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Improved Ventilatory Efficiency with Locomotor Muscle Afferent Inhibition is Strongly Associated with Leg Composition in Heart Failure

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Abstract

Background—Skeletal muscle atrophy contributes to increased afferent feedback (group III and IV) and may influence ventilatory control (high V_E/VCO_2 slope) in heart failure (HF).

Objective—This study examined the influence of muscle mass on the change in V_E/VCO_2 with afferent neural block during exercise in HF.

Methods—17 participants [9 HF (60±6 yrs) and 8 controls (CTL) (63±7 yrs, mean±SD)] completed 3 sessions. Session 1: dual energy x-ray absorptiometry and graded cycle exercise to volitional fatigue. Sessions 2 and 3: 5 min of constant-work cycle exercise (65% of peak power) randomized to lumbar intrathecal injection of fentanyl (afferent blockade) or placebo. Ventilation (V_E) and gas exchange (oxygen consumption, VO_2 ; carbon dioxide production, VCO_2) were measured.

Results—Peak work and VO_2 were lower in HF ($p<0.05$). Leg fat was greater in HF (34.4±3.0 and 26.3±1.8%) and leg muscle mass was lower in HF (63.0±2.8 and 70.4±1.8%, respectively, $p<0.05$). V_E/VCO_2 slope was reduced in HF during afferent blockade compared with CTL (−18.8±2.7 and −1.4±2.0%, respectively, $p=0.02$) and was positively associated with leg muscle mass ($r^2=0.58$, $p<0.01$) and negatively associated with leg fat mass ($r^2=0.73$, $p<0.01$) in HF only.

Conclusions—HF patients with the highest fat mass and the least leg muscle mass had the greatest improvement in V_E/VCO_2 with afferent blockade with leg fat mass being the only

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Conflicts of Interest: none

Competing Interests

None

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predictor for the improvement in V_E/VCO_2 slope. Both leg muscle mass and fat mass are important contributors to ventilatory abnormalities and strongly associated to improvements in V_E/VCO_2 slope with locomotor afferent inhibition in HF.

Keywords

Group III/IV; exercise; ventilation; fentanyl

INTRODUCTION

Adaptations within the skeletal muscle are common in heart failure with reduced ejection fraction (HFrEF) and may contribute to exercise intolerance, manifested by symptoms of dyspnea and muscle fatigue. Patients with HFrEF often have multiple physiologic system dysfunction leading to excessive sympathetic nerve activity, reduced limb blood flow, muscle deconditioning, and a number of pulmonary complications including pulmonary hypertension, impaired gas exchange and altered ventilatory control [1–4]. Thus, these changes in the periphery, in contrast to left ventricular performance itself, may be the primary mediators limiting exercise capacity in these patients [5, 6]. Along these lines, patients with HFrEF show multiple marked histological abnormalities of skeletal muscle and this condition may be perceived as ‘cardiac skeletal myopathy’. In particular, muscle fiber atrophy was observed in 68% of HFrEF patients with associated decreased capillary density [7]. Histological studies demonstrate that patients with HFrEF have a fiber type inversion, with a decrease in percentage of slow-twitch type I fibers and an increase in percentage of fast-twitch type II fibers [8]. This shift, leading to reduced oxidative capacity with greater reliance on anaerobic metabolism, may be a major contributor of exercise intolerance.

The skeletal muscle contribution to exercise intolerance in HF has been coined the ‘muscle hypothesis’. The muscle hypothesis indicates that a number of skeletal muscle factors are involved in the development of reduced peak oxygen uptake (VO_2) in patients with HFrEF, including the above noted skeletal muscle adaptations, histological abnormalities in mitochondrial structure and function, as well as oxidative stress in muscle [9]. Secondary to these skeletal muscle alterations, increased activity of afferent feedback from muscle (group III and IV afferents) has been identified as a key mediator linking the skeletal muscle myopathic changes with exercise intolerance [10]. The over-activation of afferent neurologic signals originating from the skeletal muscle is a hypothesis proposed to explain the origin of symptoms and the beneficial effect of exercise training in HFrEF.

The role of the metaboreflex has clearly been demonstrated as a contributor in the control of the ventilatory response to exercise [11, 12]. As such, stimulation of metaboreceptors by local chemical changes provokes hyperpnea. With the administration of intrathecal fentanyl as a mode for afferent blockade, Amann *et al.* (2009), demonstrated significant attenuation of the cardiovascular and ventilatory responses to high-intensity constant workload cycling with no impact on measures of efferent neuromuscular control [13]. Cyclists performed a 5K time trial with and without afferent blockade and demonstrated significant peripheral muscle fatigue when locomotor afferent feedback was blocked, suggesting that afferent feedback from working locomotor muscle may be a protective mechanism to modulate central drive

and minimize muscle fatigability development (via stimulating adequate ventilatory and circulatory responses to optimize supply and demand matching during exercise) [14]. Furthermore, as afferent feedback is fundamental in healthy individuals for appropriate exercise responses, we have demonstrated that this feedback contributes to excessive ventilation and poor ventilatory efficiency during exercise in HFrEF [4, 15].

Ventilatory efficiency is defined as the ventilatory response relative to CO₂ production. The slope generated from a plot of V_E over VCO₂ relates to HF severity, such that a greater V_E/VCO₂ slope indicates excessive ventilation for a given CO₂, and is thereby linked to increased morbidity and mortality in HFrEF [16, 17]. The purpose of this study was to determine the relationship between leg skeletal muscle mass and ventilatory efficiency (V_E/VCO₂) during cycling exercise. We hypothesized that leg muscle mass would be inversely associated with V_E/VCO₂ slope, and that blocking locomotor muscle afferent feedback would generate the greatest improvement in V_E/VCO₂ in HFrEF patients with the least muscle mass, suggesting a major role for muscle mass in the locomotor muscle afferent modulation of exercise ventilation in HFrEF.

METHODS

Subject Participation

Nine patients from the Mayo Clinic Heart Failure Service and the Cardiovascular Health Clinic (a preventive and rehabilitative center) completed the study (Table 1). Inclusion criteria included: diagnosis of ischemic or dilated cardiomyopathy with duration of HF symptoms >1 year, stable HF symptoms (>3 months), left ventricular ejection fraction 35% (from clinical records within 3 months), body mass index (BMI) <35 kg/m², and current non-smokers with a past smoking history <15 pack-years. All patients were treated with standard optimized medications for HFrEF patients at the time of the study (Table 1).

Eight control participants (CTL) were recruited via advertisement in the surrounding community with intention to match the HF group for age and sex. Control participants had normal cardiac function without evidence of exercise-induced ischemia on ECG and had no history of hypertension, lung disease or coronary artery disease.

Prior to the experiments, participants gave written informed consent. This study was conducted in accordance with the Declaration of Helsinki and approved by the Mayo Clinic Institutional Review Board. Some gas exchange variables measured in this study have been previously reported [15]. The relationships reported in this paper are novel and ventilation is only reported as it relates to leg muscle mass and fat mass.

Experimental Protocol Overview

These procedures have been described in detail previously and will be explained briefly as it relates to this study [15]. Participants underwent three days of testing procedures in an environmentally controlled physiology laboratory separated by a minimum of 48 hours. The first study visit included a dual energy x-ray absorptiometry (DEXA) (GE Healthcare) scan and a peak cycle ergometry exercise test at 65 rpms with increasing workloads of 20 watts (HFrEF patients) or 40 watts (CTL) every 3 minutes (to volitional fatigue). The second and

third study visits consisted of constant-work submaximal exercise at 65% of the previously determined peak power output and were randomized to intrathecal injection of fentanyl at the lumbar level to produce locomotor afferent neural block or placebo (sham injection) in a single blind fashion. These procedures have been described previously [15]. Ventilation (V_E) and gas exchange were measured continuously during all exercise sessions. Prior to all exercise, participants were fitted with a nose clip and standard mouthpiece attached to a PreVent Pneumotach (Medical Graphic, St. Paul, MN) worn throughout the testing procedure for measurement of V_E and pulmonary gas exchange.

Study visits 2 and 3 were identical with the exception of using fentanyl or placebo during the intrathecal injection (see below). Within 2–3 minutes after the intrathecal injection technique, the participants were seated on a recumbent cycle ergometer and V_E and gas exchange were measured for 5 minutes while resting (last minute of resting data used for analysis). After resting, participants cycled at 65% peak power output at 65 rpm for 5 minutes. At the end of the exercise session participants stopped cycling and recovered for 5 minutes. After recovery, central chemoreceptor sensitivity via CO_2 rebreathing was measured (see below).

Blockade of Afferent Neural Traffic from the Locomotor Muscles vs Placebo

Participants were seated in upright flexed position and, using an aseptic technique, the skin and subcutaneous tissue were anesthetized at the L3-L4 vertebral interspace with 2–4 mL of 1% lidocaine. During the experimental study visit, a 22 or 25g Whitaker needle was advanced to the subarachnoid space with placement confirmed by visualization of free flowing CSF. A small amount of CSF was aspirated and 1 mL of fentanyl (50 mcg/mL) injected. During the placebo visit participants were prepared in the same way; however, advancement of the needle to the subarachnoid space was simulated after subcutaneous local anesthesia.

Measurement of Ventilation and Gas Exchange

Oxygen consumption (VO_2), carbon dioxide production (VCO_2), V_E , and the ventilatory equivalent for carbon dioxide (V_E/VCO_2) were measured with a metabolic measurement system through a mouth piece and pneumotach while wearing a nose clip for the entire measurement period (MedGraphics CPX/D; Medical Graphics, St. Paul, MN).

Measurement of Central Chemoreceptor Sensitivity

Measurement of central chemoreceptor sensitivity was conducted within ten minutes after the exercise sessions using a modified rebreath method described previously [18]. Briefly, participants breathed on a mouthpiece connected to a pneumatic switching valve and 6-Liter rebreath bag that was filled with 5% CO_2 and 95% O_2 at the initiation of each session. Partial pressure of end-tidal oxygen ($P_{ET}O_2$), carbon dioxide ($P_{ET}CO_2$) and V_E were measured breath-by-breath. Dyspnea was measured via modified Borg scale (0–10). The test continued for four minutes or until one of the following stopping criteria occurred: $P_{ET}CO_2$ of 65mmHg, $P_{ET}O_2$ of 160 mmHg, V_E of 100 L/min, or participant wished to stop. The slope of V_E vs $P_{ET}CO_2$ was used as an index of central CO_2 sensitivity ($V_E/P_{ET}CO_2$).

The test was performed three times with 3–5 minutes of exposure to room air to allow V_E and $P_{ET}CO_2$ to return to baseline.

STATISTICS

Statistical analysis and graphic presentation were accomplished using SPSS v 20 and Graphpad Prism® (v 4.0), respectively. Data for each group was first identified for normal distribution. If normally distributed an independent t-test was used to determine differences between the groups. If the data was not normally distributed, a nonparametric test (Wilcoxon W) was used. A repeated measures ANOVA was used to determine the differences in V_E/VCO_2 across sessions (session $\times$ group). ANCOVA was used when necessary to determine how specific independent variables may influence the main dependent variable (change in V_E/VCO_2). Pearson product correlation was used to determine relationships between two variables. Multiple linear regression was used to test if the relationship of leg mass and change in V_E/VCO_2 was different between HFrEF and control patients (test for coincident regression lines). This test was accomplished using a multiple partial F test that simultaneously tested a main effect of patient cohort and its interaction with leg mass equal to zero (no association). In the event this test was rejected (i.e., the association of leg mass and V_E/VCO_2 was found to be different amongst cohort), separate regressions lines were fit within patient cohort to describe the relationship of leg mass with change in V_E/VCO_2 on account of collinearity between leg mass and patient cohort. This collinearity inflated the parameter standard errors and masked the strong linear relationships that were present in the data. Statistical significance was set at an alpha level of 0.05 for all analyses. Data in text and figures are presented as mean $\pm$ standard deviation.

RESULTS

Subject characteristics

Heart failure and CTLs were not different in age ($p=0.28$) or height ($p=0.64$), but BMI was greater in HFrEF ($p<0.01$). Baseline participant demographics are provided in Table 1.

Body composition

Total body mass was greater in HFrEF compared with CTLs (93.1 ± 9.0 kg vs. 76.0 ± 12.8 kg, $p=0.001$). There was no difference in leg, arm, trunk or total muscle mass in kilograms (kg) between CTLs and HFrEF ($p>0.05$, Table 2). Fat mass in kg was greater in HFrEF compared with CTLs for arms, legs, trunk and total body mass ($p<0.05$, Table 2). When normalized to total tissue (kg), muscle mass was lower in the arms and legs in HFrEF patients compared with CTLs and total muscle mass (%) was less in HFrEF ($p<0.05$, Figure 1A). Additionally, when normalized to total tissue (kg), arm, leg and total fat (%) were greater in HFrEF ($p<0.05$, Figure 1B), with trunk fat (%) marginally significant ($p=0.05$).

Exercise tolerance

Peak watts were greater for CTLs compared with HFrEF patients (185 ± 57 vs. 120 ± 35 watts, $p=0.02$). When normalized to leg muscle mass, HFrEF patients produced 4.0 ± 1.1 watt $\cdot$ kg $^{-1}$ whereas CTLs produced 7.2 ± 1.3 watt $\cdot$ kg $^{-1}$ ($p<0.001$). Peak VO_2 was less in HFrEF

compared with CTLs (18.4 ± 2.7 vs. 27.3 ± 5.0 ml·kg⁻¹·min⁻¹, $p < 0.001$). Peak $\dot{V}O_2$ normalized to leg muscle mass was greater in CTLs compared with HFrEF (86.2 ± 11.7 vs. 61.2 ± 8.4 ml·kg⁻¹·min⁻¹, $p < 0.001$).

Peak work was positively associated with leg muscle mass in HFrEF ($r^2 = 0.50$, $p = 0.04$), but not in CTLs ($r^2 = 0.18$, $p = 0.29$, Figure 2A) and was inversely associated with leg fat mass in HF ($r^2 = 0.48$, $p = 0.04$), but not in CTLs ($r^2 = 0.17$, $p = 0.1$). Peak $\dot{V}O_2$ was positively associated with leg muscle mass in HFrEF ($r^2 = 0.66$, $p = 0.007$), but not in CTLs ($r^2 < 1.0$, $p = 0.50$, Figure 2B) and was inversely associated with leg fat mass in HFrEF ($r^2 = 0.67$, $p = 0.007$), but not in CTLs ($r^2 < 1.0$, $p = 0.53$). Neither peak $\dot{V}O_2$ or peak work were associated with resting ejection fraction in HFrEF ($p > 0.05$).

Peak $\dot{V}O_2$ was associated with the reduction in $\dot{V}_E/\dot{V}CO_2$ with afferent blockade during the submaximal exercise in HFrEF ($r^2 = 0.67$, $p = 0.007$), but did not reach statistical significance in CTLs ($r^2 = 0.45$, $p = 0.07$). $P_{ET}CO_2$ was also strongly correlated with the reduction in $\dot{V}_E/\dot{V}CO_2$ with afferent neural blockade in HFrEF ($r^2 = 0.76$, $p = 0.002$), but not CTLs ($r^2 < 1.0$, $p = 0.55$).

Impact of afferent blockade on ventilatory efficiency

Heart failure patients and CTLs cycled at a relative constant-work submaximal rate (65% of peak power output), which resulted in a lower absolute workload during the exercise in patients with HFrEF (78 ± 23 W vs. 120 ± 37 W, respectively). $\dot{V}_E/\dot{V}CO_2$ slope was reduced with afferent neural blockade (session effect, $p = 0.001$) more so in HFrEF patients (session $\times$ group, $p = 0.05$, Figure 3) with no group effect ($p = 0.28$). Because BMI was different between groups, an ANCOVA was performed with BMI as the covaried factor. When this analysis is performed, a session $\times$ group interaction persists ($p = 0.03$) with no interaction between session and BMI ($p = 0.13$). In addition, a main effect of group was only present when covaried for BMI ($p = 0.01$ vs. $p = 0.36$). $\dot{V}_E/\dot{V}CO_2$ in the placebo condition appeared to be more elevated with the severity of HFrEF, although this did not reach significance [Class I (n=3): 30.00%, Class II (n=4): 31.72%, and Class III (n=2): 35.38%, ($p > 0.05$)]. In addition, the reduction in $\dot{V}_E/\dot{V}CO_2$ with afferent feedback was slightly greater, but not significant for the sicker patients [Class I: -14.05%, Class II: -17.34%, and Class III: -28.85% ($p > 0.05$)].

Relationship between ventilatory efficiency, body composition and exercise capacity

$\dot{V}_E/\dot{V}CO_2$ during the placebo condition was associated with leg muscle mass in HFrEF ($r^2 = 0.58$, $p = 0.02$), but not in CTLs ($r^2 = 0.30$, $p = 0.16$, Figure 4A). With afferent blockade the relationship between $\dot{V}_E/\dot{V}CO_2$ and leg muscle mass was abolished in HFrEF ($r < 1.0$, $p = 0.96$) and remained non-significant in CTLs ($r^2 = 0.16$, $p = 0.32$, Figure 4B). There was a differential relationship of leg muscle mass with change in $\dot{V}_E/\dot{V}CO_2$ by patient cohort (test of coincidence, $p = 0.002$). The nature of the differential relation was such that the healthy control participants did not show a significant linear relationship ($r^2 < 1.0$, $p = 0.87$) whereas HF patients did ($r^2 = 0.72$, $p = 0.004$, Figure 4C). $\dot{V}_E/\dot{V}CO_2$ during the placebo condition was associated with leg fat mass in HFrEF ($r^2 = 0.58$, $p = 0.02$), but not in CTLs ($r^2 = 0.27$, $p = 0.18$, Figure 4D). With afferent blockade the relationship between $\dot{V}_E/\dot{V}CO_2$ and leg fat mass was abolished in HFrEF ($r < 1.0$, $p = 0.96$) and remained non-significant in CTLs ($r^2 = 0.16$, $p = 0.32$,

Figure 4E). The change in V_E/VCO_2 was also inversely correlated with leg fat mass ($r^2=0.74$, $p=0.003$) in HFrEF patients but not in controls ($r^2<1.0$, $p=0.04$, Figure 4F). When groups are combined, the change in V_E/VCO_2 was associated with BMI ($r^2=0.45$, $p=0.003$), but not when separated for either group ($p>0.05$). To determine the main predictor for the reduction in V_E/VCO_2 in HFrEF patients, a stepwise multiple linear regression with variables VO_2 peak, leg fat mass (%) and leg lean mass (%) indicated leg fat mass (%) to be the major predicting variable ($y = -0.76x + 7.3$, $p=0.003$).

Influence of lumbar injection of fentanyl on central chemoreceptor sensitivity

There was no change in the hypercapnic ventilatory response in either group during locomotor muscle afferent feedback inhibition when compared to placebo suggesting no cephalad migration of the drug or other central effect of fentanyl on ventilatory control ($p>0.05$). This data has been reported previously [15].

DISCUSSION

The novel findings from this study are that 1) leg muscle mass (%) and leg fat mass (%) are closely related to V_E/VCO_2 during submaximal exercise in HFrEF and 2) the greatest reductions observed in V_E/VCO_2 with afferent blockade occurred in patients with the least leg muscle mass (%), greatest fat mass (%) and lowest peak VO_2 . Further, leg fat mass (%) was the only predictor for reduction in V_E/VCO_2 slope with afferent blockade in HFrEF. Finally, consistent with the literature, leg muscle mass was positively associated and leg fat mass was negatively associated with exercise capacity in HFrEF patients (peak workload and peak VO_2). As anticipated, when normalized to leg muscle mass, CTL participants were able to achieve a greater workload and higher peak VO_2 compared with HFrEF patients. Neither exercise capacity nor the reduction in V_E/VCO_2 with afferent blockade, were associated with ejection fraction in HFrEF.

Skeletal muscle mass and ventilatory efficiency in heart failure

A number of ventilatory and gas exchange abnormalities are known to occur at rest and during exercise in patients with HFrEF [19, 20]. The current study demonstrated an elevated ventilatory response for a given metabolic load during exercise (increased V_E/VCO_2), commonly referred to as reduced ventilatory efficiency [21]. Reduced ventilatory efficiency contributes to functional limitation and is associated with increased morbidity and mortality in HF [22]. Mechanisms related to poor ventilatory efficiency are not understood. However, there is evidence to suggest that mechanically or metabolically sensitive afferent receptors in the skeletal muscle contribute to excessive ventilation in HFrEF [4, 23, 24].

Over the past 30 years, clinical research has focused on various peripheral changes which occur in HFrEF. For example, these patients experience a loss of muscle strength [25, 26], greater muscle atrophy [25, 27], decreased oxidative capacity of the muscle [8, 12] and structural changes in skeletal muscle [8]. As a result of these changes, the exercising muscle in HFrEF relies more heavily on anaerobic metabolism thus producing an increased quantity of metabolic byproducts during muscle contraction. It is proposed that the greater anaerobic metabolism during physical activity in HFrEF will stimulate group III and IV muscle

afferents resulting in an increased ventilatory response during exercise [28, 29]. In line with this, our findings suggest that reduced leg muscle mass relative to total mass was associated with an elevated V_E/VCO_2 . Importantly, this association was only observed in HFrEF patients. Further, the patients with the most improved V_E/VCO_2 when locomotor afferent feedback was blocked were sicker patients (NYHA Class III) with the lowest leg muscle mass. This implies that the patients with the least amount of muscle tissue demonstrate the greatest influence of locomotor muscle afferent feedback on ventilatory control. It is important to note that the relationship between leg mass and V_E/VCO_2 and the change in V_E/VCO_2 were observed during submaximal exercise, suggesting that even at lower relative work rates, applicable to functional daily activities, HFrEF patients demonstrate a dysregulation in ventilatory control. Evident by the findings in this study, the observed elevation in V_E/VCO_2 was strongly linked to alterations in the skeletal muscle in mild to moderate HFrEF patients that displayed no evidence of cachexia (greater BMI and total body mass in HFrEF).

Mechanisms for muscle myopathy in heart failure

In HF, muscle wasting contributes to reduced skeletal muscle mass and strength [30]. Although the patients in this study were considered to be in mild to moderate HFrEF, they demonstrated significant relative muscle loss compared with the CTLs. Muscle atrophy is mainly a consequence of an imbalance in homeostasis of muscle protein synthesis and degradation. Hence, there is a shift of the metabolic balance to an accelerated muscle loss associated with serious illness [31]. Loss of muscle mass frequently observed in these patients has been related to stimulation of the ubiquitin-proteasome-dependent pathway, which has been suggested to be the most important pathway of protein degradation in human cells [32, 33].

Along with muscle wasting, HFrEF patients also demonstrate histological changes indicating a shift in fiber type proportion and myosin heavy chain isoform expression. Several studies have confirmed an increase in type II fibers (particularly Type IIb) and a decrease in type I fibers [6, 8]. This results in a decline in activity of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase [8] without alteration in glycolytic enzyme activity [30] suggesting an increased reliance on glycolytic metabolism leading to intramuscular acidosis and reduced muscle endurance.

One of the consequences of greater metabolic activity in the muscle is an increased activation of mechano- and/or metabo-receptors. During exercise, metabolites such as lactate, adenosine, inorganic phosphate, potassium, kinins, and cations are produced in the skeletal muscle [11, 34, 35]. These substances accumulate with increasing exercise or stress and when O_2 delivery cannot match the metabolic needs of the contracting muscle these metabolic products stimulate the muscle metaboreceptors (group IV muscle afferents). This in turn leads to a reflex-regulation commonly called the 'metaboreflex' which contributes to changes in cardiovascular and ventilatory control [28]. Moreover, mechanical contraction of skeletal muscle leads to stimulation of mechanoreceptors (group III muscle afferents), the 'mechanoreflex', which can also stimulate afferent feedback and likely contributes to cardiovascular and ventilatory control [36]. Overactivation of the group III and IV muscle

afferents relays signals to the cardiorespiratory centers in the brainstem [37], via the dorsal horn of the spinal column, to increase ventilation and blood pressure in patients with HFrEF [4, 38]. As such, our study demonstrated that when afferent feedback is inhibited (by local injection of a μ -opioid receptor agonist, fentanyl), V_E/VCO_2 was greatly reduced in HFrEF. Importantly this reduction of V_E/VCO_2 was closely related to the reduced muscle mass in these patients. Although the histology of skeletal muscle was not examined in this study, conventional theory suggests that the shift in fiber type proportion and predominance of metabolic pathway selection occurs concurrently with the loss of muscle mass in HFrEF (although not necessarily via the same mechanism).

The shift in fiber types and resultant reduction in oxidative capacity of the muscle is noted to be related to the greater fatigability of the skeletal muscle [39, 40], contributes greatly to exercise intolerance [41, 42], and therefore is likely related to symptoms of dyspnea and fatigue during exercise in HFrEF. In this light, our patients with the greatest loss in muscle mass likely had the highest afferent feedback contributing to an elevated V_E/VCO_2 slope and thus the greatest reduction in V_E/VCO_2 slope when afferent feedback was inhibited. Additionally, the reduction in V_E/VCO_2 in HFrEF patients during submaximal exercise was associated with both peak VO_2 and peak $P_{ET}CO_2$, demonstrating a close link between leg muscle mass, afferent feedback, ventilatory efficiency and exercise capacity in HFrEF.

Fat Mass Predicts the Reduction in V_E/VCO_2 with Afferent Blockade

An interesting and unexpected finding in this study was that fat mass (%) was the only predictor for the reduced V_E/VCO_2 in HFrEF. This is important as it indicates that along with muscle mass, fat composition may be a significant contributor to ventilatory abnormalities in HF. Although not much is known on how fat composition influences ventilation, it may be due to alterations in blood flow distribution in the exercising muscle. Muscle satellite cells can acquire features of adipocytes, including the ability to accumulate lipids and intermuscular fat (IMF) may compete with active muscle tissue for critical nutritive blood flow during exercise [43]. Adipose tissue blood flow adjacent to the active muscles increased sevenfold during continuous isometric knee-extension exercise in nonobese healthy sedentary women [43]. In addition, in patients with preserved ejection fraction (HFpEF), IMF was inversely related to peak VO_2 [44]. Therefore, increased thigh IMF in patients with HFpEF may “steal” blood that would normally be delivered to the active muscles during exercise and thereby, reduce perfusive oxygen delivery to the thigh muscle. Although, we did not measure IMF, the HFrEF patients in this study had a significantly higher fat content and this appears to have played a role in the improved ventilatory efficiency with afferent blockade. Lastly, fat composition appears to be of importance in this cohort of patients as a significant difference between absolute and relative fat mass is noted, but only relative leg muscle mass was significantly different between HF and controls.

Limitations

This study has limitations that should be considered when interpreting the data. The sample number of HFrEF patients and controls were small and future studies should be conducted to include a larger sample in order to confirm findings of the current study. However, even

with low sample numbers and mild to moderately sick patients, this study still observes significant differences in the relationship between V_E/VCO_2 , leg muscle and fat mass between patients and controls. Although there was no difference in absolute leg muscle mass, when normalized to total mass, relative muscle mass held a strong relationship with V_E/VCO_2 in patients only. In addition, patients that experienced the greatest reduction in V_E/VCO_2 with afferent blockade had the least muscle mass. In addition, BMI was greater in HFrEF patients compared with controls and could be a potential confounder, however, when covarying for BMI, the interaction of group and session remained significant with no interaction between BMI and session. Therefore, although BMI was elevated in HF patients we feel in these patients that it was not a confounder and has not significantly impacted the results of this study.

Conclusion

Skeletal muscle mass is an important determinant of exercise capacity in patients with HFrEF and this study indicates that leg fat mass may also be a strong contributor in determining exercise capacity [30]. Both leg mass and fat mass were associated with high V_E/VCO_2 slope during exercise. This is important because both exercise capacity and high V_E/VCO_2 are independent predictors of morbidity and mortality in HFrEF patients [16, 17, 45]. Furthermore, blocking locomotor muscle afferent feedback improves ventilatory efficiency in HFrEF and these improvements were greatest for patients with the least muscle mass, greatest fat mass and the lowest peak VO_2 , with fat mass being the major predictor of improved ventilatory efficiency. This study is clinically relevant because increasing skeletal muscle mass and reducing fat mass may be therapeutic targets for improving breathing efficiency during exercise, increasing exercise tolerance, improving symptomology, and ultimately reducing morbidity and mortality.

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Highlights

- We examined the relation between leg composition and V_E/VCO_2 during exercise in HF.
- V_E/VCO_2 was reduced with afferent inhibition (fentanyl) during submaximal exercise.
- The reduction in V_E/VCO_2 was strongly associated with leg muscle and fat mass.
- Leg fat mass was predictive of the reduced V_E/VCO_2 slope with afferent blockade.
- Greater reductions in V_E/VCO_2 were observed in patients with the lowest peak VO_2 .

Figure 1. Leg muscle mass, % (A) and leg fat mass, % (B) for CTLs and HFrEF participants
HFrEF patients had a reduced leg muscle mass and greater leg fat mass compared with CTLs (* $p < 0.05$).

Figure 2. Relationship between leg muscle mass and peak work (A) and leg muscle mass and peak VO_2 (B)

Peak work was associated with leg muscle mass in HFrEF ($r^2=0.50$, $p=0.04$), but not in CTLs ($r^2=0.18$, $p=0.29$). Peak VO_2 was associated with leg muscle mass in HFrEF patients ($r^2=0.66$, $p=0.007$), but not in CTLs ($r^2<1.0$, $p=0.50$).

Figure 3. Change in V_E/VCO_2 slope with afferent blockade
 HFrEF patients had a greater reduction in V_E/VCO_2 slope compared with CTLs (* $p < 0.05$).

Figure 4. Relationship between leg muscle mass (%) and V_E/VCO_2 slope during placebo condition (A), fentanyl condition (B) and change in V_E/VCO_2 slope with afferent blockade (C) and leg fat mass (%) and V_E/VCO_2 slope during placebo condition (D), fentanyl condition (E) and change in V_E/VCO_2 slope with afferent blockade (F)

V_E/VCO_2 during the placebo condition was associated with leg muscle mass in HFrEF ($r^2=0.58$, $p=0.02$), but not in CTLs ($r^2=0.30$, $p=0.16$). With afferent blockade the relationship between V_E/VCO_2 and leg muscle mass was abolished in HFrEF ($r<1.0$, $p=0.96$) and remained non-significant in CTLs ($r^2=0.16$, $p=0.32$). V_E/VCO_2 during the placebo condition was associated with leg fat mass in HFrEF ($r^2=0.58$, $p=0.02$), but not in CTLs ($r^2=0.27$, $p=0.18$). The change in V_E/VCO_2 was also inversely correlated with leg fat mass ($r^2=0.74$, $p=0.003$) in HFrEF patients but not in controls ($r^2<1.0$, $p=0.04$).

Table 1

Participant characteristics and medications for HF patients.

Participant Characteristics	HF (n=9)	CTL (n=8)	p-value
Age (yrs)	60±2	64±2	0.28
Sex (M/F)	7/2	7/1	
VO _{2peak} (mL·kg ⁻¹ ·min ⁻¹)	18.4±0.9	27.3±1.8	<0.001
LVEF (%)	26.7±1.8		
NYHA Class	I(3), II(4), III(2)		
Etiology (ischemic/idiopathic)	5/4		
Height (cm)	175.8±3.2	177.8±2.8	0.64
BMI (kg/m ²)	31.9±1.5	25.3±1.0	<0.05
<u>Medications (n,%)</u>			
ACE Inhibitors	6 (67)		
Angiotensin Receptor Blockers	3 (33)		
β-Blockers	9 (100)		
Aspirin	5 (56)		
Diuretics	6 (67)		

BMI, body mass index; VO_{2peak}, peak oxygen consumption; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; ACE, angiotensin converting enzyme; Angiotensin II, angiotensin II. Data are presented as Mean ± SEM, number of participants or percentage of population.

Table 2

Body Composition of HF and CTL participants.

Fat (kg)	HF	CTL	p-value
arms	3.6±0.3	2.0±0.1	0.001
legs	10.1±1.0	6.5±0.3	0.005
trunk	20.2±1.8	12.7±1.3	0.005
total	34.9±3.0	22.2±1.4	0.003
Fat (%)			
arms	33.2±3.6	26.9±2.7	0.02
legs	34.4±9.1	27.5±2.4	0.02
trunk	41.3±3.0	33.9±2.4	0.05
total	37.3±2.9	30.4±2.1	0.03
Muscle (kg)			
arms	7.3±0.6	6.3±0.6	0.22
legs	19.3±1.1	18.8±1.5	0.79
trunk	28.2±1.1	25.2±1.6	0.15
total	58.2±2.8	53.8±3.7	0.36
Muscle (%)			
arms	63.9±3.3	71.4±1.9	0.02
legs	63.0±2.8	70.4±5.0	0.02
trunk	57.5±2.9	66.4±2.4	0.16
total	60.6±2.7	68.0±1.4	0.04