Mechanisms of Exercise Intolerance in Heart Failure with Preserved Ejection Fraction: The Role of Abnormal Peripheral Oxygen Extraction

Dhakal et al: Peripheral Oxygen Extraction in Heart Failure

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Abstract

Background—Exercise capacity as measured by peak oxygen uptake (VO$_2$) is similarly impaired in patients with heart failure (HF) with preserved ejection fraction (HFpEF) and HF with reduced EF (HFrEF). However, characterization of how each component of VO$_2$ changes in response to incremental exercise in HFpEF vs. HFrEF has not been previously defined. We hypothesized that abnormally low peripheral O$_2$ extraction (arterio-mixed venous O$_2$ content difference, [C(a-v)O$_2$]) during exercise significantly contributes to impaired exercise capacity in HFpEF.

Methods and Results—We performed maximum incremental cardiopulmonary exercise testing with invasive hemodynamic monitoring on 104 patients with symptomatic NYHA II-IV HF (HFpEF, n=48, peak VO$_2$=13.9±0.5ml/kg/min, mean±SEM, and HFrEF, n=56, peak VO$_2$=12.1±0.5ml/kg/min) and 24 control subjects (peak VO$_2$ 27.0±1.7ml/kg/min). Peak exercise C(a-v)O$_2$ was lower in HFpEF compared to HFrEF (11.5±0.27 vs. 13.5±0.34 ml/dl, respectively, p<0.0001) despite no differences in age, hemoglobin level, peak RER, CaO$_2$ or cardiac filling pressures. Peak C(a-v)O$_2$ and peak HR emerged as the leading predictors of peak VO$_2$ in HFpEF. Impaired peripheral O$_2$ extraction was the predominant limiting factor to exercise capacity in 40% of HFpEF patients and was closely related to elevated systemic blood pressure during exercise (r=0.49, p=0.0005).

Conclusions—In the first study to directly measure C(a-v)O$_2$ throughout exercise in HFpEF, HFrEF, and normals, we found that peak C(a-v)O$_2$ was a major determinant of exercise capacity in HFpEF. The important functional limitation imposed by impaired O$_2$ extraction may reflect intrinsic abnormalities in skeletal muscle or peripheral microvascular function, and represents a potential target for therapeutic intervention.

Key Words: heart failure, exercise, diastole
Heart failure (HF) with preserved left ventricular (LV) ejection fraction (HFpEF) is an increasingly common condition with similar incidence and prognosis to HF with reduced LV ejection fraction (HFrEF).\textsuperscript{1-4} A major source of morbidity in both HFpEF and HFrEF is impaired functional capacity, which is best quantified by the degree of impairment in peak VO\textsubscript{2}.\textsuperscript{5-7} Mechanistic studies of exercise intolerance in HFpEF have primarily focused on central cardiovascular abnormalities, including chronotropic incompetence\textsuperscript{6} and impaired stroke volume (SV) augmentation in the setting of decreased LV compliance.\textsuperscript{5,8} More recently, impaired systolic reserve function and abnormal LV-central vascular coupling have also been implicated in causing impaired exercise capacity in HFpEF.\textsuperscript{9}

In assessing the capacity to augment VO\textsubscript{2} in HFpEF, it is important to consider relative increases in each of the three components of VO\textsubscript{2} [i.e., heart rate (HR), stroke volume (SV), and arterio-mixed venous oxygen content difference: C(a-v)O\textsubscript{2}]. In normal individuals, the degree to which peripheral oxygen extraction [i.e., C(a-v)O\textsubscript{2}] increases in response to exercise (~2.5x)\textsuperscript{10-12} is much greater than changes in stroke volume (~1.3x)\textsuperscript{11,13} and similar to increases in HR (~2.5x). Several previous studies have found that HFpEF patients are not able to increase HR and SV normally during exercise,\textsuperscript{5,6,8,14} which implies a greater reliance in the ability to increase C(a-v)O\textsubscript{2} in order to augment VO\textsubscript{2}. However, the role of C(a-v)O\textsubscript{2} in determining exercise capacity in HFpEF remains incompletely understood.\textsuperscript{15-17}

In HFpEF, two studies\textsuperscript{15,16} that derived C(a-v)O\textsubscript{2} indirectly have suggested that C(a-v)O\textsubscript{2} is abnormally low in HFpEF, while a third study found that it was not impaired.\textsuperscript{17} To date, no studies have performed direct serial measurements of C(a-v)O\textsubscript{2} throughout exercise in HFpEF and HFrEF in order to define O\textsubscript{2} extraction patterns.
Based on the heterogeneous pathogenesis of HFpEF, and the recognized role of peripheral O₂ extraction augmentation in increasing VO₂ during exercise, we hypothesized that HFpEF patients would be limited primarily by an inability to augment peripheral O₂ extraction appropriately [i.e. C(a-v)O₂ < 14 ml/dl or CvO₂ > 5 ml/dl]. To address this hypothesis, we measured respiratory gas exchange parameters, arterial and mixed venous O₂ saturations [C(a-v)O₂], as well as HR and stroke volume at one-minute intervals throughout maximum incremental exercise in patients with symptomatic HFpEF and compared them to patients with HFrEF and normal controls. The primary objective of this study was to delineate the relative contributions of each component of VO₂ to peak exercise capacity in patients with HF.

Methods

Patient population and study design
Consecutive patients who underwent cardiopulmonary exercise testing (CPET) with invasive hemodynamic monitoring at Massachusetts General Hospital and chronic NYHA class II-IV symptoms were included in the study. We classified patients based on LVEF and resting and exercise pulmonary capillary wedge pressure (PCWP) as a) HFrEF: Chronic NYHA II-IV left ventricular systolic dysfunction, LVEF < 0.45 on standard pharmacotherapy; b) HFpEF: Chronic NYHA II-IV symptoms, LVEF ≥ 0.50, and > 15 mmHg PCWP at rest. Exclusion criteria consisted of the following: 1) incomplete pulmonary arterial catheter pressure measurements; 2) documented intra-cardiac shunting; 3) severe valvular heart disease; 4) known active flow limiting CAD; 5) submaximal exercise as evidenced by peak respiratory exchange ratio (RER) < 1.0; 6) the presence of a pulmonary mechanical limitation to exercise as defined by V̇E/(forced expiratory volume in 1 second [FEV₁] x 35) > 0.7 at the anaerobic threshold. The control
group was included in order to determine the extent to which hemodynamic measurements and O\textsubscript{2} utilization during exercise in HFpEF subjects differed from normal controls. Controls consisted of subjects referred for CPET to evaluate dyspnea on exertion during the same period of time as the HFpEF group. Controls were required to have normal LV function, normal resting and exercise PCWP and normal exercise capacity as reflected by a peak VO\textsubscript{2} greater than 80% of that predicted on the basis of age, gender, and height.\textsuperscript{18}

**Cardiopulmonary exercise testing**

All patients underwent placement of a pulmonary arterial catheter via the internal jugular vein and placement of a systemic arterial catheter via the radial artery. First-pass radionuclide ventriculography of both ventricles was performed immediately prior to cycle ergometry testing as previously described.\textsuperscript{21} Subjects then underwent maximum incremental upright cycle ergometry CPET (5-25 Watts/min continuous ramp after an initial 3-minute period of unloaded exercise, MedGraphics, St. Paul, MN) with simultaneous hemodynamic monitoring (Witt Biomedical Inc, Melbourne, FL) as previously described.\textsuperscript{21, 22} None of the subjects developed angina, arrhythmia, hypotension or significant electrocardiographic changes during exercise. Right atrial pressure (RAP), mean pulmonary arterial pressure (PAP), PCWP, and systemic arterial pressures were measured in the upright position, at end-expiration, while patients were seated on the cycle, at rest, and at one-minute intervals during exercise. Fick cardiac outputs (CO)\textsuperscript{11, 23} were also determined at one minute intervals throughout exercise by measuring oxygen uptake (VO\textsubscript{2}) and simultaneous radial arterial and mixed venous O\textsubscript{2} content to calculate the C(a-v)O\textsubscript{2}. Peak VO\textsubscript{2} was defined as the highest O\textsubscript{2} uptake, averaged over 30 seconds, during the last minute of symptom-limited exercise, as previously described.\textsuperscript{22} Age-predicted maximal HR was
defined as 220 minus age in years. Chronotropic response index (CRI) was derived as the proportion of HR reserve used at peak exercise based on (HRpeak-HRrest)/(220-age)-HRrest) \times 100.^{24,25} CRI <62\% and <80\% were considered abnormal in the presence and absence of beta-blocker use, respectively.^{24, 26, 27,28}

### Arterio-mixed venous oxygen content

Arterial O₂ content (CaO₂)\(^{29}\) is the amount of O₂ carried by blood to the periphery and was calculated as (hemoglobin [Hb] \times 1.39 \times SaO₂) + (0.003 \times PaO₂). Similarly mixed venous O₂ content (CvO₂) represents the O₂ content of blood returning from the peripheral tissues to the right heart which was calculated as (Hb \times 1.39 \times SvO₂) + (0.003 \times PvO₂). Given a normal circulating hemoglobin level of \(\sim 15 \text{ g/dl}\), an arterial saturation of 96\% and mixed venous saturation of 72\%, the normal resting CaO₂ is 20 ml/dl and CvO₂ is 15 ml/dl which results in a normal resting C(a-v)O₂ value of 5 ml/dl.

During exercise, peripheral tissues extract more O₂ to maintain aerobic metabolism which leads to a decrease in mixed venous saturation to \(\sim 24\%\) with a resultant reduction in CvO₂ from 15 ml/dl at rest to 5 ml/dl at peak exercise in normal individuals.\(^{30}\) Thus peak exercise C(a-v)O₂ in a normal person with a Hb of 15 g/dl is 15 ml/dl (i.e approximately equal to the Hb level).\(^{18,31}\)

The amount of O₂ extracted by tissues at peak exercise relative to O₂ delivered (i.e. extraction ratio, peak C(a-v)O₂/CaO₂) is normally 75\%.

### Statistical methods

STATA 10 (Statacorp, College Station, Texas) was used for statistical analysis. The Wilk-Shapiro test was used to assess the normality of distribution of the data. All continuous,
normally-distributed measurements are presented as the Mean±SEM. Categorical data are reported as percentages. Group baseline characteristics were compared using either the Student t test, Mann Whitney U test, or Fisher’s exact test, as appropriate. For clinical characteristics, comparisons between groups for continuous variables were performed using ANOVA with post-hoc pairwise comparisons, unpaired two-sample t tests or the Wilcoxon signed rank test, as appropriate. Pearson or Spearman correlation coefficients were calculated, based on whether or not the data was either normally or not normally distributed, respectively. Partial R-square values were obtained from a multiple linear regression model that included age, gender, HRmax, SVmax, and C(a-v)O2max. Subgroup analysis was performed comparing HF patients with higher and lower CvO2. A p-value < 0.05 was considered significant. This study was approved by the Partners Healthcare IRB, the authors had full access to the data and take responsibility for its integrity and for the manuscript as written.

Results

Population characteristics

Baseline characteristics for all HFpEF (N=48), HFrEF (N=56) and control subjects (N=24) are reported in Table 1. All patients surpassed their ventilatory anaerobic thresholds and demonstrated an average peak RER of 1.15-1.16 in all 3 groups, indicating maximum or near maximum exercise effort across the 3 groups. HFpEF subjects had more elevated BMI and a female predominance (60%) compared to HFrEF patients, consistent with the known distinct demographic characteristics of HFpEF and HFrEF populations. 

Functional capacity as indicated by peak VO2 was reduced in HFpEF (13.9±0.5 ml/kg/min) and in HFrEF (12.1±0.5 ml/kg/min) compared to controls (27.0±1.7 ml/kg/min,
p<0.05 for both comparisons, Table 1). The measurement of VO₂, HR, CaO₂ and CvO₂ during each minute of exercise and application of the Fick Principle [i.e. \( \text{VO}_2 = \text{HR} \times \text{SV} \times C(a-v)O_2 \)] permitted analysis of each component of VO₂ during exercise in the 3 groups.

**Arterial and mixed venous oxygen content at rest and at peak exercise**

All three groups had similar CaO₂ values at rest and at peak exercise, reflecting mildly reduced Hb levels and normal systemic arterial O₂ saturations (Tables 1 and 2). Resting CvO₂ was lowest in HFrEF (9.4±0.3 ml/dl), and similar in HFpEF and controls (11.6±0.3 and 12.1±0.36 ml/dl, p=0.70, Table 2). Compared to controls and HFrEF patients, HFpEF patients had the lowest average C(a-v)O₂ and highest peak exercise CvO₂, indicating relatively impaired maximum peripheral O₂ extraction in HFpEF (Table 2, Figure 1). Maximum C(a-v)O₂ was less than the predicted value [i.e. maximum C(a-v)O₂=Hb level,\(^1\) and CvO₂ >5 ml/dl, see Methods] in 75% of HFpEF vs. 21% of HFrEF and 33% of controls (p<0.001). Peak C(a-v)O₂ was not related to peak CO in any of the groups, indicating that at peak exercise these variables are dissociated, and not reciprocally related as they are at rest and during low-level exercise.

CO/VO₂ slope, also termed exercise factor, was 5.6±0.2 in controls consistent with values reported by previous investigators.\(^{12,33,34}\) CO/VO₂ slope was 6.1±0.2 in HFpEF (p=0.068 compared to controls, p<0.0001 compared to HFrEF) and 5.0±0.17 in HFrEF (p=0.02 compared to controls). Higher CO/VO₂ slope in HFpEF compared to HFrEF is indicative of a reduced relative contribution of C(a-v)O₂ to VO₂ in HFpEF throughout exercise. Among patients in whom peak C(a-v)O₂ was below predicted and constituted the primary cause of reduced peak VO₂, the CO/VO₂ slope was 8 (Supplemental Figure 1), indicating a disproportionate reliance on CO increment throughout exercise in order to compensate for abnormal C(a-v)O₂.
Chronotropic response during exercise

HR at rest was similar in all three groups (Table 2). Failure to reach 85% of predicted HR was similarly common in HFP EF (67%) and HFrEF (75%, p=0.35). After accounting for beta-blocker use, 73% of HFP EF patients and 75% in HFrEF patients met diagnostic criteria for chronotropic incompetence, consistent with findings from previous studies of exercise response patterns in HF.6, 15, 35, 36

Stroke volume and filling pressures during exercise

Resting SV in HFP EF was higher than resting SV in HFrEF and similar to that in controls (Table 2). At peak exercise, HFP EF patients achieved higher SV than HFrEF subjects (88±3.6 ml vs. 68±2.8 ml, p<0.001) but lower than controls (103±4.3 ml, p=0.03 compared to HFP EF) (Table 2). The observed differences in stroke volumes in HFP EF and HFrEF occurred in the setting of similar resting and exercise PCWP.

Integrated responses: Cardiac output vs. extraction reserve capacity during exercise

We examined “reserve capacity” of each component of VO₂ independently of resting values by assessing change in HR, SV, and C(a-v)O₂ from rest to peak exercise in the three groups (Figure 2). In normal middle-aged controls in our study, VO₂ increased 592±42% from rest to peak exercise, consistent with previous studies.13, 31 This increase was due to a 109±8% increase in HR, a 39±4% increase in SV, and a 138±9% increase in C(a-v)O₂ during exercise. In contrast, HFP EF patients had a 311±20% increase in resting VO₂ during exercise due to a 63±5% increase in HR, a 32±5% increase in SV, and a 91±6% increase in C(a-v)O₂. HFrEF patients had a 264±14% increase in VO₂ attributable to a 53±4% increase in HR, a 40±5% increase in SV, and
a 77±5% increase in C(a-v)O2 (Figure 2). Notably, in all groups the magnitude of increase in C(a-v)O2 in response to exercise was greater than the magnitude of increase in HR or SV; thereby highlighting the important contribution of increase in C(a-v)O2 to augmenting VO2 during exercise.

Assessment of convective oxygen delivery (i.e. cardiac output x CaO2) and diffusive oxygen transport (represented by fall in CvO2) is an alternative, mechanistic way to analyze components of O2 utilization37. Multi-point plots of CvO2 vs. VO2 in the 3 groups indicates that diffusive O2 transport is most impaired in HFrEF, whereas convective O2 delivery is lowest in HFrEF (Figure 3). The extent to which VO2 would increase upon normalization of convective O2 delivery and diffusive O2 utilization in HFrEF is illustrated in Figure 3 and highlights the greater relative abnormality in diffusive O2 transport than in convective O2 delivery in HFrEF.

Predictors of Peak VO2

Partial R² values describing age and sex-adjusted relationships between peak VO2 and individual components of peak VO2 are displayed in Table 3. In HFrEF, peak VO2 related to maximum C(a-v)O2 (partial R²=0.28, p=0.0002) and peak HR (partial R²=0.35, p<0.0001) and there was a trend towards association with maximum SV (partial R²=0.07, p=0.077). In normal controls, by way of contrast, peak C(a-v)O2 tended to be more constant (mean 13.3±0.3ml/dL) and predictably related to Hb levels (mean 13.2g/dL),18 with a lower, partial R² value (0.19, p=0.056) relative to peak VO2.
**Blood pressure and diffusive oxygen transport in HFpEF**

To further investigate impaired diffusive O₂ transport in HFpEF in isolation, we stratified HFpEF patients into two groups based on median peak exercise CvO₂ of 6.8 ml/dL. The higher CvO₂ subgroup did not differ from the lower CvO₂ subgroup in age, gender, LVEF, CO or cardiac filling pressures but Hb was slightly higher in the higher CvO₂ group (Supplemental Table 1). The subset of HFpEF patients with higher CvO₂ had similar lactate and peak RER to the lower CvO₂ group which argues against reduced effort during exercise as an explanation for the attenuated fall in CvO₂ during exercise in the high CvO₂ group. The most striking difference between HFpEF CvO₂ subgroups was that elevated CvO₂ was associated with a disproportionate hypertensive response during exercise with elevation of DBP (93±4 mmHg vs. 76±3, p=0.001), SBP (196±7 vs. 171±7 mmHg, p=0.01) and MAP (127±4 vs. 107±4 mmHg, p=0.001) at peak exercise (Figure 4 and Supplemental Table 1). Among patients with peak exercise DBP in excess of 100mmHg, CvO₂ was 8.5±0.3 ml/dl, compared to CvO₂ of 6.5±0.4 ml/dl (p=0.005) in patients with exercise DBP≤100mmHg. When analyzed as a continuous variable, CvO₂ was directly correlated with peak exercise DBP (Pearson r=0.49, p=0.0005) and extraction ratio was inversely related to exercise DBP (Pearson r=-0.41, p=0.004).

**Discussion**

In comprehensively characterized cohorts with HFpEF, HFrEF, and controls we found that relative augmentation in peripheral oxygen extraction [C(a-v)O₂] exceeded that of heart rate or stroke volume during maximum incremental exercise in all three groups. Impaired peripheral O₂ extraction was present in 75% of HFpEF subjects in our study and was attributable to impaired diffusive O₂ transport and utilization (Figures 1 and 3). In contrast to the close association that
we observed between peak VO$_2$ and C(a-v)O$_2$ in HFP EF, we found relatively modest or absent associations between peak VO$_2$ and LV filling pressures or LV stroke volume in HFP EF. Taken together, our findings highlight the potentially important role of targeting peripheral O$_2$ extraction to augment impaired exercise capacity in HFP EF, particularly in light of failure of other interventions directed at central cardiac function to improve exercise capacity in HFP EF.$^{32, 38-40}$

The validity of our findings defining relative components of VO$_2$ augmentation in HFP EF, HFr EF, and normals is supported by: 1) rigorous entry criteria with confirmation of diagnoses with invasive hemodynamic assessment and ventriculography on the day of enrollment; 2) direct repeated measurements of CaO$_2$, CvO$_2$, and CO at 1 min intervals throughout exercise; 3) use of physiologically-relevant upright exercise with maximum effort confirmed by mean RERs>1.15 in each group; 4) consistency of our findings with other studies with regard to demographic variables of HF subgroups and absolute levels of peak C(a-v)O$_2$ during exercise in normals.

**Exercise Capacity in HF**

Limitation in exercise capacity is a cardinal manifestation of HF that is closely related to poor quality of life and mortality.$^{41, 42}$ The degree of reduction in exercise capacity in HFP EF in our study was similar to that reported in previous studies,$^{3, 5, 15, 16, 32}$ and was intermediate between two recent interventional trials in HFP EF with rigorous entry criteria.$^{43, 44}$ In HFr EF exercise capacity was also similar to that reported in previous studies,$^{45, 46}$ confirming that the peak VO$_2$ values measured in our study were representative of the broader HF populations.
Association of central cardiac function with exercise capacity in HF

Our finding that 73% of HFpEF and 75% of HFrEF patients had chronotropic incompetence, after accounting for beta-blocker use, and that peak HR was strongly associated with peak VO₂ in HFpEF, confirms previous studies demonstrating an important influence of chronotropic response on exercise capacity in HF.6,35,36,47,48 By confining our study to individuals who exceeded their ventilatory anaerobic threshold and an RER of 1.0 we can be confident that impaired chronotropic responses did not reflect lack of maximum effort or premature cessation of exercise due to pulmonary or orthopedic limitations.

Stroke volume in HFpEF patients compared to controls was similar at rest but lower at peak exercise. Previous elegant studies have elucidated mechanisms by which stroke volume is impaired in HFpEF at rest and during exercise, including abnormal ventriculo-vascular coupling,6 impaired relaxation,8,46 and impaired augmentation in systolic function.5,49 However, not all studies to date have found impaired stroke volume responses to exercise in HFpEF50,51 and we found that within HFpEF patients, peak SV was not significantly related to peak VO₂. Furthermore, the percentage increases in SV from rest to peak exercise within the groups were modest and similar between HF patients and controls (39±4% in controls, 32±5% in HFpEF and 40±5% in HFrEF, Figure 2).13,31 The modest increments in SV in response to exercise (32-40%) across the 3 groups indicates that the range of SV reserve capacity is more narrow than that for HR (53-109%) or C(a-v)O₂ (77-138%) (Figure 2). Hence, targeting impaired SV augmentation in response to exercise may be of limited benefit in a broad population of HFpEF patients.
Peripheral oxygen extraction in HFpEF

Reduced C(a-v)O₂ was the leading cause of impaired exercise capacity (i.e. the degree of impairment in C(a-v)O₂ was greater than that in cardiac output as a % of predicted) in 40% of HFpEF patients in our study and in only 2% of HFrEF patients. Furthermore, in HFpEF patients, we found that normalization of impaired O₂ diffusion would result in a greater increment in peak VO₂ than normalization of convective O₂ delivery (Figure 3).

Following convective delivery of O₂ to skeletal muscle, diffusive O₂ transport and utilization is dependent on the pathway consisting of skeletal muscle tissue microcirculatory O₂ exchange vessels (i.e. arterioles, venules, capillaries) and muscle units. O₂ is transported passively by diffusion in this physically short pathway. In light of the large-scale blood flow redistribution to skeletal muscles during exercise, our finding that impaired diffusive O₂ transport in HFpEF was closely related to an exaggerated systemic blood pressure increment during exercise (Figure 4), suggests a potential role of impaired skeletal muscle vasodilatory capacity in small resistance vessels in mediating reduced peak C(a-v)O₂ in HFpEF.

Vasoconstrictor sympathetic tone and intrinsic microvascular control mechanisms have been shown to modulate the balance between O₂ delivery an O₂ demand within organs, which suggests that skeletal muscle sympatholysis during exercise may be dysregulated in HFpEF patients with impaired O₂ extraction. In further support of sympathetic dysregulation and poorly coordinated vasoconstriction, elevated norepinephrine levels have been reported in HFpEF patients at rest. Alternatively, diffusing capacity of the microvascular network may be limited by heterogeneity in microcirculatory blood flow recognized to occur in pro-inflammatory states. Finally, morphologic and histochemical changes in skeletal muscle have also been described in HFrEF, including marked abnormalities in skeletal muscle mass, composition, capillary
density, fiber type, oxidative metabolism, mitochondrial mass, and mitochondrial function as reviewed by Clark et al.\textsuperscript{55} These pathological peripheral abnormalities are distinct from the influence of deconditioning alone.\textsuperscript{56,57} Detailed investigations of skeletal muscle in HFpEF are limited, though intriguing in that Bhella et al first reported reduced oxidative metabolism by MRI in 2 HFpEF patients\textsuperscript{15} and more recently abnormal skeletal muscle mass, adiposity, fiber type, and capillary density have been observed in HFpEF.\textsuperscript{58,59}

Previous HFpEF studies in which C(a-v)O$_2$ was estimated via non-invasive CO measurement have led to widely variable estimates of C(a-v)O$_2$ levels in normals and in HFpEF.\textsuperscript{15,16} Peak exercise C(a-v)O$_2$ values should be equal to Hb levels in normal individuals.\textsuperscript{18,31} In one previous study that directly measured C(a-v)O$_2$ in a subset of patients studied, C(a-v)O$_2$ levels in controls and HFpEF were similarly low (10.1±0.3 vs. 9.9±0.3 ml/dL, p=0.7).\textsuperscript{17} However, the study by Abudiab et al. relied on exercise in a semi-supine position and control subjects only exercised to 80 Watts, which may not have elicited maximum C(a-v)O$_2$ since we observed C(a-v)O$_2$ to increase in a linear fashion throughout maximum incremental exercise in our study (data not shown). In other HFpEF studies with a control group, the peak C(a-v)O$_2$ values in controls \textsuperscript{7,15,17} were also 30\% lower than their Hb levels, which is much lower than to the ~6\% reduction in C(a-v)O$_2$ expected with deconditioning alone.\textsuperscript{18} In previous small studies that deployed maximum upright exercise, C(a-v)O$_2$ is consistently depressed.\textsuperscript{5,50,51} Our findings of an inverse initial relationship between C(a-v)O$_2$ and CO that is no longer present at peak exercise points to the importance of performing maximum effort exercise to ascertain peak O$_2$ extraction capacity in study populations.
Clinical Implications

Within the constraints of currently applied definitions of HFpEF, a single dominant pathophysiologic mechanism governing exercise intolerance in HFpEF is unlikely to exist. The heterogeneity of the HFpEF population poses a major challenge to development of therapies to treat the entire HFpEF population. A potential pathway forward is to carefully identify subjects in whom the majority of reduction in peak VO₂ is attributable to an abnormality in one component of peak VO₂. In this study, CPET with invasive hemodynamic measurements permitted us to probe the reserve capacity of each component of VO₂ to subphenotype patients on the basis of the dominant mechanism limiting exercise capacity. This approach may refine patient selection for targeted HFpEF therapeutics, for example, HFpEF could be subclassified into those with primarily "impaired peripheral O₂ extraction", "chronotropic incompetence" or "impaired stroke volume" among patients able to complete maximum incremental exercise without orthopedic or pulmonary mechanical limitation.

This study highlights the significant role of impaired C(a-v)O₂ augmentation in contributing to exercise intolerance in approximately 40% of a HFpEF population similar to those recently studied in HFpEF trials. Further studies are needed to determine the relative impact of targeting different aspects of the O₂ diffusion unit. A recent study by Haykowsky et al. found that improved peripheral function [estimated C(a-v)O₂] primarily accounted for observed improvements in peak VO₂ following exercise training in a HFpEF cohort. In light of the plasticity of skeletal muscle, targeting oxygen diffusion abnormalities in HF is particularly attractive. Positive studies with iron repletion in HFrEF, which promotes aerobic enzymatic activity and O₂ storage in myoglobin offer promise for the possibility of extending this intervention to HFpEF. With regard to improving diffusional O₂ transport to muscle in HF,
decreasing O₂ affinity (right shifting the O₂ dissociation curve) has been shown to improve exercise capacity in mice with HF. Alternatively, HFpEF patients with an exaggerated BP response to exercise and impaired O₂ diffusion may be particularly amenable to treatment with vasodilator interventions (i.e. with nitrates, such as the NHLBI Heart Failure Network NEAT Trial, NCT02053493) to target skeletal muscle resistance vessels.

In contrast, patients in whom the dominant component of VO₂ impairment is chronotropic incompetence, pacing or reduction in heart-rate lowering medications may promote improved exercise capacity, as will be tested in the Restoration of Chronotropic CompEtence in Heart Failure PatientS (RESET) Trial. Finally, while stroke volume emerged as the least dynamic of the three Fick variables, if stroke volume remains fixed due to a non-compliant ventricle, then attempting to promote improvement in myocardial relaxation properties during exercise may be warranted.

Limitations

Our study has several limitations. Results were derived from small patient cohort referred to a tertiary care center which may not be representative of the general HFpEF patients found in the community, and we tested multiple hypotheses regarding associations between C(a-v)O₂ and physiological parameters, increasing the chance of type 1 error. Our control population was limited in size (n=24) because of the infrequency with which subjects without significant cardiopulmonary disease undergo CPET with invasive hemodynamic monitoring. Using clinically referred patients who were physiologically normal as controls may underestimate differences between HF patients and controls. The sampling of systemic venous blood does not permit localization of the peripheral abnormality in oxygen utilization in HFpEF. However, the
majority of blood is directed to skeletal muscle during exercise and splanchnic and renal vasoconstriction have been shown to occur normally in HFrEF. Although none of the HFpEF patients in our study had a known diagnosis of a mitochondrial disease or muscular dystrophy, it is possible that some of these patients may have had underlying conditions other than HFpEF that impaired skeletal muscle oxygen extraction. Finally, direct assessments of skeletal muscle and its perfusion were not available in our study to investigate potential histopathological correlates of impaired O₂ diffusion. This will be an important topic of future investigations aimed at further characterizing impaired O₂ diffusion in HFpEF.

**Conclusion**

HFpEF patients demonstrated abnormally low peripheral oxygen extraction [C(a-v)O₂] during exercise compared to HFrEF subjects and normal controls. This finding highlights the importance of looking beyond impairments in LV-function and CO in evaluating functional limitations in patients with HFpEF. Our findings further indicate that improving abnormal O₂ extraction may be an important therapeutic target in the notoriously difficult-to-treat HFpEF patient population.

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**Disclosures**

None.
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Table 1. Demographics of Heart Failure and Control subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HFpEF (48)</th>
<th>HFrEF (56)</th>
<th>Controls (24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, Years</td>
<td>63±12†</td>
<td>59±12</td>
<td>55±18</td>
</tr>
<tr>
<td>Male Sex (number, %)</td>
<td>20 (40)†</td>
<td>45 (81)‡*</td>
<td>15 (62)</td>
</tr>
<tr>
<td>Race (White %)</td>
<td>46 (96)</td>
<td>50 (88)</td>
<td>23 (96)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.7±7.6†</td>
<td>27.8±6*</td>
<td>27.6±3.0</td>
</tr>
<tr>
<td>Comorbidities %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>29 (60)†</td>
<td>34 (61)‡</td>
<td>9 (37)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>14 (25)†</td>
<td>12 (21)‡</td>
<td>0</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>25 (52)†</td>
<td>32 (57)‡</td>
<td>6 (25)</td>
</tr>
<tr>
<td>Atrial Fibrillation</td>
<td>12 (26)†</td>
<td>11 (19)‡</td>
<td>0</td>
</tr>
<tr>
<td>Heart Failure Pharmacotherapy %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>30 (63)†</td>
<td>48 (86)‡*</td>
<td>1 (4)</td>
</tr>
<tr>
<td>ACE Inhibitor or ARB</td>
<td>14 (29)</td>
<td>45 (80)‡</td>
<td>7 (29)</td>
</tr>
<tr>
<td>β-adrenergic receptor blocker</td>
<td>25 (52)†</td>
<td>51 (91)‡*</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
<td>4 (8)</td>
<td>30 (54)‡</td>
<td>0</td>
</tr>
<tr>
<td>Digoxin</td>
<td>6 (12)†</td>
<td>28 (50)‡</td>
<td>0</td>
</tr>
<tr>
<td>LVEF %</td>
<td>62±7†</td>
<td>29±6‡</td>
<td>67±6</td>
</tr>
<tr>
<td>Resting Supine PCWP, mmHg</td>
<td>20±2.7†</td>
<td>22±8.9‡</td>
<td>10±3.9</td>
</tr>
<tr>
<td>Hemoglobin, gm/dl</td>
<td>13.2±1.4</td>
<td>12.9±2.2</td>
<td>13.2±1.5</td>
</tr>
<tr>
<td>Peak VO₂, ml/kg/min</td>
<td>13.9±3.5†</td>
<td>12.4±3.7‡*</td>
<td>27.2±8.3</td>
</tr>
<tr>
<td>Max watts achieved</td>
<td>82±32†</td>
<td>75±37‡</td>
<td>166±57</td>
</tr>
<tr>
<td>Peak Exercise RER</td>
<td>1.15±0.07</td>
<td>1.16±0.14</td>
<td>1.15±0.05</td>
</tr>
<tr>
<td>Peak Exercise Lactate, mM</td>
<td>5.3±2.7†</td>
<td>4.8±1.5‡</td>
<td>7.6±1.5</td>
</tr>
</tbody>
</table>

† indicates p<0.05 between HFpEF and controls, ‡ indicates p<0.05 between HFrEF and controls, * indicates p<0.05 between HFpEF and HFrEF
Table 2. Hemodynamic, gas exchange and ventriculography measurements of HF and Control subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HFpEF</td>
<td>HFrEF</td>
</tr>
<tr>
<td>VO₂ ml/min</td>
<td>307±11</td>
<td>281±9‡</td>
</tr>
<tr>
<td>VO₂ ml/kg/min</td>
<td>3.4±0.1</td>
<td>3.4±0.1‡</td>
</tr>
<tr>
<td>CO, liters/min</td>
<td>5.1±0.2</td>
<td>3.7±0.1‡*</td>
</tr>
<tr>
<td>CI, l/min/m²</td>
<td>2.7±0.1</td>
<td>1.9±0.1‡*</td>
</tr>
<tr>
<td>SV, ml</td>
<td>69±2.6</td>
<td>51±2‡*</td>
</tr>
<tr>
<td>SVI, ml/m²</td>
<td>36.6±1.3</td>
<td>25.6±1.0‡*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>75±2</td>
<td>75±0.5</td>
</tr>
<tr>
<td>CaO₂, ml/dl</td>
<td>17.8±0.3</td>
<td>17.1±0.4</td>
</tr>
<tr>
<td>CvO₂, ml/dl</td>
<td>11.6±0.3</td>
<td>9.4±0.3‡*</td>
</tr>
<tr>
<td>C(a-v)O₂, ml/dl</td>
<td>6.2±0.2</td>
<td>7.8±0.2‡*</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>150±4</td>
<td>123±3.2‡*</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>74±2</td>
<td>67±1.5‡*</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>99±2</td>
<td>86±2.3‡*</td>
</tr>
<tr>
<td>RVEF (%)</td>
<td>50±1</td>
<td>38±1‡*</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>62±1†</td>
<td>30±1‡*</td>
</tr>
<tr>
<td>LVDEV, ml</td>
<td>133±5</td>
<td>264±12‡*</td>
</tr>
<tr>
<td>LVEDVI, ml/m²</td>
<td>65±2.4</td>
<td>133±5.4‡*</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>10±0.4†</td>
<td>9±0.7‡</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>30±0.9†</td>
<td>35±1.7‡*</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>20±0.4†</td>
<td>22±1.2‡</td>
</tr>
<tr>
<td>Elastance (Ea), mmHg/ml</td>
<td>2.2±0.1†</td>
<td>2.4±0.1‡</td>
</tr>
</tbody>
</table>

† indicates p<0.05 between HFpEF and controls, ‡ indicates p<0.05 between HFrEF and controls, * indicates p<0.05 between HFpEF and HFrEF.
Table 3.  Heart rate (HR), Stroke volume (SV), and C(a-v)O₂ association with peak VO₂

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HFpEF Partial R-squared</th>
<th>HFpEF P value</th>
<th>HFpEF Partial R-squared</th>
<th>HFpEF P value</th>
<th>HFpEF Partial R-squared</th>
<th>HFpEF P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR max</td>
<td>0.350</td>
<td>&lt;0.0001</td>
<td>0.307</td>
<td>&lt;0.0001</td>
<td>0.342</td>
<td>0.006</td>
</tr>
<tr>
<td>SV max</td>
<td>0.072</td>
<td>0.077</td>
<td>0.379</td>
<td>&lt;0.0001</td>
<td>0.436</td>
<td>0.001</td>
</tr>
<tr>
<td>C(a-v)O₂ max</td>
<td>0.281</td>
<td>0.0002</td>
<td>0.253</td>
<td>0.0001</td>
<td>0.187</td>
<td>0.056</td>
</tr>
</tbody>
</table>

HR, SV and C(a-v)O₂ are adjusted for age and sex both and VO₂ is indexed to body weight.
Figure Legends

Figure 1. Arterial oxygen content (CaO2) and mixed venous oxygen content (CvO2) at peak exercise in patients with HFpEF, HFrEF, and controls, * indicates p<0.05 for comparison to HFrEF and controls.

Figure 2. Percentage increase in VO2 and each of its components, heart rate (HR), stroke volume (SV) and arterio-mixed venous saturation difference (C(a-v)O2) from rest to peak exercise, * indicates p<0.05.

Figure 3. Illustration of the convective and diffusive components that interact to determine exercise capacity (VO2) in HF and controls. Mean values for CvO2 and VO2 at rest, 30W, and peak exercise are used to construct Fick principal lines, which indicate convective O2 delivery and are curvilinear because they directly reflect the hemoglobin dissociation curve. The vertical lines extending from the origin to the VO2-CvO2 plot at peak exercise indicate maximum diffusive oxygen delivery as determined by the Fick law, with a steeper relationship indicating better O2 diffusion. ▪ Indicates the increment in peak VO2 in HFpEF if convective O2 delivery was corrected to that of normal controls. ⇔ Indicates the increment in peak VO2 if O2 diffusion was normalized in HFpEF.

Figure 4. Diastolic blood pressure at rest and during incremental exercise in two subgroups of HFpEF stratified by median mixed venous oxygen content at peak exercise.
Oxygen content in ml/dl

- **HFPpEF**: 
  - CaO2: \( \Delta 11.5 \pm 0.3^* \)
  - CvO2: 

- **HFrEF**: 
  - CaO2: \( \Delta 13.5 \pm 0.3 \)
  - CvO2: 

- **Controls**: 
  - CaO2: \( \Delta 13.3 \pm 0.3 \)
  - CvO2: 

(CaO2: Cardiac Oxygen Saturation, CvO2: Cerebral Oxygen Saturation, HFPpEF: Heart Failure with Preserved Ejection Fraction, HFrEF: Heart Failure with Reduced Ejection Fraction)
Panel A: ΔVO2

Panel B: ΔHR

Panel C: ΔSV

Panel D: ΔC(a-v)O2

*Indicates p < 0.05 compared to controls
Convective O₂ supply into skeletal muscles

Diffusive O₂ conductance from blood to mitochondria

CvO₂ in ml/dl

VO₂ in ml/min

- Controls
- HFpEF
- HFrEF
Mechanisms of Exercise Intolerance in Heart Failure with Preserved Ejection Fraction: The Role of Abnormal Peripheral Oxygen Extraction
Bishnu P. Dhakal, Rajeev Malhotra, Ryan M. Murphy, Paul P. Pappagianopoulos, Aaron L. Baggish, Rory B. Weiner, Nicholas E. Houstis, Aaron S. Eisman, Stacyann S. Hough and Gregory D. Lewis

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SUPPLEMENTAL MATERIAL

Supplemental Table 1: Demographics and hemodynamics of HFpEF subclasses*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Higher CvO\textsubscript{2} (n=24)</th>
<th>Lower CvO\textsubscript{2} (n=24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in Years</td>
<td>60±3</td>
<td>66±3</td>
<td>0.10</td>
</tr>
<tr>
<td>Male Sex %</td>
<td>42</td>
<td>37</td>
<td>0.60</td>
</tr>
<tr>
<td>BMI</td>
<td>34.2±1.5</td>
<td>33.1±1.7</td>
<td>0.64</td>
</tr>
<tr>
<td>Heart Failure Pharmacotherapy %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretic</td>
<td>65</td>
<td>71</td>
<td>0.04</td>
</tr>
<tr>
<td>ACE Inhibitor or ARB</td>
<td>42</td>
<td>24</td>
<td>0.6</td>
</tr>
<tr>
<td>β-Adrenergic Receptor Antagonist</td>
<td>54</td>
<td>50</td>
<td>0.79</td>
</tr>
<tr>
<td>Digoxin</td>
<td>0</td>
<td>21</td>
<td>0.02</td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
<td>0</td>
<td>21</td>
<td>0.02</td>
</tr>
<tr>
<td>Resting Supine PCWP, mm Hg</td>
<td>20±0.6</td>
<td>20±0.6</td>
<td>0.68</td>
</tr>
<tr>
<td>LVEF at rest %</td>
<td>63±1</td>
<td>62±1</td>
<td>0.56</td>
</tr>
<tr>
<td>Mean Hb, gm/dl</td>
<td>13.9±0.2</td>
<td>12.6±0.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Peak VO\textsubscript{2}, ml/kg/min</td>
<td>14.2±0.7</td>
<td>13.5±0.7</td>
<td>0.47</td>
</tr>
<tr>
<td>Peak VO\textsubscript{2} % predicted</td>
<td>70±3.3</td>
<td>72±3.9</td>
<td>0.74</td>
</tr>
<tr>
<td>Resting C(a-v)O\textsubscript{2}, ml/dl</td>
<td>6.0±0.2</td>
<td>6.4±0.3</td>
<td>0.23</td>
</tr>
<tr>
<td>Peak Exercise C(a-v)O\textsubscript{2}, ml/dl</td>
<td>10.8±0.3</td>
<td>12.2±0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Extraction ratio</td>
<td>57±1.2</td>
<td>69±1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resting CO, liters/min</td>
<td>5.2±0.3</td>
<td>4.6±0.2</td>
<td>0.10</td>
</tr>
<tr>
<td>Peak CO, liters/min</td>
<td>11.7±0.7</td>
<td>9.6±0.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Peak CO, % predicted</td>
<td>88±3.5</td>
<td>77±4.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>72±3</td>
<td>78±3</td>
<td>0.11</td>
</tr>
<tr>
<td>Peak HR, beats/min</td>
<td>129±5</td>
<td>113±5</td>
<td>0.02</td>
</tr>
<tr>
<td>Peak HR, % predicted</td>
<td>80±3</td>
<td>73±3</td>
<td>0.11</td>
</tr>
<tr>
<td>DBP rest, mm Hg</td>
<td>78±2</td>
<td>70±3</td>
<td>0.02</td>
</tr>
<tr>
<td>SBP rest, mm Hg</td>
<td>156±5</td>
<td>143±6</td>
<td>0.10</td>
</tr>
<tr>
<td>MAP rest, mm Hg</td>
<td>104±3</td>
<td>94±3</td>
<td>0.02</td>
</tr>
<tr>
<td>DBP max, mm Hg</td>
<td>93±4</td>
<td>76±3</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP max, mm Hg</td>
<td>196±7</td>
<td>171±7</td>
<td>0.01</td>
</tr>
<tr>
<td>MAP max, mm Hg</td>
<td>127±4</td>
<td>107±4</td>
<td>0.001</td>
</tr>
<tr>
<td>Peak exercise Lactate</td>
<td>5.7±0.5</td>
<td>4.8±0.5</td>
<td>0.21</td>
</tr>
<tr>
<td>Peak RER</td>
<td>1.15±0.03</td>
<td>1.15±0.02</td>
<td>0.96</td>
</tr>
<tr>
<td>Peak pH</td>
<td>7.38±0.01</td>
<td>7.40±0.01</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Stratification based on median CvO\textsubscript{2} of 6.8 mg/dl*
Supplemental Figure 1: Graphical representation of the linearity of the cardiac output-VO₂ relationship during exercise in 2 HFpEF subgroups.

Figure Legend: The group with impaired peak C(a-v)O₂ demonstrates a steep CO-VO₂ slope upon initiation of exercise in comparison to other patients with HFpEF.