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Metabo and Mechanoreceptor Expression in Human Heart Failure: Relationships with the Locomotor Muscle Afferent Influence on Exercise Responses

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Abstract

Introduction: Heart failure patients with reduced ejection fraction (HFrEF) exhibit abnormal locomotor group III/IV afferent feedback during exercise; however, the underlying mechanisms are unclear. Therefore, the purpose of this study was to determine 1) metabo and mechanoreceptor expression in HFrEF and controls and 2) relationships between receptor expression and changes in cardiopulmonary responses with afferent inhibition.

Methods: Ten controls and 6 HFrEF performed 5 min of cycling exercise at 65% peak workload with lumbar intrathecal fentanyl (FENT) or placebo (PLA). Arterial blood pressure (BP) and catecholamines were measured via radial artery catheter. A vastus lateralis muscle biopsy was performed to quantify cyclooxygenase-2 (COX-2), purinergic $2X_3$ ($P2X_3$), transient receptor potential vanilloid type 1 (TRP_V1), acid-sensing ion channel 3 ($ASIC_3$), Piezo 1, and Piezo 2 protein expression.

Results: TRP_V1 and COX-2 protein expression were greater in HFrEF than controls (both, $p<0.04$), while $P2X_3$, $ASIC_3$, and Piezo 1 and 2 were not different between groups (all, $p>0.16$). In all participants, COX-2 protein expression was related to the % change in ventilation ($r= -0.66$) and MAP ($r= -0.82$) (both, $p<0.01$) with FENT (relative to PLA) during exercise. In controls, TRP_V1 protein expression was related to the % change in SBP ($r=-0.77$, $p=0.02$) and MAP ($r= -0.72$, $p=0.03$) with FENT (relative to PLA) during exercise.

Conclusion: TRP_V1 and COX-2 protein levels are elevated in HFrEF compared to controls. These findings suggest that the elevated TRP_V1 and COX-2 expression may contribute to the exaggerated locomotor muscle afferent feedback during cycling exercise in HFrEF.

Keywords

blood pressure; exercise pressor reflex; fentanyl; group III/IV muscle afferents; heart failure with reduced ejection fraction

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Introduction

Cardiopulmonary and autonomic adjustments to exercise, which include increases in blood pressure, ventilation ($\dot{V}_E$), and heart rate, are mediated by central command and the exercise pressor reflex, while modulated by the arterial baroreceptors (Fisher *et al.*, 2015; Smith *et al.*, 2019). The afferent arm of the exercise pressor reflex is comprised of group III (predominantly mechanically sensitive) and group IV (predominantly metabolically sensitive) afferents located within the interstitum of the contracting muscle (McCloskey & Mitchell, 1972; Adreani *et al.*, 1997). Importantly, inhibition of locomotor muscle group III/IV afferents via intrathecal fentanyl (FENT) results in attenuated increases in $\dot{V}_E$, cardiac output, and blood pressure during exercise in healthy young adults (Amann *et al.*, 2010; Amann *et al.*, 2011b) indicating that locomotor muscle afferent feedback significantly contributes to the cardiopulmonary and autonomic adjustments to exercise.

Exercise intolerance is a hallmark symptom of heart failure with reduced ejection fraction (HFrEF). One of the primary mechanisms responsible for the deterioration of exercise tolerance is exaggerated locomotor muscle neural afferent feedback (Sinoway & Li, 2005; Smith *et al.*, 2006; Piepoli *et al.*, 2008). In fact, inhibition of locomotor muscle group III/IV afferents via FENT results in greater ventilatory efficiency (i.e. ventilatory equivalent for carbon dioxide slope (V_E/VCO_2 slope)), oxygen uptake kinetics, and locomotor muscle blood flow during exercise in HFrEF (Amann *et al.*, 2014; Olson *et al.*, 2014; Van Iterson *et al.*, 2017). One of the underlying mechanisms responsible for the enhanced locomotor muscle neural feedback in HFrEF is differential expression of receptors associated with the metabolic component of the exercise pressor reflex (i.e. metaboreflex). Specifically, previous studies in the rat-infarct model of HF have investigated expression of receptors associated with the metaboreflex and found greater expression of cyclooxygenase-2 (COX-2) and purinergic $2X_3$ ($P2X_3$), and lower expression of transient receptor potential vanilloid type 1 (TRP_V1) and acid-sensing ion channel 3 ($ASIC_3$) compared to controls (Smith *et al.*, 2005; Wang *et al.*, 2010a; Morales *et al.*, 2012; Wang *et al.*, 2012; Xing *et al.*, 2015; Xing & Li, 2016). However, it is unclear if these findings in animals translate to humans and whether the differential protein expression is associated with cardiopulmonary and neural responses during exercise with locomotor muscle group III/IV afferent inhibition.

Therefore, the purpose of this study was to determine if differences exist in the protein expression of TRP_V1, COX-2, $P2X_3$, and $ASIC_3$ between healthy humans and patients with HFrEF. Further, we wanted to determine if differences existed in Piezo 1 and 2, mechano-gated channels associated with mechanoreflex sensitivity (Copp *et al.*, 2016), between groups. Lastly, we sought to determine if relationships were present between protein expression and changes in cardiopulmonary and neural responses with locomotor muscle group III/IV afferent inhibition (via FENT) during cycling exercise. Based on animal literature, we hypothesized that patients with HFrEF would exhibit 1) greater protein expression of COX-2 and $P2X_3$ and lower protein expression of TRP_V1 and $ASIC_3$ compared to age-matched controls and 2) significant relationships between increased protein expression and changes (i.e. decreases) in blood pressure and $\dot{V}_E$ during exercise with locomotor muscle afferent feedback inhibition.

Methods

Ethical approval:

All aspects of this study were approved by the Mayo Clinic Institutional Review Board (approval no. 09–000032) and conformed to the standards set forth by the latest revision of the Declaration of Helsinki except for registration in a database. All participants were informed about the experimental procedures and potential risk involved, and provided written and verbal informed consent.

Participants:

Six patients with HFrEF and 10 healthy matched control participants (CTL) were recruited for this study and provided written informed consent. The patients with HFrEF were recruited from the Mayo Clinic Heart Failure Service and the Cardiovascular Health Clinic. Inclusion criteria for the patients with HFrEF included diagnosis of ischemic or dilated cardiomyopathy with duration of >1 year of symptoms, stable HF symptoms (>3 months), left ventricular ejection fraction ≤35%, body mass index <35 kg/m^2, non-smokers with a smoking history of <15 pack-years, and no diagnosis of coexisting pulmonary disease. Patients with HFrEF performed all testing, while remaining on standard pharmacological therapy. CTL participants were matched for sex, age, and height to the patients with HFrEF and free of cardiovascular, pulmonary, and muscular diseases.

Experimental protocol:

For this single-blind case-control study, participants performed all protocols and measurements during four study visits. On the first study visit, participants were familiarized with all experimental measurements and protocols and then performed an incremental exercise test to volitional fatigue to determine peak oxygen uptake ($\dot{V}O_2$peak). On the second and third study visits, participants were randomized to lower lumber intrathecal injection of fentanyl (FENT) or placebo (PLA) and then performed constant workload submaximal exercise at 65% of peak workload. At rest and during exercise, ventilatory and metabolic variables and arterial blood pressure were measured and arterial blood sampling occurred. On the fourth study visit, a skeletal muscle biopsy was performed of the vastus lateralis for quantification of protein expression levels of TRP_V1, COX-2, $P2X_3$, $ASIC_3$, Piezo 1 and Piezo 2.

$\dot{V}O_2$peak:

Participants performed an incremental cycling test to volitional fatigue to determine $\dot{V}O_2$peak using an electronically braked upright cycle ergometer (Lode Corival, Groningen, the Netherlands). The incremental step test consisted of increasing workloads of 20 and 40 W increments for HFrEF and CTL, respectively every 3 min. During the incremental test, participants maintained a pedal frequency of 60 rpm and remained seated. Ventilatory and gas exchange variables were collected during the incremental cycling test (CPX/D, MGC Diagnostics, St. Paul, MN) and averaged over 30 s. Peak workload was determined as the highest workload achieved at $\dot{V}O_2$peak.

Intra-arterial blood pressure and blood sampling:

Following local anesthesia (1% lidocaine), a 20 gauge Teflon catheter (FA-04020; Arrow International Inc., Reading, PA) was placed in the non-dominant radial artery for blood sampling and arterial pressure measurement. Arterial pressure recordings from the pressure transducer (PX-MK099; Edwards Lifesciences, Irvine, CA) were exported to a digital oscilloscope for offline analysis of systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP). At rest and during the last min of exercise, arterial blood was drawn anaerobically over 10–15 s. Epinephrine (Epi) and norepinephrine (NE) plasma concentrations were analyzed via high performance liquid chromatography. Arterial partial pressure of carbon dioxide ($PaCO_2$) was measured in duplicate, averaged, and temperature corrected at a temperature of 37˚C (ABL825 Flex Blood Gas/CO-ox analyzer, Radiometer America Inc. Westlake, OH, USA).

Fentanyl lower lumbar intrathecal injection:

As previously described (Olson *et al.*, 2014), participants were seated in an upright flexed position and the skin and subcutaneous tissues were anaesthetized at the L3-L4 vertebral interspace with 2–4 mL of 1% lidocaine under aseptic technique. During the FENT study visit, a 22g Whitaker needle was advanced to the subarachnoid space, with placement confirmed by visualization of free-flowing cerebrospinal fluid. A small amount of free-flowing cerebrospinal fluid was aspirated and 1 mL of fentanyl (0.05 mg/mL) was injected. The participants remained in the seated position to minimize the cephalad migration of fentanyl. The PLA study visit was identical to the FENT study visit except the advancement of the needed to the subarachnoid space was simulated after subcutaneous local anesthesia.

Submaximal cycling exercise:

Within 2–3 min of placement of the radial intra-arterial catheter and the intrathecal injection (described above), resting data was collected for 5 min. Then, the participants exercised at 65% of peak power for 5 min followed by 5 min of recovery. Immediately following recovery, central chemosensitivity was assessed via CO_2 rebreathing. At rest and during the last min of exercise, arterial blood was sampled for $PaCO_2$, Epi, and NE. Ventilatory and gas exchange variables were collected at rest and during exercise using the same methodology used during the incremental test with the average of the last min of rest and exercise reported. $\dot{V}_E/\dot{V}CO_2$ slope was calculated using resting data and the last min of submaximal exercise (Olson *et al.*, 2014; Smith & Olson, 2019).

CO_2 rebreathing testing:

Following the submaximal cycling exercise, central chemosensitivity was assessed via CO_2 rebreathing testing as previously described (Olson *et al.*, 2014). Briefly, the participants breathed on a mouthpiece connected to a pneumatic switching valve and 6 L rebreathing bag (5% CO_2 and 95% O_2). Participants breathed room air for 2 min and were then switched to the rebreathing bag for 4 min or until one of the stopping criteria was reached. Stopping criteria included $P_{ET}CO_2$ of 65 mmHg, $P_{ET}O_2$ of 160 mmHg, $\dot{V}_E$ of 100 L/min, or the participants desire to stop. The slope of $\dot{V}_E$ versus $P_{ET}CO_2$ was used an index of central CO_2 chemosensitivity (Olson *et al.*, 2014). The test was performed 2–3 times (with the averaged

reported) with 3–5 min between allowing for cardiopulmonary variables to return to baseline levels.

Skeletal muscle biopsy:

Under aseptic technique and following local anesthesia (2% lidocaine), muscle biopsy samples (~50–100 mg) were obtained from the superficial portion of the left vastus lateralis by the percutaneous muscle biopsy technique (Han *et al.*, 2001). The skeletal muscle biopsy samples were cleaned of visible adipose and connective tissue then immediately frozen in liquid nitrogen and stored in a freezer at −80°C for subsequent analysis.

Western blotting:

Frozen muscle samples were homogenized in cold RIPA lysis buffer and protease inhibitor cocktail and the homogenates were centrifuged and supernatants were isolated. Proteins (68 μg) were electrophoretically separated using a 10% Bis Tris NuPage precast gel using NuPAGE SDS Running Buffer for 40 min at 200 V (Criterion Cell (Bio-Rad Laboratories, Hercules, CA) and then transferred to a nitrocellulose membrane (Thermo Scientific, Waltham, MA) for 75 min at 17 V. The membrane was blocked with 5% dried milk for 60 min and then incubated with 1) polyclonal anti-$TRPV_1$ antibody diluted 1:500 (Sigma-Aldrich, Cat# SAB3501027, RRID: AB_2810269), 2) monoclonal anti-COX-2 antibody diluted 1:500 (Cayman Chemical, Cat# 160112, RRID: AB_10078980), 3) monoclonal anti-$P2X_3$ antibody diluted 1:500 (Santa Cruz Biotechnology, Cat# sc-390572, RRID: AB_2810268) and 4) polyclonal anti-$ASIC_3$ antibody diluted 1:200 (Abcam, Cat# ab190638, RRID: AB_2810270) in 10x Tris-buffered saline (TBS), 0.1% Tween-20 (TBS-T) with 0.5% dried milk at 4 C overnight. Membranes were washed and then incubated for 60 min at room temperature with either horseradish peroxidase (for $TRPV_1$ and $ASIC_3$ diluted 1:5,000 and 1:1,000, respectively; ThermoFisher Scientific, Waltham, MA) or IRDye (for COX-2 and $P2X_3$ diluted 1:10,000; LiCor, Lincoln, NE) secondary antibodies in 10x Tris-buffered saline (TBS), 0.1% Tween-20 (TBS-T) with 0.5% dried milk. The same membranes were then stripped and used to determine glyceraldeyde-3-phosphate dehydrogenase (GAPDH) protein expression as an internal control (1:5,000 dilution; ab9485; Abcam, Cambridge, UK) and the $TRPV_1$, COX-2, $P2X_3$, and $ASIC_3$ protein expression reported herein is relative to GAPDH protein expression. Digital images were captured with chemiluminescence for $TRPV_1$ and $ASIC_3$ and infrared fluorescence for COX-2 and $P2X_3$ (Odyssey FC, LiCor, Lincoln, NE). To control for intra-assay variability, the HFrEF and CTL samples were alternated on the blot.

Piezo 1 and 2 total protein expression were performed with the Wes System (Protein Simple, San Jose, CA). The protein samples, primary and secondary antibodies, blocking reagent, wash buffer, and chemiluminescent substrate were loaded on the provided microplate according to the manufacturer's instructions. Specifically, skeletal muscle samples were diluted to 29 ng/μL in sample buffer (100x diluted '10x Sample Buffer'), then mixed with Fluorescent Master Mix and heated at 95°C for 5 min. All reagents: samples, blocking reagent (antibody diluent), primary antibodies (i.e. 1) polyclonal anti-Piezo 1 antibody diluted 1:160 (Novus Biologicals, Cat# NBP1–78537, RRID: AB_11003149) and 2) anti-Piezo 2 antibody diluted 1:100 (a gift from Dr. Ardem Patapoutian of The Scripps Research

Institute, La Jolla, CA) (Woo *et al.*, 2014)), HRP-conjugated secondary antibodies and chemiluminescent substrate were pipetted into a plate. Instrument default settings were used (e.g. separation time, temperature). The following criteria are routinely used to distinguish between signal and background: the peak signal-to-noise (S/N) ratio given by the software must be ≥10, and the peak height/baseline ratio (calculated manually from the peak height and baseline values given by the software) must be ≥3. To control for differences in signal between experiments, a 5-point calibration curve of a CTL muscle tissue was included in all runs. Each calibration curve must display a linearity of r^2>0.90. Data analysis was performed with Compass software (Protein Simple, San Jose, CA).

Statistical analyses:

Values are reported as mean±standard deviation (SD). Statistical analyses were performed using SigmaStat 2.0 (Jandel Scientific, San Rafael, CA). Normality and equal variance were assessed using the Shapiro-Wilk and Levene tests, respectively and non-parametric tests were used when appropriate. Participant characteristics, protein expression, and % change in cardiopulmonary variables with FENT (relative to PLA) were compared using unpaired *t*-tests. Cardiopulmonary variables as well as $PaCO_2$, Epi, and NE were compared within (PLA vs. FENT) and between (CTL vs. HFrEF) groups using mixed factorial analysis of variance and Tukey's post-hoc test when appropriate. Relationships were determined via linear regression. An influential outlier was detected in Figure 3 via Cook's distance, thus the data point was shown for transparency, but not included in the correlation. Statistical significance was set at $p<0.05$.

Results

Participant characteristics:

Age, height, hemoglobin, peak workload, and submaximal workload were not different between HFrEF and CTL (all, $p>0.12$) (Table 1). HFrEF had a greater BMI and lower $\dot{V}O_2$peak compared to CTL (both, $p<0.02$).

Protein expression:

Protein expression of TRP_V1 and COX-2 were greater for HFrEF compared to CTL (both, $p<0.04$), while $P2X_3$ and $ASIC_3$ were not different between groups ($p>0.21$) (Figure 1). Further, protein expression of Piezo 1 and 2 were not different between HFrEF and CTL (both $p>0.16$) (Figure 2).

Rest and submaximal exercise responses:

At rest with PLA, HFrEF had greater $\dot{V}_E$ and lower SBP and MAP than CTL (all, $p<0.05$). With FENT compared to PLA, arterial NE was greater in CTL at rest ($p<0.01$). With FENT compared to PLA, $PaCO_2$ was greater in HFrEF at rest ($p<0.01$). In addition, HFrEF had greater % decreases in $\dot{V}_E$ and f_B as well as increase in $PaCO_2$ than CTL with FENT at rest (all, $p<0.05$).

During exercise with PLA, HFrEF had lower RER, HR, SBP, DBP, and MAP than CTL (all, $p<0.05$), but higher arterial NE ($p=0.03$) (Table 2). With FENT compared to PLA, HR, SBP,

DBP, and MAP were lower during exercise for CTL (all, $p<0.04$). With FENT compared to PLA, $\dot{V}_E$, f_B, V_E/VCO_2 slope, SBP, DBP, and MAP were lower and $PaCO_2$ was higher during exercise for HFrEF (all, $p<0.02$). Further, HFrEF had greater % decreases in $\dot{V}_E$, f_B, and V_E/VCO_2 slope with FENT during exercise than CTL (all, $p<0.03$), but a greater % increase in $PaCO_2$ ($p=0.01$).

Relationships:

At rest, the % change in MAP and $\dot{V}_E$ from PLA to FENT were negatively related to TRP_V1 protein expression in CTL ($r= -0.72$, $p=0.02$ and $r= -0.80$, $p<0.01$, respectively). Further, the % change in MAP was negatively related to $ASIC_3$ protein expression ($r= -0.65$, $p=0.04$) in CTL at rest. No other relationships were present between protein expression and % change in cardiopulmonary variables from PLA to FENT in CTL or HFrEF at rest ($p>0.05$).

During submaximal exercise, the % change in MAP ($r= -0.72$, $p=0.03$) and SBP ($r= -0.77$, $p=0.02$) from PLA to FENT were negatively associated with TRP_V1 protein expression in CTL (Figure 3). Further, $\dot{V}_E/\dot{V}CO_2$ slope from PLA to FENT was positively related to TRP_V1 protein expression in CTL ($r= 0.81$, $p<0.01$). In all participants, the % changes in MAP ($r= -0.82$, $p<0.01$) and $\dot{V}_E$ ($r= -0.66$, $p<0.01$) from PLA to FENT were negatively associated with COX-2 protein expression (Figure 4). Further, these negative relationships between the % changes in MAP and $\dot{V}_E$ from PLA to FENT with COX-2 protein expression remained with the removal of the HFrEF patient with the greatest COX-2 protein expression ($r= -0.51$, $p=0.05$ and $r= -0.53$, $p=0.04$, respectively). Lastly, the % change in $PaCO_2$ from PLA to FENT was positively associated with TRP_V1 protein expression in all participants ($r= 0.60$, $p=0.02$). No other relationships were present during submaximal exercise between protein expression and % change in cardiopulmonary variables from PLA to FENT in CTL or HFrEF ($p>0.05$).

Central chemosensitivity:

There were no changes in the $V_E/P_{ET}CO_2$ slope between PLA and FENT in CTL (PLA: 2.3±0.8 vs. FENT: 2.1±0.9 L/min/mmHg, $p=0.15$) or HFrEF (PLA: 2.4±0.7 vs. FENT: 2.4±0.6 L/min/mmHg, $p=0.69$)

Discussion

The major novel findings of the present study are threefold. First, patients with HFrEF exhibited greater protein expression of TRP_V1 and COX-2 than CTL, while no differences were present in protein expression of $P2X_3$, $ASIC_3$, Piezo 1, or Piezo 2 between groups. Second, the changes in MAP and $\dot{V}_E$ with FENT (relative to PLA) during submaximal exercise were associated with COX-2 protein expression in all participants. Third, the changes in MAP and SBP with FENT (relative to PLA) during submaximal exercise were associated with TRP_V1 protein expression in CTL. These findings suggest that HFrEF-induced increases in metaboreceptors influence the cardiopulmonary and neural responses during locomotor muscle exercise.

Protein expression in HFrEF:

In the present study, we found that TRP_V1 and COX-2 protein expression levels were elevated in HFrEF compared to CTL, while $P2X_3$ and $ASIC_3$ were not different between groups. To date, there have been minimal studies in humans quantifying protein expression levels of receptors associated with the metabolic and mechanical components of the exercise pressor reflex. Specifically, Antunes-Correa et al found that COX-2 protein expression decreased from pre- to post-exercise training in patients with HFrEF (Antunes-Correa *et al.*, 2014). However, since no control group was included in the study, it is unclear if these protein expression levels were initially elevated in HFrEF compared to healthy individuals. Previous studies using the rat-infarct model of HF have found differential protein expression of TRP_V1, COX-2, $P2X_3$, and $ASIC_3$ when compared to healthy rats (Smith *et al.*, 2005; Wang *et al.*, 2010a; Morales *et al.*, 2012; Wang *et al.*, 2012; Xing *et al.*, 2015; Xing & Li, 2016). For example, TRP_V1 and $ASIC_3$ have been found to be lower, while COX-2 and $P2X_3$ have been found to be greater in the rat-infarct model of HF compared to control rats (Smith *et al.*, 2005; Wang *et al.*, 2010a; Morales *et al.*, 2012; Wang *et al.*, 2012; Xing *et al.*, 2015; Xing & Li, 2016). The TRP_V1, $P2X_3$, and $ASIC_3$ findings presented herein were surprising as previous animal models have shown that hindlimb intra-arterial infusion of capsaicin and lactic acid resulted in blunted increases in group IV afferent activity and blood pressure (Smith *et al.*, 2005; Wang *et al.*, 2010a; Wang *et al.*, 2010b; Xing *et al.*, 2015), while hindlimb intra-arterial infusion of α,β-methylene ATP increased the blood pressure response in HF compared to control rats (Gao *et al.*, 2007). Likely explanations for the discrepant findings in TRP_V1 and $P2X_3$ include species differences, length of disease, age (i.e. generally young rats are used), and/or medication use. Further, obesity has been shown to influence the underlying mechanisms of the metaboreflex (Negrao *et al.*, 2001; Milia *et al.*, 2015). Future studies are necessary to determine the independent effect of obesity on protein expression of receptors associated with the metaboreflex. It is important to note that other receptors (not investigated in the present study) associated with the metaboreflex have also been reported to be differentially expressed between HFrEF and CTL (e.g., kinin 2 receptors) (Xing & Li, 2016). For the first time, we also quantified Piezo 1 and 2, mechano-gated channels associated with the mechanoreflex (Copp *et al.*, 2016), protein expression levels in HFrEF and CTL and found no differences between groups. Taken together, these preliminary findings indicate that pathophysiologic mechanisms associated with HFrEF elevate TRP_V1 and COX-2 protein expression in humans.

Relationships between cardiopulmonary responses and protein expression in HFrEF:

Neural afferent feedback arising from the locomotor muscles significantly contributes to blood pressure and ventilatory regulation during exercise in humans (Amann *et al.*, 2010, 2011a). To this point, previous studies have demonstrated attenuated increases in blood pressure and $\dot{V}_E$ during exercise in HFrEF and CTL participants by inhibition of locomotor muscle group III/IV afferents via intrathecal fentanyl injection (Amann *et al.*, 2010; Amann *et al.*, 2011b; Amann *et al.*, 2014; Olson *et al.*, 2014). Consistent with these previous studies, the present study found that $\dot{V}_E$ was reduced at rest and during exercise in patients with HFrEF and blood pressure was reduced during exercise in both groups with FENT.

A secondary purpose of the present study was to determine if relationships were present between the cardiopulmonary variables with FENT (relative to PLA) and metabo- and mechanoreceptor protein expression. We found that the % change in MAP and $\dot{V}_E$ with FENT during exercise were associated with COX-2 protein expression levels in all participants. These findings suggest that as COX-2 protein expression increases in patients with HFrEF, there is greater locomotor muscle group III/IV afferent feedback during cycling exercise. Consistent with these relationships, previous studies have found that HFrEF patients have greater prostaglandin production during handgrip exercise and isolated metaboreflex activation compared to age-matched controls (Scott *et al.*, 2002; Scott *et al.*, 2004). Further, previous studies have found that COX inhibition attenuates the exaggerated ventilatory response during isolated metaboreflex activation as well as the blood pressure and sympathetic activity responses to mechanoreflex activation and mechanoreflex sensitization in HFrEF patients and animal models (Scott *et al.*, 2004; Middlekauff *et al.*, 2008; Morales *et al.*, 2012).

We found that the % changes in SBP and MAP during exercise with FENT were associated with TRP_V1 protein expression in CTL. Consistent with our findings, recent studies have found that TRP_V1 contributes to the blood pressure response during handgrip exercise in healthy adults via the metabolically-sensitive component of the exercise pressor reflex (Dawson *et al.*, 2004; Notay *et al.*, 2018; Vianna *et al.*, 2018). For example, Vianna et al found that topical application of capsaicin-based analgesic balm in healthy men resulted in attenuated increases in blood pressure and muscle sympathetic nerve activity via TRP_V1 desensitization when the skeletal muscle metaboreflex was activated (Vianna *et al.*, 2018). Future studies using an interventional approach are necessary to determine the impact of these receptors on the integrative response to whole-body exercise in humans.

Methodological considerations:

There are several methodologic considerations that may have influenced our results. First, while this is the first study in humans comparing a clinical population with matched healthy control participants, we acknowledge the relatively small sample size of the HFrEF group, which may have limited our ability to determine differences in some outcomes (e.g., $P2X_3$ protein expression). Second, the protein expression of TRP_V1, COX-2, $P2X_3$, $ASIC_3$, Piezo 1, and Piezo 2 were determined from skeletal muscle biopsy samples, which included neuronal, vascular, muscular, and connective tissue. Third, the relationships between changes in cardiopulmonary and neural responses with FENT presented herein provide essential insight as to the underlying mechanisms contributing to the locomotor muscle group III/IV afferent feedback during large muscle (i.e. whole-body) exercise in humans. However, future interventional studies are necessary to confirm the findings of the present study.

Conclusions:

Patients with HFrEF exhibit greater expression of TRP_V1 and COX-2 compared to CTL. Future studies using an interventional approach are necessary to determine if changes in locomotor muscle TRP_V1 and COX-2 expression helps to minimize the exaggerated locomotor muscle group III/IV afferent feedback during exercise in HFrEF patients.

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New Findings

What is the central question of this study?

The goal of this study was to compare locomotor muscle metabo and mechanoreceptor expression in heart failure patients and controls. Further, we investigated if relationships existed between the protein expression and cardiopulmonary responses during exercise with locomotor muscle neural afferent feedback inhibition.

What is the main finding and its importance?

The novel findings were that heart failure patients exhibited greater protein expression of TRP_V1 and COX-2 than controls. These findings are important as they identify receptors that may underlie the augmented locomotor muscle neural afferent feedback in heart failure.

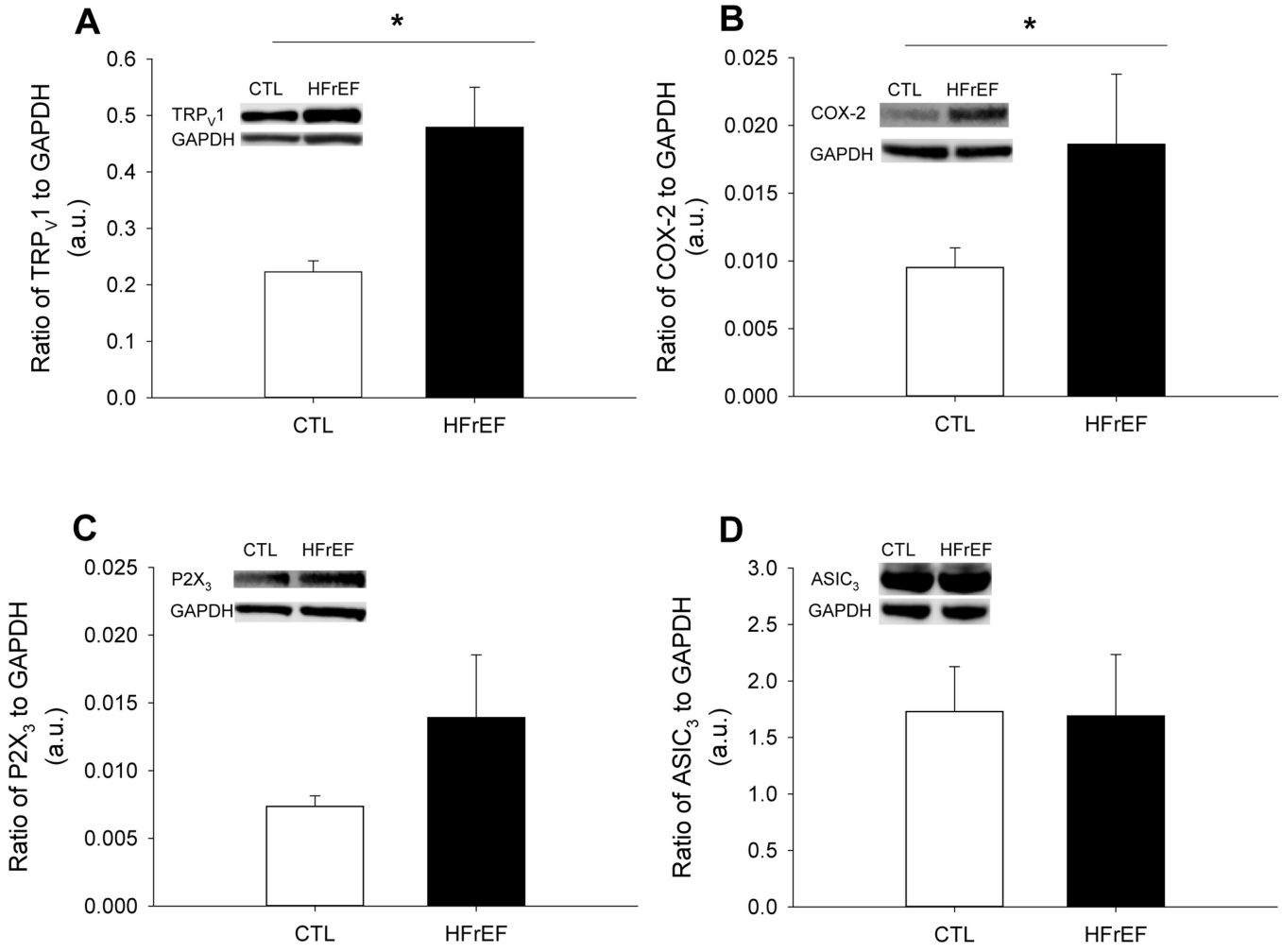

Figure 1: Metaboreceptor protein expression in CTL and HFrEF

TRP_V1 (A), COX-2 (B), $P2X_3$ (C), and $ASIC_3$ (D) protein expression normalized to GAPDH in CTL and HFrEF. TRP_V1 and COX-2 protein expression were greater in HFrEF compared to CTL ($p<0.04$), while $P2X_3$ and $ASIC_3$ were not different (both, $p>0.21$). Data reported as mean±SD.

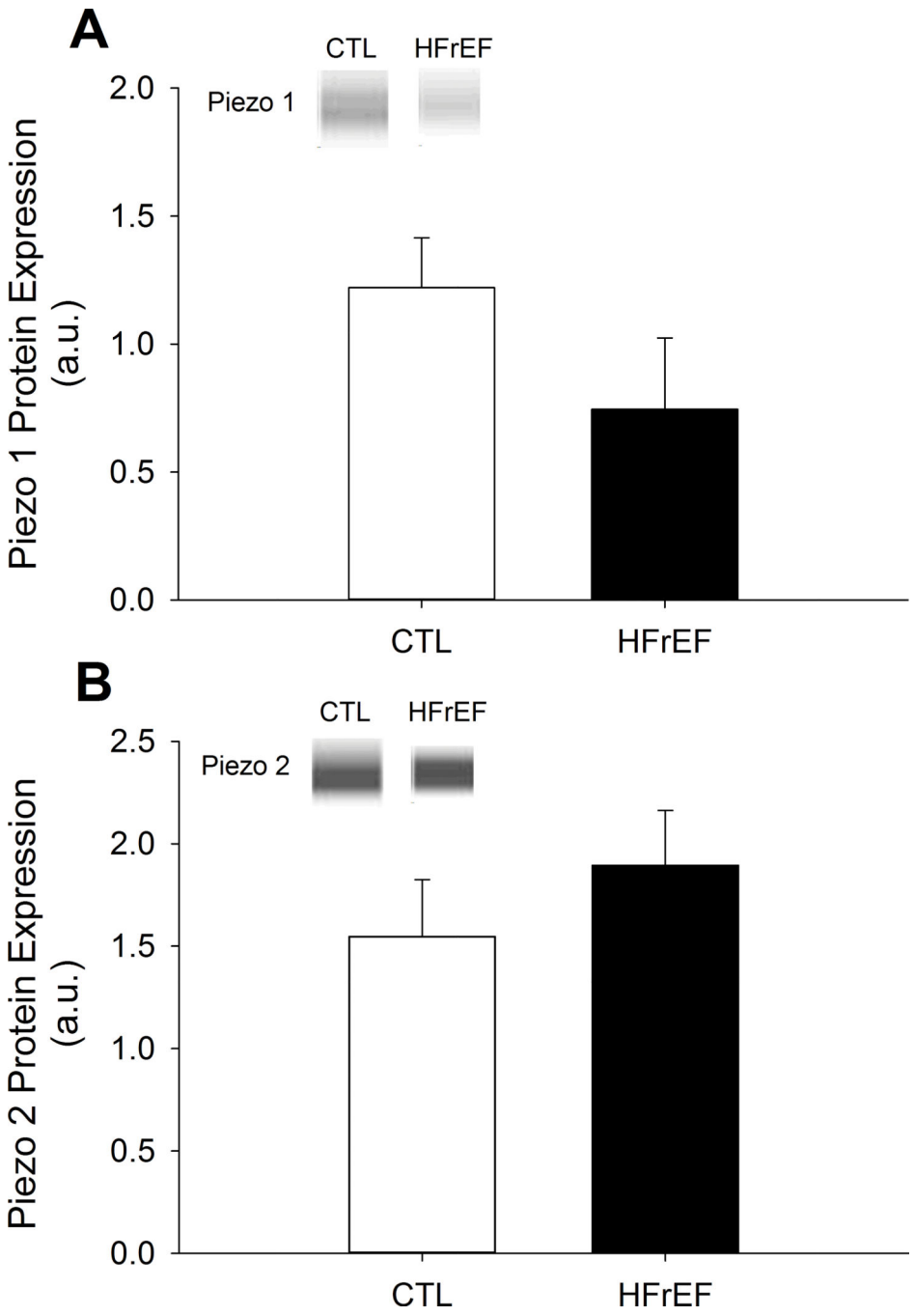

Figure 2: Mechanoreceptor protein expression in CTL and HFrEF

Piezo 1 (A) and 2 (B) protein expression in CTL and HFrEF. There were no differences in Piezo 1 and 2 protein expression between CTL and HFrEF (both, $p>0.16$). Data reported as mean±SD.

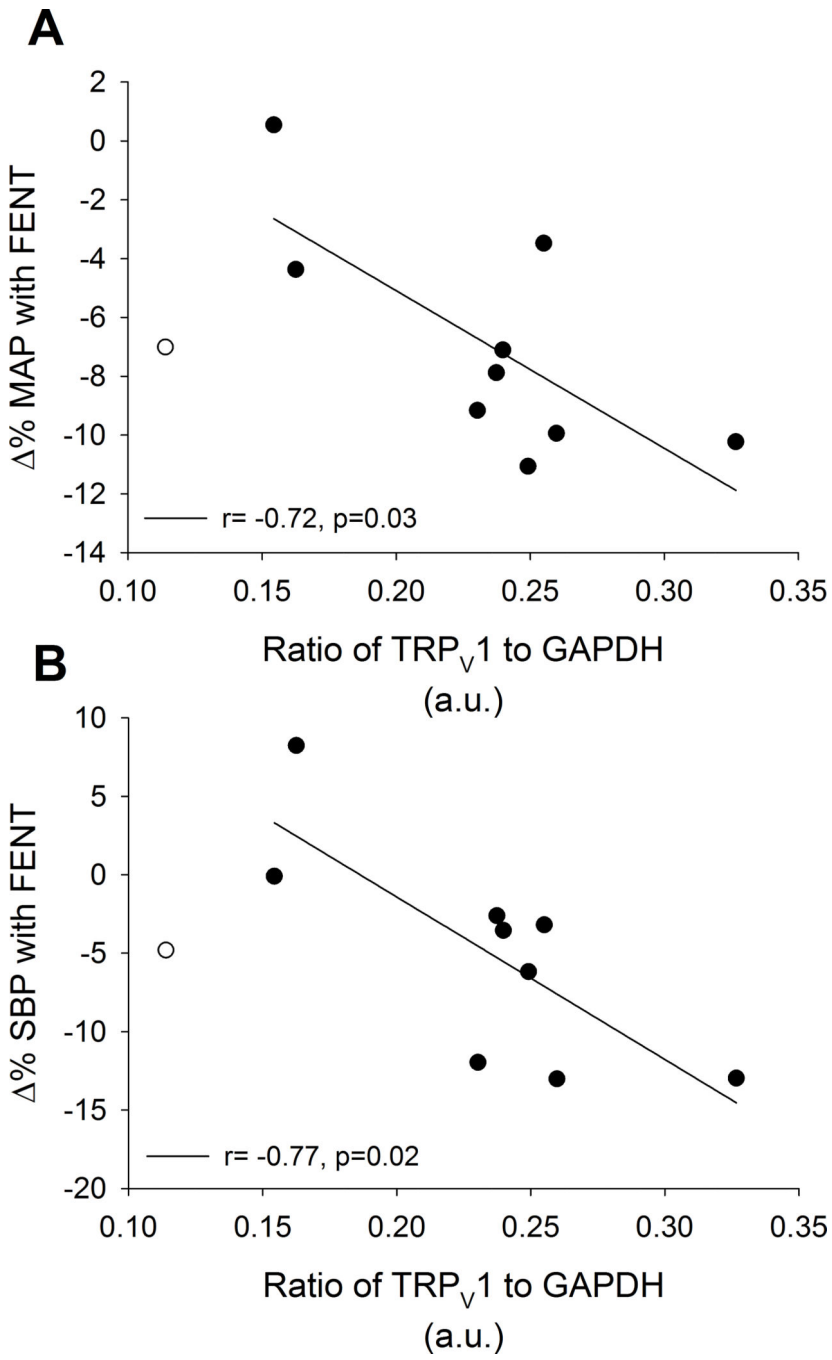

Figure 3: Relationships between TRP_V1 and % change in blood pressure with FENT in CTL during exercise

In CTL during exercise, TRP_V1 protein expression was negatively related to the % change with FENT in MAP (A; r= −0.72, p=0.03) and SBP (B; r= −0.77, p=0.02). An influential outlier was detected and shown for transparency (open circle), but not included in the correlation (see Methods).

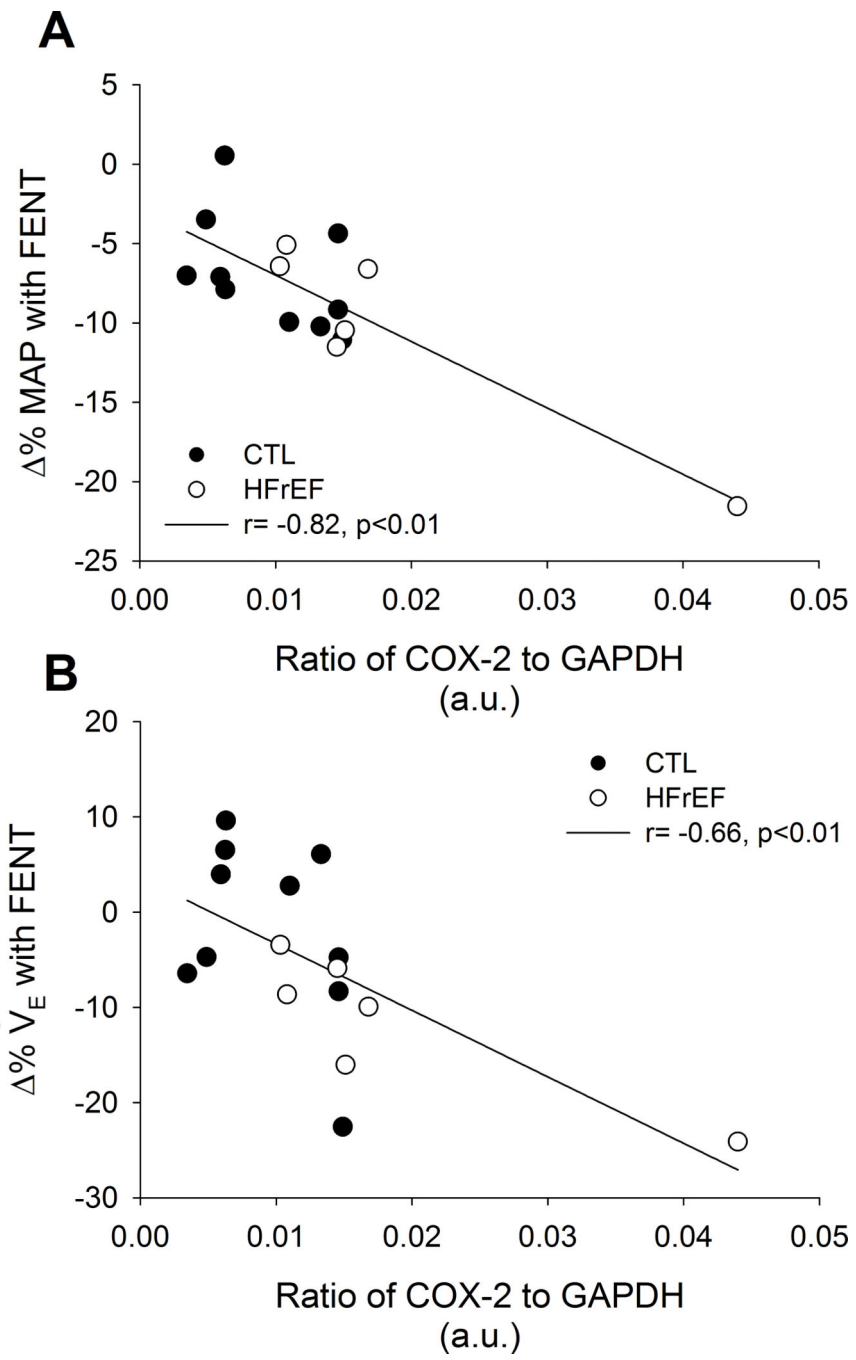

Figure 4: Relationships between COX-2 and % change in blood pressure and ventilation with FENT in CTL and HFrEF during exercise

In all participants during submaximal exercise, COX-2 protein expression was negatively related to the % change with FENT in MAP (A; r= −0.82, p<0.01) and $\dot{V}_E$ (B; r = −0.66, p<0.01). CTLs and HFrEF are presented in closed and open circles, respectively.

Table 1:

participant characteristics

	CTL	HFrEF
n	**10**	**6**
Age (years)	63 ± 8	60 ± 4
Sex (men/women)	8/2	5/1
Height (cm)	175 ± 9	179 ± 5
Weight (kg)	79 ± 12	100 ± 11 *
Body mass index (kg•m^2)	26 ± 3	31 ± 5 *
Haemoglobin (g•dL^{-1})	14.1 ± 1.5	14.1 ± 1.1
VO_2peak (mL•kg^{-1}•min^1)	27.2 ± 5	19.0 ± 3.1 *
Peak workload (W)	176 ± 55	137 ± 34
Submaximal workload (W)	116 ± 38	87 ± 13
LV ejection fraction (%)		27 ± 6
NYHA class: I/II/III		3/2/1
ACE I or ARBs	0 (0)	6 (100)
β-blocker	0 (0)	6 (100)
Aspirin	0 (0)	3 (50)
Diuretics	0 (0)	5 (83)

Mean±SD. VO_2, oxygen uptake; W, watts; LV, left ventricular; NYHA, New York Heart Association; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blockers.

*, significantly different than CTL.

Table 2:

Resting cardiopulmonary data with PLA and FENT

	CLT		HFrEF		CLT	HFrEF
	PLA	**FENT**	**PLA**	**FENT**	**Δ%**	**Δ%**
$\dot{V}O_2$ (L•min^{-1})	0.34 ± 0.03	0.33 ± 0.07	0.36 ± 0.04	0.39 ± 0.05	−1.9 ± 14.1	6.8 ± 9.7
$\dot{V}CO_2$ (L•min^{-1})	0.30 ± 0.03	0.29 ± 0.05	0.33 ± 0.03	0.35 ± 0.05 *	−1.8 ± 13.7	6.7 ± 13.2
$\dot{V}_E$ (L•min^{-1})	11 ± 2	12 ± 2	14 ± 2 *	13 ± 2	5.6 ± 13.9	−7.1 ± 11.9 *
V_T(L)	0.8 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	−1.3 ± 19.3	1.2 ± 8.4
f_B (breaths•min^{-1})	16 ± 4	17 ± 4	19 ± 2	18 ± 2	9.3 ± 19.8	−8.3 ± 8.1 *
HR (beats•min^{-1})	72 ± 12	71 ± 11	67 ± 11	67 ± 8	0.2 ±11.5	0.6 ± 6.5
SBP (mmHg)	143 ± 11	141 ± 14	119 ± 11 *	114 ± 10 *	−1.2 ± 8.5	−4.0 ± 6.8
DBP (mmHg)	68 ± 10	64 ± 8	60 ± 8	59 ± 8	−4.8 ± 8.8	−1.1 ± 7.8
MAP (mmHg)	93 ± 9	90 ± 9	80 ± 8 *	77 ± 8 *	−3.0 ± 8.3	−2.7 ± 6.4
$PaCO_2$ (mmHg)	38 ± 3	39 ± 4	39 ± 3	42 ± 5 †	2.0 ± 5.0	7.3 ± 3.6 *
Arterial Epi (pg•mL^{-1})	99 ± 43	159 ± 189	132 ± 95	127 ± 58	75.0 ± 207.5	16.4 ± 66.0
Arterial NE (pg•mL^{-1})	498 ± 149	675 ± 249 †	658 ± 161	785 ± 203	38.0 ± 31.6	21.3 ± 28.9

Mean±SD. VO_2, oxygen uptake; VCO_2, carbon dioxide production; RER, respiratory exchange ratio; V_E, ventilation; V_T, tidal volume; f_B, breathing frequency; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; $PaCO_2$, arterial carbon dioxide pressure; Epi, epinephrine; NE, norepinephrine.

*, signficantly different than CTL.

†, significantly different than PLA.

Table 3:

Cardiopulmonary responses with PLA and FENT during exercise

	CTL		HFrEF		CTL	HFrEF
	PLA	FENT	PLA	FENT	Δ%	Δ%
$\dot{V}O_2$ (L•min^{-1})	1.6 ± 0.4	1.7 ± 0.4	1.6 ± 0.3	1.7 ± 0.3	4.8 ± 9.9	1.6 ± 5.5
$\dot{V}CO_2$ (L•min^{-1})	1.9 ± 0.5	1.9 ± 0.5	1.7 ± 0.3	1.7 ± 0.3	3.1 ± 8.2	3.1 ± 10.5
$\dot{V}_E$ (L•min^{-1})	62 ± 16	61 ± 18	54 ± 9	48 ± 10†	−1.8 ± 9.6	−11.4 ± 7.6*
V_T(L)	2.1 ± 0.5	2.1 ± 0.5	1.8 ± 0.2	1.8 ± 0.3	−1.7 ± 6.1	−0.9 ± 9.2
f_B (breaths•min^{-1})	30 ± 5	30 ± 5	30 ± 6	27 ± 6†	0.0 ± 6.6	−10.3 ± 4.8*
$\dot{V}_E/\dot{V}CO_2$ slope	31 ± 3	29 ± 4	31 ± 4	26 ± 2†	−2.6 ± 5.7	−15.2 ±10.3*
HR (beats/min)	129 ± 16	123 ± 20†	102 ± 23*	96 ± 18*	−4.5 ± 6.7	−4.5 ± 6.5
SBP (mmHg)	243 ± 16	232 ± 27†	165 ± 30*	148 ± 28*†	−5.0 ± 6.6	−10.3 ± 8.8
DBP (mmHg)	76 ± 9	69 ± 6†	63 ± 10*	57 ± 11*†	−9.8 ± 7.3	−10.2 ± 5.8
MAP (mmHg)	132 ± 6	123 ± 7†	97 ± 14*	87 ± 15*†	−10.3 ± 6.1	−7.0 ± 3.6
$PaCO_2$ (mmHg)	38 ± 4	39 ± 4	37 ± 3	43 ± 4†	4.0 ± 5.9	14.7 ± 9.0*
Arterial Epi (pg•mL^{-1})	208 ± 115	206 ± 134	206 ± 67	253 ± 66	4.0 ± 36.2	33.8 ± 56.4
Arterial NE (pg•mL^{-1})	1506 ± 365	1670 ± 517	2127 ± 853*	2154 ± 748	10.2 ± 33.7	5.4 ± 27.1

Mean±SD. VO_2, oxygen uptake; VCO_2, carbon dioxide production; RER, respiratory exchange ratio; V_E, ventilation; V_T, tidal volume; f_B, breathing frequency; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; $PaCO_2$, arterial carbon dioxide pressure; Epi, epinephrine; NE, norepinephrine.

*, signficantly different than CTL.

†, significantly different than PLA.