these studies could include investigation of interaction between the metaboreflex and the arterial baroreflex in the control of ventricular-vascular coupling, the role of coronary vasoconstriction in control of cardiac relaxation dynamics, and whether chronic ablation of skeletal muscle afferents could lower the exaggerated increases in sympathetic activity seen during exercise in heart failure. The arterial baroreflex is known to modulate both parasympathetic and sympathetic function through changes in heart rate, and sympathetic outflow to modulate arterial pressure. Furthermore, this control has been observed to be exerted on muscle metaboreflex activation of the vasculature (13, 98-100). To what extent the baroreflex is capable of attenuating healthy muscle metaboreflex induced increases in effective arterial elastance is unknown. Furthermore, baroreflex function is altered in heart failure (12, 25, 37, 60, 68, 73, 77-79, 83, 98, 132, 182) and to what extent this effects baroreceptor unloading or muscle metaboreflex induced changes in effective arterial elastance and therefore ventricular-vascular coupling is unknown. However, it is likely that the baroreflex a more vascular associated reflex would have a greater impact before and likely enhanced effect after heart failure on effective arterial elastance as a result of baroreceptor unloading.

In this study we observed muscle metaboreflex activation significantly enhances relaxation rate, and that heart failure attenuates these responses likely through enhanced coronary vasoconstriction. However, this study did not assess muscle metaboreflex control of diastolic function in regard to how these alterations in relaxation impact filling, or the end diastolic pressure volume relationship. It is likely that there is significant impairment in diastolic dynamics and that this also contributes to the inability to raise cardiac output in heart failure and other cardiovascular pathologies.

Intrathecal resiniferatoxin administration achieved approximately a 50% inhibition of muscle metaboreflex activation and shows promise as a potential therapy for attenuating the profound vasoconstriction observed during reflex activation in heart failure and other pathologies. In this study complete attenuation was not achieved and thus evaluation of possible compensatory mechanisms such baroreflex activation, or mechanosensitive afferents polymodality must be explored. Furthermore, TRPV1 receptors are not the only receptors located on metabosensitive afferents and thus there may be competition for receptor availability on the cell surface limiting toxin efficacy. Finally, within the data two distinct populations were observed after administration of RTX, animals whose baseline blood pressure at rest was reduced and those whom increased. Could this be an effect of baroreflex compensation due to the loss of some form of feedback from skeletal muscle afferents even in the absence of reduced perfusion. Furthermore, studies have shown that attenuation of skeletal muscle afferents do not affect baroreflex activity (60, 75), but those studies did not involve acute or chronic ablation and thus more studies are warranted.

Other future directions include the evaluation of all of these studies within various cardiovascular pathologies such as peripheral artery disease, hypertension, diabetes, and metabolic syndrome. Additionally, in regard to metaboreflex mechanisms that potentiate reflex activation, no study has assessed the impact of muscle metaboreflex activation on energy availability in the muscle I.E. the ability to enhance glucose efflux into active muscle, or the impact of metaboreflex sympathetic activity on pancreatic or immunological function.

In summary, these studies unveiled multiple aspects of the muscle metaboreflex that warrant further study in addition to adding more knowledge to the understanding of

the reflex arc as a whole.

APPENDIX A

IUCAC Protocol Approval Letters

WAYNE STATE UNIVERSITY

Institutional Animal Care
And Use Committee
87 East Canfield, Second Floor
Detroit, Michigan 48201
Phone: (313) 577-1629

ANIMAL WELFARE ASSURANCE # A3310-01

TO: Donal O'Leary
Physiology

FROM: Institutional Animal Care and Use Committee

DATE: January 27, 2017

SUBJECT: Approval of Protocol 16-10-155

TITLE: Blood pressure control during exercise in heart failure

Protocol Effective Period: January 27, 2017 - January 26, 2020

Your animal research protocol has been approved by the Wayne State University Institutional Animal Care and Use Committee (IACUC).

Be advised that this protocol must be reviewed by the IACUC on an annual basis to remain active. Any changes (e.g. procedures, lab personnel, strains, additional numbers of animals) must be submitted as amendments and require prior approval by the IACUC. Any animal work on this research protocol beyond the expiration date will require the submission of a new IACUC protocol application for committee review and approval.

The *Guide for the Care and Use of Laboratory Animals* (the Guide, NRC 2011) is the primary reference used for standards of animal care at Wayne State University. The University has submitted an appropriate assurance statement to the Office for Laboratory Animal Welfare (OLAW) of the National Institutes of Health. The animal care program at Wayne State University is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

WAYNE STATE UNIVERSITY

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ANIMAL WELFARE ASSURANCE # D16-00198 (A3310-01)

TO: Donal O'Leary
Physiology

FROM: Institutional Animal Care and Use Committee

DATE: January 29, 2020

SUBJECT: Approval of Protocol IACUC-19-11-1493

TITLE: Blood pressure control during exercise in heart failure

Protocol Effective Period: January 29, 2020 - January 28, 2023

Your animal research protocol has been approved by the Wayne State University Institutional Animal Care and Use Committee (IACUC).

Be advised that this protocol must be reviewed by the IACUC on an annual basis to remain active. Any changes (e.g. procedures, lab personnel, strains, additional numbers of animals) must be submitted as amendments and require prior approval by the IACUC. Any animal work on this research protocol beyond the expiration date will require the submission of a new IACUC protocol application for committee review and approval.

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APPENDIX B

Copyright License Agreement for Chapter 2

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TERMS AND CONDITIONS

Aug 10, 2020

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ABSTRACT

CAUSES AND POTENTIAL TREATMENT FOR ALTERED MUSCLE METABOREFLEX CONTROL OF VENTRICULAR VASCULAR INTERACTIONS IN HEART FAILURE

by

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Muscle Metaboreflex Activation occurs as a result of metabolic accumulation within active skeletal muscle that stimulates type III and IV afferents. This reflex in healthy subjects causes increased ventricular contraction, tachycardia, enhanced central blood volume mobilization, and β_2 mediated vasodilation as a means to increase mean arterial pressure and thereby improve perfusion pressure of active skeletal muscle. However, to date no study has evaluated the interaction between the observed changes in ventricular and vascular dynamics or how the reflex impacts contraction relaxation dynamics before and after induction of heart failure Furthermore, no study has evaluated the impact of chronic selective ablation of skeletal muscle afferents responsible for reflex activation. In this study we addressed three questions: 1) Does muscle metaboreflex activation increase effective arterial elastance and maintain ventricular vascular coupling before and after induction of heart failure, 2) Are ventricular contraction and relaxation dynamics influenced by muscle metaboreflex activation and what is the effect of heart failure, 3) What is the contribution of TRPV1 expressing skeletal muscle afferents to muscle metaboreflex activation. We used a chronically instrumented canine model in a

longitudinally designed study in which each animal serves as its own control against either induction of heart failure or treatment with intrathecal resiniferatoxin a potent dominant negative TRPV1 agonist that causes afferent ablation to assess these questions. We observed that: 1) Muscle metaboreflex activation does increase effective arterial elastance and this increase sustains ventricular vascular coupling promoting optimal energy transfer from the left ventricle to the systemic circulation and thereby increasing stroke work. In heart failure baseline levels of elastance are increased and the ventricular vascular relationship is uncoupled. Metaboreflex activation only worsens this relationship by further increasing effective arterial elastance with little to no improvements in ventricular maximal elastance. 2) The ventricular contraction and relaxation ratio is sensitive to muscle metaboreflex activation and is primarily affected by large reflex induced increases in relaxation rate. In heart failure metaboreflex activation does not alter the ratio from exercise conditions and this is due to a significant attenuation in the ability to increase relaxation rate. 3) TRPV1 sensitive skeletal muscle afferents account for 50-70% of metaboreflex induced changes in cardiovascular performance. Maximal hemodynamic responses to muscle metaboreflex activation are significantly attenuated. Based on these effects intrathecal administration of resiniferatoxin poses as a potential therapy to attenuate over sympatho-activaton during muscle metaboreflex activation in heart failure.

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PUBLICATIONS

1. **Mannozzi J**, Kaur J, Spranger MD, Al-Hassan MH, Lessanework B, Alvarez A, Chung CS, and O'Leary DS. Muscle Metaboreflex-Induced Increases in Effective Arterial Elastance: Effect of Heart Failure. *Am J Physiol Regul Integr Comp Physiol* 2020.
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