EFFECT OF PRIMING EXERCISE ON OXYGEN UPTAKE KINETICS IN HEART FAILURE WITH PRESERVED EJECTION FRACTION

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ABSTRACT

The cardinal symptom of heart failure with preserved ejection fraction (HFpEF) is exercise intolerance, which is associated with fatigue and reduced quality of life. HFpEF patients exhibit skeletal muscle dysfunction related to reduced peak oxygen uptake (peak $\dot{V}O_{2p}$). Prior heavy exercise speeds pulmonary oxygen uptake ($\dot{V}O_{2p}$) kinetics in older adults and in HF patients with reduced ejection fraction (HFrEF), presumably through increased oxygen delivery to the exercising muscle. We tested the hypothesis that prior heavy exercise would not speed $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF, suggesting HFpEF exercise intolerance stems primarily from muscle dysfunction. Eight HFpEF patients, 4 high-fit (CTL-HF), and 5 low-fit age-matched controls (CTL-LF) underwent echocardiography and peak exercise testing (cycle ergometer). Subjects performed 3 separate repetitions of 2 exercise transitions: MOD1, transition from rest to 4-min moderate intensity cycling (work rate corresponding to 90% ventilatory threshold); and MOD2, MOD1 preceded by 2-min of heavy cycling (Δ50% intensity; halfway between ventilatory threshold and peak) and 5-min of inter-transition rest. $\dot{V}O_{2p}$ (breath-by-breath gas exchange), heart rate (HR, ECG), stroke volume (SV, ModelFlow), cardiac output (CO, calculated), total peripheral resistance (TPR, calculated), and tissue oxygenation of the vastus lateralis (TOI, near-infrared spectroscopy) on-kinetics were measured. $\dot{V}O_{2p}$, HR, and CO data were linearly interpolated, time-aligned, averaged into 5-s time bins, and curve-fitted using a monoexponential equation. ΔSV, ΔTPR, and ΔTOI were calculated from baseline at 15s, 30s, and end-exercise to represent time-course changes. Analysis included repeated measures ANOVA, and SNK post-hoc, break-down analyses, and non-parametric testing where appropriate. Significance was $P<0.05$. HFpEF $\dot{V}O_{2p}$ on-kinetics were slower than CTL-HF but similar to CTL-LF, pooled across conditions ($P=0.008$). MOD2 $\dot{V}O_{2p}$ on-kinetics were faster compared to MOD1, pooled across groups ($P=0.039$). CTL-HF had a greater reduction in TPR across all time points compared to HFpEF (all $P<0.038$) and at 30s and end-exercise compared to CTL-LF (all $P<0.032$), pooled across conditions. HFpEF patients and CTL-LF had decreased TOI at 15-30s (all $P\leq0.024$) in MOD1. By visual inspection, HFpEF TOI remained depressed while CTL-LF TOI increased to baseline levels, with no group difference at end-exercise ($P=0.086$) in MOD1. CTL-HF TOI increased at 15-30s (all $P\leq0.024$) and decreased toward baseline levels. All groups had slower HR on-kinetics in MOD2 compared to MOD1 ($P=0.001$). TOI showed a greater reduction in MOD2 than MOD1 in HFpEF at 30s

(P=0.033) and CTL-HF throughout MOD2 (all P<0.05). The results of this study indicate that HFpEF patients have impaired $\dot{V}O_{2p}$ on-kinetics and a speeding response of $\dot{V}O_{2p}$ on-kinetics to prior exercise, suggesting O_2 delivery may be an important rate-limiting factor of $\dot{V}O_{2p}$ on-kinetics in HFpEF.

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DEDICATION

This thesis is dedicated to my wonderful, loving, and supportive fiancé, Kyle Mostat. Thank you for supporting me in every way while I pursued my dream. You knew, from the outside looking in, that this path made me happy and gave me fulfillment. You reminded me that I am strong when I felt weak. You knew that every challenge was worth it, and you shared in every achievement and excitement I felt. I hope that someday I can make your dream come true, too.

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CHAPTER ONE: LITERATURE REVIEW

1.1 Background

Heart failure (HF) is defined by the American Heart Association and American College of Cardiology Foundation as "a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood" (59, 105). HF is associated with increased morbidity and mortality rates and thus places a heavy economic burden on Canada's healthcare system (43, 44). Between 2012 and 2030, HF prevalence is expected to increase by 46% in America (67). Recently, two phenotypes of HF have been identified: heart failure with reduced ejection fraction (EF $\leq$ 35%; termed HFrEF), and heart failure with preserved ejection fraction (EF $\geq$ 45%; termed HFpEF). The incidence of HF is roughly 50% HFrEF and 50% HFpEF, although these vary depending on world location (17, 74). HFrEF, previously known as systolic HF, typically arises from volume overload. In most cases, HFrEF can be managed with heart transplantation being curative (98), and survival rates have improved over time (2, 9, 78, 85, 96, 97, 106). Conversely, effective treatment strategies for HFrEF have proven ineffective for improving survival in HFpEF (previously referred to as diastolic HF) and survival rates continue to decline (43). Many HF patients exhibit both systolic and diastolic dysfunction (105) and it is therefore the EF that determines HF phenotype. Phenotype differentiation is crucial for appropriate clinical treatment and management of HF, therefore phenotype-specific pathophysiology must continue to be scrupulously assessed. However, as many of the classic signs and symptoms of HF can be observed in other chronic diseases (*e.g.,* pulmonary hypertension, chronic lung disease, renal insufficiency, cirrhosis (101)), HFpEF diagnosis can be difficult due to the preserved ejection fraction. HFpEF, generally caused by pressure overload or chronic systemic inflammation, has only recently been commonly accepted as a distinct type of HF and diagnosed as more than diastolic dysfunction. Coincidentally, HFpEF is the fastest growing type of HF and is mostly found in older adults, particularly older women (41, 43).

1.2 Introduction

HF, independent of phenotype, causes significant economic burden to Canada's healthcare system and directly costs over $2.8 billion per annum (44). Expenses include emergency hospitalization, clinic visits, medications, surgeries, and rehabilitation. HFrEF has some effective neurohormonal (19, 77, 104, 107) and cardiac resynchronization (98) therapies that improve

prognosis, mortality rates, and quality of life. Unfortunately, these same therapies have failed to improve mortality rates in patients with HFpEF (6, 7, 61, 71, 86, 91), further illustrating the stark physiological differences between phenotypes. The mortality rate for HFpEF is 25% within 3 years from diagnosis (adjusted for age, gender, etiology, hypertension, diabetes, and atrial fibrillation) (64). Accordingly, our current understanding of HFpEF pathophysiology must be improved for future HFpEF therapies to be successfully designed. Exercise therapy coupled with caloric restriction has been proven to improve exercise tolerance and body composition in patients with HFpEF (50). As exercise intolerance is the cardinal symptom of HF, and cardiorespiratory fitness is correlated with mortality (70), the mechanisms behind exercise intolerance in HFpEF should be revealed. Recently, focus has turned to investigating peripheral mechanisms of exercise intolerance in HFpEF patients as opposed to central, as seen in HFrEF.

The current thesis was specifically designed to investigate the integrative cardiovascular physiology in HFpEF during exercise. The following is a review of the most updated literature concerning the clinical presentation and pathophysiology of HFpEF, pulmonary oxygen uptake ($\dot{V}O_{2p}$) on-kinetics in healthy young and older adults and in HFrEF, the effects of priming exercise in healthy older adults, and other physiological responses to the onset of exercise such as the on-kinetics of cardiac output, heart rate, stroke volume, total peripheral resistance (TPR), and muscle tissue oxygenation (TOI).

1.3 HFpEF Clinical Diagnosis and Presentation

Historically, the Framingham criteria (63) have functioned well for early and accurate diagnosis of congestive HF. The Framingham criteria consists of well-documented signs and symptoms, both of major (paroxysmal nocturnal dyspnea, neck vein distention, rales, increased cardiac size on chest radiography, hepatojugular reflux, to name a few) and minor importance (bilateral ankle edema, nocturnal cough, dyspnea on ordinary exertion, to name a few) in a clinical diagnosis for HF, regardless of phenotype (63). As these criteria do not account for EF, more sophisticated criteria have been proposed by multiple agencies (*e.g.,* European Society of Cardiology, American College of Cardiology Foundation/American Heart Association), however powered trials validating these criteria are still needed (101). Generally, four requirements exist: 1. symptoms of HF, 2. signs of HF, 3. preserved EF (≥45%) and left ventricle not dilated, and 4. relevant structural heart disease and/or diastolic dysfunction (101). Clinicians must also ensure the

signs and symptoms of HF cannot be explained by pulmonary hypertension, chronic lung disease, renal insufficiency, cirrhosis, etc., (101).

The clinical presentation of HFpEF differs substantially to HFrEF (71). Compared to HFrEF, patients with HFpEF tend to be older, more obese, are more likely to have hypertension, diabetes, and atrial fibrillation, and less likely to have coronary artery disease (71). Population studies and meta-analyses indicate the gender distribution in HFpEF is roughly equal (16), or female dominant (55-70% female) (17, 18, 46, 74, 92). Accordingly, females are more likely to develop HFpEF than HFrEF (16). Noncardiac comorbidities are also common, although potentially not more common in HFpEF than HFrEF, and include chronic lung disease, anemia, chronic kidney disease, and cancer, all of which contribute to morbidity and mortality in HFpEF (3, 18, 71, 84). For example, renal dysfunction alone can affect the morphology of the left ventricle (62), diabetes further increases the risk of hospitalization in HFpEF and further reduces exercise capacity (57), and atrial fibrillation (history or current) increases the risk of stroke (73).

Echocardiography is a pivotal tool in diagnosing and determining prognosis in HFpEF (101). The most common measure of left ventricular end-diastolic pressure and stiffness is E/é (early-diastole pulse-wave velocity divided by early-diastole tissue-wave velocity), which is a key indicator of diastolic function. E/é >15 indicates increased end-diastolic pressure, leading to HFpEF diagnosis in patients with HF symptoms (101). Left ventricular size and dimension in HFpEF are highly varied; in a large sample size, a 10-15% increase from healthy controls and hypertensive controls was found, but did not preclude a significant portion of individuals having normal or slightly reduced left ventricular diastolic diameter, even when adjusted for age, gender, body size, and race (62). Left ventricular hypertrophy is well-documented in HFpEF (20, 26), although often not present (20). Further, indices of left ventricular hypertrophy (relative left ventricular wall thickness, end-diastolic volume/mass ratio) were also seen in hypertensive controls, with no difference between groups (62). Valvular function should also be assessed, as leaky or sclerotic valves can lead to fluid overload in the cardiac chambers (101). Increased left atrial size and stiffness are commonly present in HFpEF (101). Diastolic dysfunction is related to increased serum levels of B-type natriuretic peptide (BNP), but this is also common with a reduced ejection fraction (17). However, *N*-terminal pro-BNP >220 pg/mL or BNP levels >200 pg/mL

coupled with echocardiogram-indicated diastolic dysfunction aids to yield a HFpEF diagnosis (101).

1.4 HFpEF Pathophysiology

Exact mechanisms underpinning HFpEF development are varied, but a primary cause of the characteristic peripheral dysfunction in HFpEF is thought to be chronic systemic inflammation (58, 76, 93). Suggested contributors to chronic systemic inflammation by Shah et al. (93) include aging and common comorbidities, such as metabolic syndrome (obesity (84% of patients) (37), arterial hypertension (60-80%) (29), type 2 diabetes mellitus (20-45%) (29)), chronic obstructive pulmonary disease (76), and renal insufficiency (93). Some biomarkers of systemic inflammation (*i.e.,* soluble interleukin 1 receptor-like 1, C-reactive protein, and growth differentiation factor 15) were recently observed to be higher in HFpEF compared to HFrEF (87). Systemic inflammation has multi-organ effects that disrupt resting and exercise physiology (93). Of focus for the current thesis were the role of cardiac function (cardiac output, heart rate, and stroke volume on-kinetics), vascular function (TPR on-kinetics), and skeletal muscle function ($\dot{V}O_{2p}$ and TOI on-kinetics).

Initially proposed by Paulus and Tschöpe in 2013 (76), Shah et al. (93) summarize a signalling cascade beginning with coronary microvascular endothelial dysfunction (33, 76). Macrophages infiltrate the myocardium, which induces reactive interstitial fibrosis (102), in turn leading to disrupted communication between adjacent endothelial and myocardial cells (33). The alteration of intercellular signalling causes increased activation of myofibroblasts that propagate an inflammatory response. Nitric oxide bioavailability and protein kinase G activity are reduced in the coronary microvascular endothelium due to chronic systemic inflammation (76). Through cross-talk between the endothelium and cardiomyocytes (58), low protein kinase G activity increases resting tension in the myocardium due to hypophosphorylation of titin, leading to myocardial hypertrophy (76). The resulting stiff cardiomyocytes and fibrosis contribute to a stiff left ventricle in diastole, propagating HF development. This proposed paradigm is different from previously assumed increased afterload on the heart that favours stiffening of the myocardium, and is supported by similar tissue remodeling in all cardiac chambers instead of only the left ventricle (76). These details are important to differentiate the HF phenotypes; HFpEF development entails stiffening and fibrosis of cardiomyocytes, whereas HFrEF development may progress secondary to the loss of cardiomyocytes (76).

1.5 Current HFpEF Therapies

Current HFpEF therapy is dictated by the unique clinical presentation phenotype (*e.g.,* lung congestion, chronotropic incompetence, pulmonary hypertension, skeletal muscle weakness, atrial fibrillation, or a combination thereof) and any predisposing comorbidities (metabolic syndrome or any of its constituents, arterial hypertension, renal dysfunction, coronary artery disease, or a combination thereof) (93). Therapies will depend on the specific combination of the above factors, and can include exercise training, caloric restriction, diuretics (*e.g.,* spironolactone, or loop diuretics in diabetes), statins, anti-hypertensives (*e.g.,* sacubitril), angiotensin-converting enzyme inhibitors, rate adaptive atrial pacing (chronotropic incompetence only), revascularization (coronary artery disease only), pulmonary vasodilators, cardioversion (atrial fibrillation only), anticoagulation (atrial fibrillation only) and ultrafiltration (renal dysfunction only) (93). Although large neurohormonal outcome-focused trials have failed to yield clinical benefit similar to the success found in HFrEF (29, 71), this may be due to the heterogeneity of the HFpEF sample; the therapy must be specific to the predisposition/presentation combination (29, 54, 93). The process of "phenomapping" patients with HFpEF, *i.e.,* categorizing very large datasets of patients with significant heterogeneity of clinical presentation into smaller, homogeneous subgroups, is underway (92) and presents a promising future avenue for determining ideal therapies for a unique patient with HFpEF.

1.6 HFpEF Exercise Physiology

Normal cardiovascular physiology at rest does not support the assumption that reserve capacity exists for times of physiological stress, the most common of which is physical activity (10). Understanding exercise physiology in HFpEF yields critical insights into the complex clinical syndrome of HFpEF and what limits functional capacity in these patients (10). Exercise intolerance in HFpEF is not completely understood, but significant limitations to exercise tolerance have been documented, covering the full O_2-cascade spectrum from cardiac and hemodynamics to metabolic inefficiencies (11). Although not precluding the significance of cardiac reserve and vascular reserve dysfunction (12, 14, 31, 53, 54), a focus of study has brought supportive evidence to suggest peripheral limitations (*e.g.,* microvascular O_2 delivery and O_2 utilization in the skeletal muscle) play a larger role in limiting exercise tolerance in HFpEF compared to healthy controls (8, 38, 39, 42, 51, 52, 66) and HFrEF (43). Notably, exercise training in HFpEF increased peak

$\dot{V}O_{2p}$, ventilatory threshold, and self-reported quality of life without any improvements in left ventricular morphology or neuroendocrine function (52). Molina et al. (66) recently reported invasive measurements of mitochondrial proteins (porin expression and mitofusin content – regulate mitochondria quality control) and oxidative enzyme activity (citrate synthase) to be markedly reduced (46%, 54%, and 29% lower, respectively) in HFpEF compared to controls. Further, the indices of mitochondria content (porin expression) and quality (mitofusins) were positively correlated with peak $\dot{V}O_{2p}$ and 6-min walk distance (indices of aerobic capacity and exercise tolerance). These findings therefore suggest that adverse changes in mitochondrial function may be related to $\dot{V}O_{2p}$ abnormalities observed in patients with HFpEF. Interestingly, Mettauer et al. (65) assessed $\dot{V}O_{2p}$ and muscle biopsies and reported the same deficiency in mitochondrial intrinsic oxidative capacity and regulation in patients with HFrEF and sedentary matched controls. In comparison, physically active controls had markedly greater muscle oxidative capacity and regulation of oxidative phosphorylation, suggesting that deconditioning has a separate role in limiting physiology than disease-mediated limitations (65). Indeed, the only differences found between the patients with HFrEF and sedentary adults was reduced $\dot{V}O_{2p}$, citrate synthase levels, creatine kinase levels, and lactate dehydrogenase levels (65). The latter two substrates are usually indices of fewer type II muscle fibers, which was not found in these subjects, leading the authors to suggest the creatine kinase and lactate dehydrogenase levels may be key in the pathophysiology of exercise intolerance in HFrEF.

$\dot{V}O_{2p}$ is the rate of O_2 the body consumes per minute, which is used to aerobically generate adenosine triphosphate (ATP), the body's energy currency. Peak $\dot{V}O_{2p}$ is thus the maximal amount of O_2 the body can use per minute during exercise. The Fick principle estimates that $\dot{V}O_2$ = cardiac output × arterial-venous oxygen content difference (AVO_2Diff) (79). Cardiac output is the volume of blood pumped from the left ventricle per minute and is the product of stroke volume and heart rate (75). Arterial-venous O_2 content difference (AVO_2Diff) represents O_2 extraction at the muscle (43). Severe exercise intolerance is objectively measured through reduced peak $\dot{V}O_{2p}$ as measured at the mouth during whole body exercise (32). Peak $\dot{V}O_{2p}$ is a strong indicator of disease prognosis (79, 80) and a low peak $\dot{V}O_{2p}$ is associated with reduced quality of life (41). HFpEF patients have peak $\dot{V}O_{2p}$ values 40% lower compared to healthy older adults (41). In healthy men, $\dot{V}O_{2p}$ at maximal exercise increases 7.7-fold; this is a result of a 3.1-fold increase in cardiac output and a 2.5-fold increase in AVO_2Diff (45). Thus, limitations to exercise tolerance can be differentiated

into central (affecting cardiac output) and peripheral (AVO_2Diff) factors. AVO_2Diff adaptation from rest to maximal exercise was reported to be the strongest independent predictor of peak $\dot{V}O_{2p}$ in HFpEF patients compared to controls (39), and HFpEF patients have lower maximal cardiac output compared to controls (43).

Cardiac properties are dictated, in part, by the sympatho-vagal balance (*i.e.,* autonomic control of heart rate and contractility), afterload (*i.e.,* the forces that oppose ejection of blood from the left ventricle; ideally measured by effective arterial elastance (Ea)), and preload (amount of myocardial shortening during a contraction; determined by the magnitude of stretching of the ventricular cardiomyocytes prior to contraction as a result of blood accumulation; ideally measured by left ventricular end-diastolic volume) (10). Cardiac function can be globally characterized by its ability to eject blood (systolic function; myocardial contraction), and fill with blood (diastolic function; myocardial relaxation). Increased sympathetic tone enhances contractility, chronotropy (rate), and lusitropy (relaxation) of the heart, and peripherally increases venous return through vasoconstriction of the capacitance vessels (10). Conversely, increased parasympathetic tone reduces heart rate (10).

HFpEF patients often exhibit mild systolic dysfunction at rest and decreased systolic reserve, *i.e.,* an impaired ability to increase contractility during exercise (10). HFpEF patients typically have decreased diastolic reserve stemming from the impaired ability of the ventricle to relax during diastole, thereby decreasing the pressure gradient from atria to ventricle, lowering suction and ultimately filling (10). This is seen with decreased ventricular compliance, lower end-diastolic volumes, and increased left ventricular filling pressures (10). Chronotropic incompetence is often observed in patients with HFpEF as a result of sinus node dysfunction and/or atrial fibrillation (10), and decreases the ability to rapidly increase heart rate in response to exercise. When pooled together, these impaired cardiac properties decrease cardiac output reserve. In addition to the above listed factors, there is commonly tissue ischemia during exercise as a result of coronary artery disease (although more common in HFrEF), consequently reducing contractility (10).

During large muscle mass exercise, such as cycling, patients with HFpEF typically have lower maximal exercise cardiac output and heart rate (43). Interestingly, there is contradicting evidence on whether stroke volume is decreased (28, 53) or similar (8, 39) compared to healthy

controls, as is the case with end-diastolic volume (decreased (39, 53); similar (28)), and AVO_2Diff (decreased (8, 28, 39); similar (1, 53)). Likewise, end-systolic volume has been shown the be both increased (28) or similar (39, 53) to healthy controls. Mean arterial pressure (MAP) has been similar to healthy controls (43).

Chronic increases in sympathetic activity to the periphery has significant effects on cardiac function. HF patients in general have increased sympathetic tone which enhances vasoconstriction at rest and impairs vasodilation of the arterioles in response to exercise, thus increasing afterload on the heart and reducing venous return, ultimately lowering maximal stroke volume and cardiac output (10). Vascular dysfunction in HFpEF is in part caused by the above mentioned sympathetic tone, but also endothelial dysfunction caused by chronic systemic inflammation that is prominent in HFpEF (10, 76, 93). Additionally, arterial stiffening is also common in HFpEF, further increasing afterload on the heart, increasing cardiac metabolic demand during exercise, and reducing diastolic and systolic reserve (48). Interestingly, 16 weeks of endurance exercise training in HFpEF patients did not improve vascular function, as measured by arterial flow-mediated dilation and arterial stiffness, despite increased peak $\dot{V}O_{2p}$ (51). This suggests that vascular function, although typically impaired in HFpEF, may not be a critical determinant of exercise intolerance in this disease. Albeit, only the vascular response to an increase in flow was studied, and other factors may cause an appropriate vasodilatory response to exercise.

The primary determinant of the severe exercise intolerance in HFpEF patients may not be related to cardiac dysfunction, but rather peripheral factors (41, 43). Despite evidence showing lower maximal cardiac output in HFpEF patients compared to controls (43), Haykowsky et al. (39) reported that peripheral O_2 utilization may play a greater role in HFpEF, as compared to HFrEF, in which cardiac dysfunction plays the dominant role in exercise intolerance. Likely coupled with vascular and autonomic dysfunction, patients with HFpEF have lower AVO_2Diff reserve (10), as evidenced by the following: persons with HFpEF characteristically have reduced microvascular endothelial function, muscle dysfunction, reduced leg lean mass, and more intermuscular adipose tissue (40–43). In particular, HFpEF exercise intolerance may be particularly limited by muscle dysfunction in the form of lower muscle quality (40, 42), lower ratio of oxidative to glycolytic muscle fiber type (41, 43), lower oxidative enzymes (41, 43) and lower muscle capillary density (41, 43). Indeed, peak $\dot{V}O_{2p}$ was not only correlated with percent leg lean mass but the slope of

the relationship of peak $\dot{V}O_{2p}$ and percent leg lean mass was significantly lower in HFpEF compared to controls (40). Although evidence supporting various hemodynamic and peripheral limitations is growing, the specific mechanisms causing exercise intolerance in HFpEF remains inadequately understood.

1.7 Introduction to $\dot{V}O_{2p}$ On-Kinetics

Patients with HFpEF have a substantial reduction in peak $\dot{V}O_{2p}$, which causes the traditional exercise intensity domains of moderate, heavy, and severe to be compressed in HFpEF, making daily life more challenging. For example, performing activities of daily living that would have previously been of moderate intensity (*e.g.,* carrying groceries, making a bed, walking upstairs, etc.) now reside within the heavy intensity domain (47). This extra effort can compromise the ability of patients with HFpEF to perform daily activities and could potentially reduce their quality of life.

Transport of O_2 and its delivery to the working cells is a key component of $\dot{V}O_{2p}$. $\dot{V}O_{2p}$ on-kinetics is the change in rate of $\dot{V}O_{2p}$. In a step-transition from rest to exercise (a "square-wave" transition), ATP demand increases instantaneously (80). Ideally, this demand would be met via aerobic metabolism (*i.e.,* using O_2 to generate ATP); however, $\dot{V}O_{2p}$ increases exponentially rather than instantaneously (80). The resultant O_2 deficit requires ATP to be supplied anaerobically, thereby increasing metabolic perturbations (lower pH, increased intracellular and systemic lactate concentration, increased ADP concentration, etc.) and contributing to fatigue. $\dot{V}O_{2p}$ on-kinetics during moderate intensity exercise has three phases: phase I, also known as the time delay (TD), is a rapid increase in $\dot{V}O_{2p}$ related to a sudden increase in cardiac output and pulmonary perfusion (not related to skeletal muscle O_2 uptake); phase I amplitude is greatest when beginning from rest (15, 56, 80, 103). Phase II, also known as the primary or fundamental component, reflects the exponential rise in skeletal muscle O_2 uptake (35, 60, 80, 82, 99). Phase III, also known as steady-state when the target exercise intensity is below the ventilatory threshold, occurs when O_2 demand is met by aerobic metabolism (15). Phase II $\dot{V}O_{2p}$ on-kinetics are determined by the following monoexponential equation: $Y\,(t) = Y(b) + A \bullet [1 - e-(t - TD)/\tau]$, where Y (t) is the $\dot{V}O_{2p}$ (ml/kg/min) at time point (t) from exercise onset, Y (b) is the $\dot{V}O_{2p}$ (ml/kg/min) at the pre-exercise transition baseline, A is the change in amplitude (ml/kg/min) of $\dot{V}O_{2p}$ at the end of the TD to

steady-state exercise $\dot{V}O_{2p}$, tau (τ) is the time constant (s) and represents the rate of $\dot{V}O_{2p}$ increase in phase II, and TD (s) represents the time delay that is phase I.

Speeding phase II $\dot{V}O_{2p}$ on-kinetics would decrease the O_2 deficit, ultimately decreasing fatigue and increasing exercise tolerance (80). The speed of phase II pulmonary $\dot{V}O_{2p}$ on-kinetics is assessed primarily via a time constant "tau" (τ) during square-wave transitions and reflects muscle $\dot{V}O_2$ (60, 99). The time constant is the time from rest to 63% of the steady-state $\dot{V}O_{2p}$ (80). Phase II $\dot{V}O_{2p}$ on-kinetics are quite fast in young healthy adults ($\tau \sim$ 19-28 s (23, 69, 89)), slower in older healthy adults ($\tau \sim$ 38-50 s (23, 81, 89)), and very slow in individuals with HFrEF ($\tau \sim$ 49-80 s (15, 49)). In diseased states, phase II $\dot{V}O_{2p}$ on-kinetics may have great value in prognosis, more so than above-mentioned peak $\dot{V}O_{2p}$ (80). Indeed, a faster mean response time (τ + TD) in $\dot{V}O_{2p}$ was associated with increased survival rate in patients with HFrEF (88). To the best of our knowledge, phase II $\dot{V}O_{2p}$ on-kinetics during the on-transition to exercise have not been assessed in HFpEF. Using phase II $\dot{V}O_{2p}$ on-kinetics for prognosis in HF has several advantages over peak $\dot{V}O_{2p}$; for example, kinetics testing is less time consuming, less demanding, and less dependent on motivation, all of which are more attractive to patients with HF (88).

1.8 Priming Effects of Heavy Intensity Exercise on $\dot{V}O_{2p}$ On-Kinetics

The characteristic $\dot{V}O_{2p}$ on-kinetics response during exercise differs greatly between the three common exercise intensity domains: moderate, heavy, and severe (80). The moderate intensity domain represents exercise below the individual's ventilatory threshold. The ventilatory threshold, or gas exchange threshold, is the intensity at which the ratio between carbon dioxide production ($\dot{V}CO_{2p}$) and $\dot{V}O_{2p}$ increases, indicating that the rate of $\dot{V}CO_{2p}$ increase surpasses the rate of $\dot{V}O_{2p}$ increase (4). It is also the point at which ventilation increases at a faster rate than $\dot{V}O_{2p}$ (4). The heavy intensity exercise domain includes exercise above the individual's ventilatory threshold and below the respiratory compensation point. The respiratory compensation point occurs when ventilation, previously closely coupled to $\dot{V}CO_{2p}$, rises more rapidly than $\dot{V}CO_{2p}$ (4). The severe intensity domain is exercise above the respiratory compensation point, and exercise in this domain is soon limited by extreme fatigue (80).

During a square-wave transition from rest or easy warm-up to moderate intensity exercise, phase II $\dot{V}O_{2p}$ on-kinetics rises exponentially and rapidly achieves steady-state or plateau (Fig 1-1) (80). During a square-wave transition to heavy intensity exercise, $\dot{V}O_{2p}$ on-kinetics rises

exponentially at first, but the achievement of steady-state is delayed by an additional, less rapid increase in $\dot{V}O_{2p}$ called the slow component (Fig 1-1) (80). This slow component is exaggerated when transitioning to severe intensity exercise, and steady-state is not achieved at all as exercise is rapidly limited by extreme fatigue (Fig 1-1) (80).

Prior heavy exercise (or "priming" exercise) can speed $\dot{V}O_{2p}$ on-kinetics during subsequent heavy exercise in young healthy adults (34). A "priming" effect occurs when the $\dot{V}O_{2p}$ on-kinetics are faster (80). Possible mechanisms behind this speeding or "priming" effect have been reviewed by Poole & Jones (80), and are dichotomized as falling within either the O_2 delivery or O_2 utilization domain. Beginning with O_2 delivery, an increase in muscle temperature from the heightened energy output and a decrease in muscle and blood pH from the elevated anaerobic lactic metabolism may contribute to speeding of $\dot{V}O_{2p}$ on-kinetics, both of which cause a rightward shift in the oxygen dissociation curve (80). However, a rise in temperature alone does not seem to account for priming in $\dot{V}O_{2p}$ on-kinetics as studies using passive warming of the primary muscle groups or core temperature had no effect on $\dot{V}O_{2p}$ on-kinetics (80). Acidosis-mediated increases in muscle perfusion remain plausible explanations and increased muscle oxygenation has been observed following prior heavy exercise (80). Increases in heart rate, cardiac output, and muscle blood flow also result from prior heavy-intensity exercise (80), however these seem less likely explanations as speeded bulk O_2 delivery is generally not associated with speeded phase II $\dot{V}O_{2p}$ on-kinetics (80). In terms of O_2 utilization-dependent effects, elevated activity of the pyruvate dehydrogenase complex coupled with speeding of phase II $\dot{V}O_{2p}$ on-kinetics has been demonstrated following priming exercise in young adults (36, 80). Finally, it is entirely plausible that prior exercise speeds $\dot{V}O_{2p}$ on-kinetics via a coupled effect of increased muscle O_2 delivery and increase O_2 utilization (80). In any case, the mechanism(s) behind priming exercise have yet to be fully understood.

In addition to priming $\dot{V}O_{2p}$ on-kinetics, Rossiter et al. (83) measured phosphocreatine kinetics in the muscle via magnetic resonance spectroscopy in young adults and observed priming effects as the decrement in phosphocreatine during exercise was attenuated, indicating increased oxidative phosphorylation. Since then, several studies, originating from Scheuermann et al. (89), have demonstrated that phase II $\dot{V}O_{2p}$ on-kinetics can be accelerated in older adults during a transition to moderate intensity exercise as a result of prior heavy exercise (24, 68, 81, 89). With

prior heavy intensity exercise, priming effects at the onset of subsequent moderate exercise were not observed in young healthy adults (34, 89), until recently, although it was a small effect and seen only in those with slower baseline phase II $\dot{V}O_{2p}$ on-kinetics (68). It is hypothesized that the priming effects are a result of increased O_2 delivery and muscle perfusion when moderate exercise is performed shortly (~6 minutes) after heavy exercise (24, 34), indicating that muscle O_2 perfusion may be a more prominent rate limitation during moderate exercise in older adults compared to their younger counterparts. The distribution of blood flow within the exercising limb or muscle, not bulk conduit blood flow, may be a primary cause for slower phase II $\dot{V}O_{2p}$ on-kinetics in older adults, and speeding of $\dot{V}O_{2p}$ on-kinetics following priming exercise (22–25, 30, 34). This effect may occur via vasodilation, by increased sheer stress on the arterial wall (15). Indeed, Murias et al. (68) reported that in young adults, a heavy exercise "warm-up" improves local muscle O_2 perfusion during subsequent moderate exercise, possibly contributing to the slight speeding of phase II $\dot{V}O_{2p}$ on-kinetics. De Roia et al. (81) provided evidence that the prior bout of heavy intensity exercise allowed enhanced matching of local muscle O_2 delivery to O_2 utilization in older adults, thus speeding phase II $\dot{V}O_{2p}$ on-kinetics in the subsequent moderate intensity exercise bout. The effect of a heavy intensity "warm-up" on $\dot{V}O_{2p}$ on-kinetics has not been studied in any HF population. However, Bowen et al. (15) performed a moderate-intensity "warm-up" protocol (6 min on, 6 min recovery, 6 min on) in HFrEF using the same moderate-intensity exercise in lieu of heavy intensity priming. The second bout of moderate intensity exercise was reported to be significantly faster than the first bout ($\tau\dot{V}O_{2p}$ 41 ± 16 s vs. 49 ± 19 s, respectively), suggesting muscle function (*e.g.,* potentially enhanced O_2 extraction or decreased oxidative phosphorylation inertia) can be improved by an acute increase in local muscle O_2 perfusion in HFrEF (which is also supported by the simultaneously collected muscle deoxygenation data from near-infrared spectroscopy (NIRS)) (15). Likewise, Sperandio et al. (95) reported slower phase II $\dot{V}O_{2p}$ on-kinetics in HFrEF compared to controls, yet an exaggerated O_2 delivery response (*i.e.,* overshoot in deoxygenated hemoglobin at exercise onset). As mentioned above, individuals with HFpEF exhibit muscle dysfunction and reduced microvascular endothelial function (41, 43). The question remains if these patients would benefit from increased muscle blood flow, or if their exercise limitation is within muscle mitochondrial O_2 utilization itself. This information would improve upon our poor understanding of HFpEF pathophysiology, and thus may aid in treatment development to improve exercise intolerance and declining survival rates in this population.

1.9 Role of Muscle Oxygenation on $\dot{V}O_{2p}$ On-Kinetics

Muscle oxygenation, determined by the tissue oxygenation index (TOI), can be measured using NIRS and has been successfully measured in HF patients in several recent studies (15, 72, 100), and has demonstrated test-retest reliability (72). Bowen et al. (15) used a monoexponential curve fit to analyse TOI on-kinetics in HFrEF patients. The authors demonstrated that prior moderate intensity exercise (a surrogate for priming exercise in HFrEF) increased resting baseline TOI, had a smaller compensatory "overshoot" in the TOI response (analogous to classic deoxygenation overshoot), increased end-exercise steady state TOI, but slowed τTOI. These data were interpreted as low resting skeletal muscle oxygenation in HFrEF, and that prior moderate intensity "priming" exercise caused an increase in muscle oxygenation throughout the subsequent exercise on-transient in healthier patients (15). As well, the increased muscle oxygenation was associated with priming of phase II $\dot{V}O_{2p}$ on-kinetics in these patients, while those patients with markedly slower $\tau\dot{V}O_{2p}$ exhibited slowed muscle oxygenation on-kinetics (15). The latter response demonstrates a skeletal muscle limitation independent of muscle O_2 perfusion in HFrEF, that which may be regulated by disease severity (15). Spee et al. (94) also reported tissue saturation index (TSI; also known as TOI) in HFrEF patients during moderate intensity exercise. A similar TSI profile was exhibited in these patients that suggested an impairment in O_2 delivery rather than utilization: at exercise onset, TSI drops rapidly below baseline to a minimum value and transiently increases until end-exercise (although usually not surpassing baseline levels) (94).

1.10 Cardiac Output and Heart Rate On-Kinetics

Cardiac output on-kinetics slow with age such that an increase in cardiac output for a given $\dot{V}O_{2p}$ is attenuated compared to young healthy controls (25). Using continuous non-invasive blood pressure (NIBP) measurements, an algorithm (ModelFlow method) exists to estimate beat-by-beat cardiac output. ModelFlow cardiac output (cardiac $output_{MF}$) on-kinetics during moderate intensity exercise are typically twice as fast as phase II $\dot{V}O_{2p}$ on-kinetics in young healthy men, and can account for the $\dot{V}O_{2p}$ phase I amplitude or TD (56). Cardiac $output_{MF}$ on-kinetics are typically not improved following heavy intensity exercise in healthy older adults (81). In HFrEF patients (EF <35%), cardiac output on-kinetics were markedly slower compared to myocardial infarction patients with EF above 35% (63 ± 13 vs. 50 ± 12 s) (55). Cardiac output on-kinetics (radial pulse contour analysis) were estimated in HFrEF during moderate-intensity exercise and yielded an

average time constant of 62 ± 25 s, with no comparison to healthy controls (49). The authors did however couple the cardiac output data with $\dot{V}O_{2p}$ on-kinetics, and determined that, due to the sameness between the two signals, $\dot{V}O_{2p}$ was likely limited by convective O_2 delivery and not utilization in their HFrEF patients (49). Indeed, Spee et al. (94) reported a significant correlation between cardiac output and $\dot{V}O_{2p}$ on-kinetics in HFrEF patients. Cardiac output τ and TOI (minimum value) were correlated in patients with relatively slow cardiac output on-kinetics, suggesting central hemodynamics (cardiac output) may limit muscle oxygenation (TOI) during moderate intensity exercise in HFrEF, ultimately limiting $\dot{V}O_{2p}$ on-kinetics (94). Further, the authors confirmed their hypothesis that in HFrEF patients with delayed cardiac output increase, there is a compensatory increase in O_2 extraction at the active muscle (94). To our knowledge, the effect of priming on cardiac output on-kinetics has not been assessed in a HF population, but such information would enhance our understanding of key limitations to $\dot{V}O_{2p}$ on-kinetics and thus exercise tolerance in the HF population.

Heart rate on-kinetics are classically slower in older versus younger adults (5, 21, 23, 27, 89), attributed to, at least in part, slowing of vagal withdrawal with age and decreased beta-adrenergic responsiveness (90). In older adults, heart rate on-kinetics should approximate cardiac output on-kinetics during moderate intensity exercise (25). Priming exercise causes heart rate on-kinetics to adapt differently in young and older adults (24); while both groups tend to increase pre-transition heart rate and decrease heart rate amplitude, τ slows in young adults only following priming exercise, equalizing the post-priming heart rate τ between young and older adults. These observations are interpreted as suggesting a similar increase in muscle O_2 delivery (assumed by the elevated baseline heart rate) following priming exercise in both young and older adults, but no speeding of heart rate adaptation. In HFrEF patients, heart rate on-kinetics are slower than $\dot{V}O_{2p}$ on-kinetics without priming and were not speeded by prior moderate intensity ("priming" surrogate) exercise (15), suggesting that cardiac output kinetics had minimal impact on limiting $\dot{V}O_{2p}$ on-kinetics in those HFrEF patients.

1.11 TPR On-Kinetics

In young healthy men, TPR decreased rapidly at the onset of moderate intensity exercise, and remained markedly reduced for the duration of the 5-minute exercise protocol, achieving steady-state within 1-2 minutes of start. (56). De Roia et al. (81) estimated TPR on-kinetics in

healthy older adults before and after priming exercise. Before priming exercise, τTPR (effective τ; *i.e.,* τ + TD) was exceptionally faster than $\dot{V}O_{2p}$ effective τ (9 ± 6 vs. 39 ± 7 s). After priming, TPR effective τ was not significantly speeded (8 ± 4 s), but $\dot{V}O_{2p}$ effective τ decreased (36 ± 4 s). These results indicate that systemic vascular resistance (*i.e.,* TPR) does not hinder $\dot{V}O_{2p}$ on-kinetics in older adults, and priming exercise does not enhance TPR on-kinetics. Tomczak et al. (98) reported systemic vascular resistance in HFrEF patients at rest, during steady-state moderate intensity exercise, and at peak exercise, all before and after cardiac resynchronization therapy. Resting systemic vascular resistance was not significantly reduced, but both steady-state exercise and peak exercise systemic vascular resistance values were markedly reduced following therapy, indicating a coupling between the ventricular and vascular function (98). While TPR on-kinetics have not been studied in HFpEF (to our knowledge), HFpEF patients tend to have higher resting TPR and a lower reduction in TPR during exercise compared to controls with non-cardiac related dyspnea (13).

1.12 Purpose, Outcomes, and Hypotheses

Cardiopulmonary dynamics, particularly $\dot{V}O_{2p}$, provide a holistic understanding of key limitations to exercise; better understanding of peripheral limitations to $\dot{V}O_{2p}$ in HFpEF is required for disease characterization and treatment. Phase II $\dot{V}O_{2p}$ on-kinetics illustrate the efficiency of exercising muscle metabolism; by studying the physiological components affecting $\dot{V}O_{2p}$ (globally, a factor may be considered either O_2 delivery (convective or diffusive) or O_2 utilization), one may determine the rate-limiting factor for $\dot{V}O_{2p}$ on-kinetics, which, in the diseased state, should then be targeted with treatment to improve exercise tolerance and quality of life. The rate-limiting factor for $\dot{V}O_{2p}$ varies between populations; active young adults are typically limited by O_2 utilization; older adults tend to be limited by microvascular muscle O_2 delivery; and HFrEF patients are commonly limited by either bulk O_2 delivery (*i.e.,* slow cardiac output and/or vascular on-kinetics) or microvascular muscle O_2 delivery, or a combination of both. The rate-limiting factor for $\dot{V}O_{2p}$ in HFpEF is still debated and cardiopulmonary dynamics and effects of priming in these patients have not been investigated.

1.12.1 Purpose

The objectives of this study were twofold; the primary objective was to compare phase II $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF to their healthy high-fit and low-fit counterparts. The

secondary objective was to determine if prior "priming" heavy exercise improves phase II $\dot{V}O_{2p}$ on-kinetics during moderate-intensity exercise in HFpEF compared to high-fit and low-fit age-matched controls. Priming exercise is thought to increase bulk and/or microvascular O_2 delivery to exercising muscles. If priming exercise were to speed phase II $\dot{V}O_{2p}$ on-kinetics in HFpEF, this would suggest that local muscle O_2 delivery was a potential key contributing rate-limiting factor in this disease. Measurement of cardiac, vascular and microvascular function (*i.e.,* factors that determine O_2 delivery) will enable further understanding of how priming exercise may/may not alter phase II $\dot{V}O_{2p}$ in HFpEF compared to controls. Given emerging evidence that peripheral factors may be the primary cause for impaired exercise capacity in HFpEF, our muscle oxygenation (TOI) measurement may be key in providing insight into the microvascular role of aerobic impairment in HFpEF. Specifically, if TOI were to demonstrate an "overshoot" following an initial reduction at exercise onset, this would suggest that diffusive O_2 delivery may be limiting phase II $\dot{V}O_{2p}$ on-kinetics. If there were to be no subsequent overshoot in TOI, this would suggest that O_2 utilization in the muscle cell may limit phase II $\dot{V}O_{2p}$ on-kinetics. If the TOI response profiles are different between no priming and after priming, this would suggest a locus shift in the rate-limiting factor of phase II $\dot{V}O_{2p}$ on-kinetics.

1.12.2 Primary Outcome

The primary outcome was phase II $\dot{V}O_{2p}$ on-kinetics (τ) during the transition to moderate intensity exercise before and after heavy intensity ("priming") exercise.

1.12.3 Secondary Outcomes

Secondary outcomes were cardiac output τ, heart rate τ, and the remaining on-kinetics parameters (pre-transition baseline, TD, amplitude change, and steady-state) for $\dot{V}O_{2p}$, cardiac output, and heart rate, all during moderate intensity exercise before and after heavy intensity ("priming") exercise. Additional outcomes were time course changes of stroke volume, TPR, and TOI during moderate exercise before and after priming exercise.

1.12.4 Primary Hypotheses

1) We hypothesized that patients with HFpEF would have slower phase II $\dot{V}O_{2p}$ on-kinetics than their healthy high-fit and low-fit counterparts.

2) We hypothesized that prior heavy exercise would improve phase II $\dot{V}O_{2p}$ on-kinetics in the healthy high-fit and low-fit age-matched controls but not in the HFpEF group because of their intrinsic severe muscle dysfunction and reduced oxidative capacity.

1.12.5 Secondary Hypotheses

1) We hypothesized that HFpEF patients would have similar cardiac output and heart rate on-kinetics and stroke volume time course changes to high-fit and low-fit control subjects, slower and smaller TPR reduction compared to controls, and a smaller change in TOI after the onset of exercise compared to controls, indicating reduced O_2 extraction and thus utilization.

2) We hypothesized that in HFpEF patients, priming exercise would not improve cardiac output on-kinetics, heart rate on-kinetics, stroke volume time course changes, or TOI time course changes (as reflecting muscle oxygen extraction) but would speed and increase the reduction in TPR, and would lower TOI at exercise onset, indicating an increase in microvascular O_2 delivery. We also hypothesized the high-fit and low-fit control subjects would have no speeding of cardiac output on-kinetics, heart rate on-kinetics, or TPR time course changes as consistent with previous reports, no change in stroke volume time course changes as inferred from previous cardiac output data, but a more rapid reduction in TOI with a smaller transient increase following the initial reduction (indicative of increased O_2 delivery, thus transferring the rate-limiting step to O_2 utilization).

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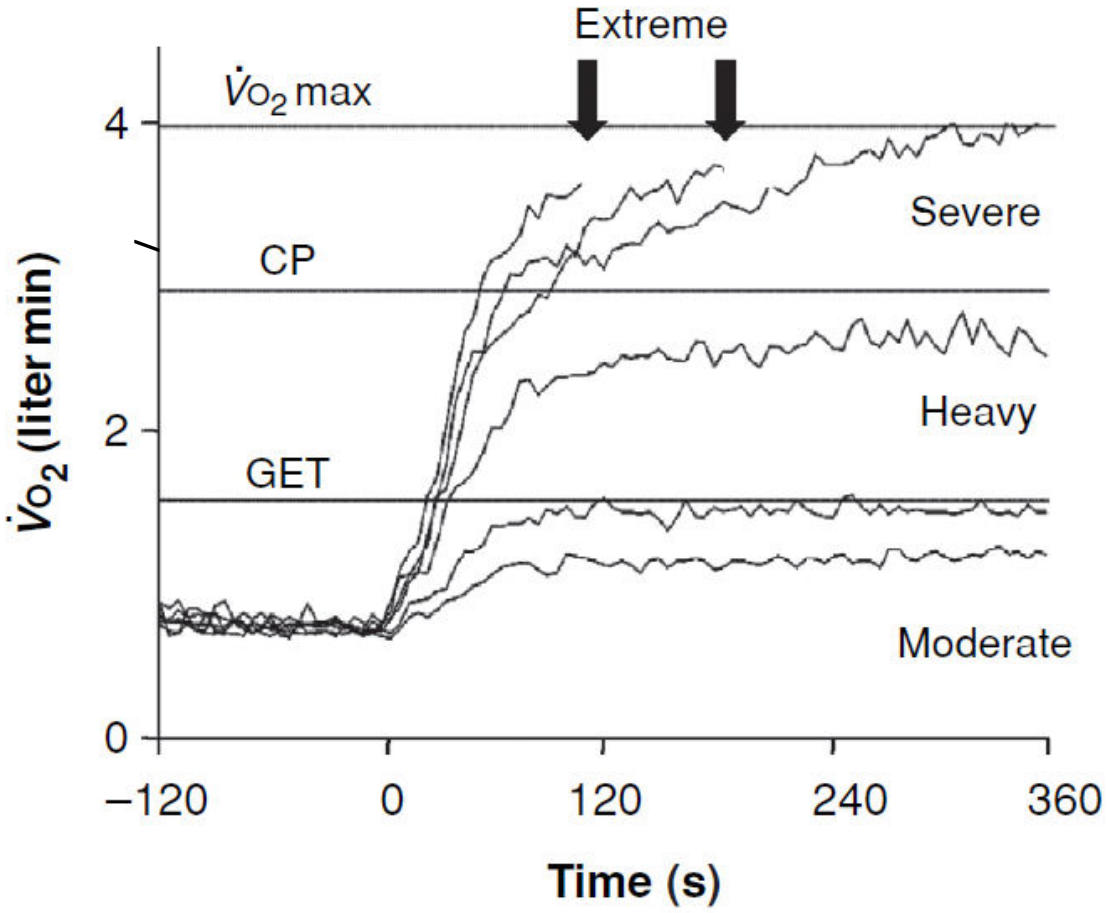

Fig 1-1. Exercise Intensity Domains. Figure from Poole & Jones (80) without permission. Briefly, the $\dot{V}O_2$ responses differ between moderate, heavy, and severe exercise intensities. Notably, a steady-state is rapidly achieved in moderate exercise, whereas heavy and severe exercise have a delay (termed the slow-component). Steady-state $\dot{V}O_2$ is reached sooner during heavy exercise vs. severe or extreme. Exercise at extreme exercise intensities are halted prior to reaching $\dot{V}O_2$ max due to extreme fatigue and therefore do not display a plateau. CP, critical power (the highest $\dot{V}O_2$ that can be sustained for a long period, above which is the "severe" intensity domain (80)); GET, gas-exchange threshold (otherwise known as the ventilatory threshold, above which is the "heavy" intensity domain until CP (80)); $\dot{V}O_2$, oxygen uptake; $\dot{V}O_2$ max, maximum oxygen uptake.

CHAPTER TWO: MANUSCRIPT

2.1 Introduction

The cardinal symptom of heart failure (HF), regardless of phenotype, is severe exercise intolerance (50), which is consequently associated with premature fatigue and reduced quality of life (19, 21). Related to exercise intolerance in patients with HF and preserved ejection fraction (HFpEF) is muscle dysfunction in the form of reduced oxidative enzymes (19, 32), reduced volume density and surface density (*i.e.,* fewer cristae) of mitochondria (19, 32), lower slow twitch to fast twitch muscle fiber ratio (19, 21), reduced muscle fiber capillary density (19, 21), and increased intermuscular adipose tissue (19, 21). Severe exercise intolerance can be objectively measured using peak pulmonary oxygen uptake (peak $\dot{V}O_{2p}$), which is ~40% lower in patients with HFpEF compared to their healthy counterparts (19). Indeed, peak $\dot{V}O_{2p}$ indexed to lean muscle mass remains lower in HFpEF patients compared to controls (18). Further, training-induced increases in peak $\dot{V}O_{2p}$, indexed to increases in percent lean leg mass, are attenuated in HFpEF compared to controls (18). The latter indicates impaired muscle quality in HFpEF (21). Arterial-venous O_2 content difference (AVO_2Diff; common index of O_2 extraction by active skeletal muscle tissue) has been reported to be the strongest independent predictor of peak $\dot{V}O_{2p}$ in HFpEF (17), and accounts for reduced peak $\dot{V}O_{2p}$ in HFpEF compared to controls in the absence of group differences in stroke volume or cardiac output (5). The predominant role of muscle dysfunction in HFpEF is further highlighted by training-related increases in peak $\dot{V}O_{2p}$ being attributed to increased AVO_2Diff, not cardiac morphology or neuroendocrine improvements (26). It is thus apparent that peripheral impairments significantly limit $\dot{V}O_{2p}$ in HFpEF; however, there is controversy on whether the primary limitation lies within microvascular O_2 delivery (perfusion or diffusion), or within O_2 utilization in the mitochondria, or a tandem between both. Given the prior reports of peripherally-mediated limitations to exercise in patients with HFpEF, it may follow that the rate of pulmonary O_2 uptake ($\dot{V}O_{2p}$ on-kinetics) would be impaired in patients with HFpEF – however, this has not been studied. Without this information, we are limited in our understanding of key factors truly limiting exercise tolerance in this population. Further, the role of deconditioning should be separated from disease-specific physiological impairments. Indeed, Mettauer et al. (31) found that patients with HF and reduced ejection fraction (HFrEF) had similarly reduced mitochondrial intrinsic oxidative capacity (via muscle biopsy) to sedentary

matched controls, while active controls exhibited far greater mitochondrial intrinsic oxidative capacity. However, despite the similarities in muscle oxidative capacity, whole body $\dot{V}O_{2p}$ was still greater in sedentary controls than HFrEF, suggesting potential delivery limitations in HFrEF that further exacerbate exercise impairment in addition to muscle dysfunction (31).

Prior heavy exercise ("priming"; above the ventilatory threshold) may increase O_2 delivery during subsequent moderate intensity exercise (below the ventilatory threshold), leaving $\dot{V}O_{2p}$ on-kinetics more-so under the control of oxidative metabolism in the mitochondria. In older adults, $\dot{V}O_{2p}$ on-kinetics are acutely improved in moderate exercise when closely preceded by priming heavy exercise, likely due to increased blood perfusion to the active muscles, thus maximizing convective O_2 delivery (9, 37). A decrease in muscle oxygenation (via tissue oxygenation index; TOI) measured by near-infrared spectroscopy (NIRS) may indicate increased local muscle diffusive O_2 delivery (7). Patients with HFrEF with relatively normal $\dot{V}O_{2p}$ on-kinetics exhibit increased muscle O_2 delivery after priming exercise, while HFrEF patients with abnormally slow $\dot{V}O_{2p}$ on-kinetics show a decrease in muscle O_2 delivery following priming (7). The latter suggests that as disease severity worsens, intrinsic muscle dysfunction limits O_2 uptake to a greater degree (7). As the primary peripheral limitation of $\dot{V}O_{2p}$ in HFpEF is unclear (microvascular O_2 delivery or O_2 utilization), priming exercise may maximize convective and/or diffusive O_2 delivery in HFpEF patients, thus revealing the possible role of O_2 utilization on limiting $\dot{V}O_{2p}$ on-kinetics in the HFpEF population. Further, $\dot{V}O_{2p}$ on-kinetics predicts survival better than peak $\dot{V}O_{2p}$ in HFrEF patients (39), therefore similar data on HFpEF should be obtained.

The objectives of this study were twofold; the primary objective was to compare $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF to healthy age-matched low-fit and high-fit controls. The secondary objective was to determine if prior "priming" heavy exercise improves $\dot{V}O_{2p}$ on-kinetics during moderate-intensity exercise in HFpEF compared to low-fit and high-fit controls. Our primary hypothesis was that patients with HFpEF would have slower $\dot{V}O_{2p}$ on-kinetics than their healthy low-fit and high-fit counterparts. Our secondary hypothesis was that prior heavy exercise would improve $\dot{V}O_{2p}$ on-kinetics in the healthy high-fit and low-fit controls but not in the HFpEF group, because of their intrinsic severe muscle dysfunction and reduced oxidative capacity. Such a finding would suggest that the primary limitation to aerobic exercise in HFpEF may be due to muscle O_2 utilization.

2.2 Methods

2.2.1 Subjects

The study subjects included 8 patients with HFpEF and 9 healthy age- and sex-matched controls, the latter of which were split into groups of 4 high-fit and 5 low-fit controls based on peak $\dot{V}O_{2p}$ score, similar to Mettauer et al. (31). The split between high-fit and low-fit controls facilitated the assessment of the effect of deconditioning in the absence of disease on exercise physiology (31). Subjects were clinically stable and able to perform cycle ergometry. Healthy controls required an EF > 50%, no systolic or diastolic dysfunction, and no known cardiovascular disease for study inclusion. A cardiologist adjudicated the HFpEF diagnosis for the patient group based on HF signs and symptoms, EF, and diastolic function (30). Exclusion criteria for all subjects included a pacemaker or implantable cardioverter-defibrillator, a change in medication prescription within the last 3 months, an inability to perform brief cycle ergometry, and respiratory disease or impairment. Patients with HFpEF were cleared to participate in the study by a cardiologist prior to study commencement and on each day of exercise testing. In addition, a cardiologist supervised peak $\dot{V}O_{2p}$ tests for HFpEF patients and control subjects and all square-wave exercise protocols for the HFpEF group. Written informed consent was obtained prior to study commencement and ethical approval for this study was obtained by the University of Saskatchewan Research Ethics Board.

2.2.2 Study Design

The study is a parallel group, quasi-experimental design. Subjects underwent the same six tests: a resting echocardiogram; a muscle density assessment; a peak $\dot{V}O_{2p}$ test; a moderate-intensity, control square-wave exercise protocol (MOD1); and an experimental square-wave exercise protocol (MOD2) involving a bout of heavy intensity exercise prior to a moderate intensity square-wave exercise protocol. Both MOD1 and MOD2 protocols were repeated three times each. Subjects reported to the laboratory after consuming only a light meal and abstaining from heavy exercise and consuming caffeine during the previous 12 hours before the test. Similar to Scheuermann et al. (40), and to condense visits to the laboratory, subjects performed one repetition of MOD1 followed by one repetition of MOD2 during the same visit, with a 30-min minimum break between the protocols, for a total of 2 exercise protocols per visit. All exercise protocols were performed on an upright, electronically braked cycle ergometer (Ergoline 800S,

SensorMedics, Yorba Linda, CA). The square-wave exercise protocols were similar to prior reports (36), but with a modification to the exercise duration and commencing exercise from rest (not an exercise 20 W baseline, for example) in order to accommodate the severe exercise intolerance of patients with HFpEF. Our procedure also served to maximize the amplitude of the $\dot{V}O_{2p}$ response to square-wave exercise so as to improve the confidence of our $\dot{V}O_{2p}$ kinetic parameter estimates (28). We have previously employed similar protocol modifications in patients with HF (45), as have others (7).

2.2.3 Echocardiography

A resting echocardiogram was performed on the first day of testing by the same trained sonographer. Parameters collected included end-diastolic volume, end-systolic volume, stroke volume, ejection fraction (EF), left atrial volume index, left atrial diameter, left ventricular early diastolic filling measured by early mitral inflow velocity (E), left ventricular late diastolic filling measured by late mitral inflow velocity (A), E/A ratio (an index of diastolic filling and ventricle recoil), myocardial diastolic motion velocity (e'), estimated left ventricular filling pressure (E/e'), left ventricular mass index, posterior wall thickness, and left ventricular diastolic score (grade I, II, or III).

2.2.4 Peak Exercise Testing

A peak exercise test to volitional fatigue was completed following the echocardiogram. Subjects sat quietly on the electronically-braked cycle ergometer (Ergoline 800S, SensorMedics, Yorba Linda, CA) for 5-min to obtain resting baseline data before the test. There was no warm-up in either group due to anticipation of early fatigue in the HFpEF patients. HFpEF patients pedaled at 60 rpm during a 10 W/min ramp protocol until exhaustion or failure to maintain 50 rpm. Control subjects completed the same peak exercise protocol but at 20-25 W/min, depending on their estimated fitness. Subjects were provided with standard verbal checkups at regular intervals by the investigator. A 12-lead electrocardiogram monitored heart rate and brachial blood pressure (Tango+ Stress Blood Pressure, SunTech Medical Inc., Morrisville, NC, USA) was taken before and after the test. Breath-by-breath gas exchange and ventilation were measured. Peak $\dot{V}O_{2p}$ and corresponding gas exchange and ventilation parameters were determined as the highest 30-s values within the last 1-min of exercise. Data from this test were used to determine each subject's work rates approximating 90% ventilatory threshold (moderate-intensity) and the half-way point

between the ventilatory threshold and peak exercise (Δ50%, heavy-intensity) and were subsequently used for the MOD1 and MOD2 square-wave protocols. The ventilatory threshold was identified as the $\dot{V}O_{2p}$ at which CO_2 production ($\dot{V}CO_{2p}$) increased at a greater rate than the increase in $\dot{V}O_{2p}$ described by Beaver et al. (2). This point coincides with a rise in the minute ventilation/$\dot{V}O_{2p}$ ratio while the minute ventilation/CO_2 ratio remains stable. The peak test also served as medical screening with a cardiologist present and monitoring the 12-lead ECG.

2.2.5 Moderate-Intensity Exercise $\dot{V}O_{2p}$ On-Kinetics Protocol (MOD1)

The control square-wave exercise protocol (MOD1; Fig 2-1) began with subjects sitting quietly on the electronically-braked cycle ergometer for 5-min followed by verbal instruction to begin pedaling (at 60 rpm) for 4-min of moderate intensity exercise. Subjects were specifically coached on obtaining a pedaling rate of 60 rpm as fast as possible and on strictly maintaining the target pedaling rate. Following exercise, subjects were instructed to stop and sit quietly for 5-min of recovery (data not reported here). Brachial blood pressure was measured before and after MOD1.

2.2.6 Heavy-Intensity "Priming" Exercise on Subsequent Moderate-Intensity Exercise $\dot{V}O_{2p}$ On-Kinetics Protocol (MOD2)

Similar to MOD1, the experimental square-wave exercise protocol (MOD2; Fig 2-1) began with subjects sitting quietly on the electronically-braked cycle ergometer for 5-min followed by verbal instruction to begin pedaling (60 rpm) for 2-min of heavy intensity exercise. At 2-min, subjects were instructed to stop and sit quietly for 5-min of recovery (data not reported here) until verbal instruction to resume pedaling (60 rpm) for 4-min of moderate intensity exercise. Subjects were specifically coached on obtaining a pedaling rate of 60 rpm as fast as possible and on strictly maintaining the target pedaling rate. Following exercise, subjects were instructed to stop and sit quietly for 5-min of recovery (data not reported here). Brachial blood pressure was measured before and after MOD2.

2.2.7 Outcome Measurements

The primary outcome was phase II $\dot{V}O_{2p}$ on-kinetics during the transition from rest to moderate exercise, as measured by breath-by-breath gas exchange analysis at the mouth. Secondary outcomes included continuous measurement of heart rate, model flow stroke volume

(stroke volume$_{MF}$), cardiac output (from heart rate and stroke volume$_{MF}$), mean arterial pressure (MAP), total peripheral resistance (TPR; from MAP and cardiac output), and TOI (NIRS).

2.2.8 $\dot{V}O_{2p}$

$\dot{V}O_{2p}$ was measured using breath-by-breath gas exchange analysis (SensorMedics Vmax Encore, VIASYS Healthcare Respiratory Technologies, Yorba Linda, CA). The metabolic cart sampled continuously to yield breath-by-breath data, and allowed real-time monitoring of $\dot{V}O_{2p}$, $\dot{V}CO_{2p}$, and respiratory exchange ratio. Prior to each test, the gas analysing system was calibrated using known gas concentrations of O_2 (16%) and carbon dioxide (4%), and flow volume was calibrated using a 3L syringe across a range of expected breathing frequencies.

2.2.9 Heart Rate

Beat-by-beat heart rate was measured continuously throughout each session using a 12-lead ECG during the $\dot{V}O_{2p}$ peak test and 3-lead ECG for both MOD1 and MOD2 protocols (Bio Amp, ADInstruments, New South Wales, Australia) using a lead II configuration.

2.2.10 Stroke Volume$_{MF}$ and Cardiac Output$_{MF}$

Stoke volume$_{MF}$ was estimated from blood pressure waveforms recorded from a finger plethysmograph (Human NIBP, ADInstruments, New South Wales, Australia) (22). The finger cuff was instrumented on a middle phalanx of the left hand. The left arm and hand was secured on a custom padded table adjacent to the cycle ergometer, allowing the subject to maintain a restful and still position of their NIBP-instrumented arm. The model flow approach for stroke volume estimation has been previously described (48) and used in a similar exercise protocol (37). Briefly, the model uses beat-by-beat MAP, age, sex, and body surface area to estimate stroke volume. We understand that absolute stroke volume values may not be reliable using the model flow estimation as this method assumes aortic compliance, which in any given individual may differ substantially from the algorithm. As such, we used this method to account for relative changes from baseline, in lieu of not having an echocardiographic calibration value. We calibrated the NIBP system using resting blood pressure values obtained with an automated stress blood pressure system (Tango+ Stress Blood Pressure, SunTech Medical Inc., Morrisville, NC, USA). Beat-by-beat cardiac output$_{MF}$ was calculated from time-aligned stroke volume$_{MF}$ and heart rate.

2.2.11 MAP and TPR

Beat-by-beat MAP was measured and monitored real-time during testing using finger plethysmography (Human NIBP, ADInstruments, New South Wales, Australia) (22). Brachial blood pressure of the right arm was taken before and after the protocols for offline calibration of the NIBP signal. Beat-by-beat TPR was estimated from time-aligned MAP and calculated cardiac output using the following equation: $\text{TPR} = 80 \bullet (\text{MAP} - \text{mean right atrial pressure})/\text{cardiac output}$, where mean right atrial pressure was assumed to be zero. TPR was expressed in relative units of force/second, dyn·s/cm^5.

2.2.12 Hemoglobin On-Kinetics

Continuous-wave near-infrared spectroscopy (NIRO-200NX, Hamamatsu Photonics K.K., Hamamatsu City, Shizuoka Pref., Japan) was used to assess the hemoglobin O_2 content of the right vastus lateralis during cycling. This technique is based on the modified Lambert-Beer law; the proximal diode emits light into the tissue at three wavelengths (735, 810, and 850 nm), which are absorbed differently by hemoglobin depending on the O_2 content. The second diode measures the returning light wavelengths, and the amount of light absorbed by HHb and O_2Hb are separately calculated by the NIRS system (incorporating all three wavelengths for both HHb and O_2Hb). The NIRS signals represent the average O_2 saturation of hemoglobin in the directly underlying vascular bed (small arteries, arterioles, capillaries, venules, and small veins) and of myoglobin in the muscle fibers (15). The greatest contribution to the NIRS signals are capillaries (15). Five signals are gained from this measure: HHb, O_2Hb, total hemoglobin (CHb), TOI, and normalized total hemoglobin index (nTHI). All five signals are relative to an initial value set equal to zero, determined during a period of rest with no bodily movement. A pair of non-stick electrodes placed on the skin surface of the vastus lateralis of the right leg recorded signals of O_2 content in the muscle and was monitored live on a computer screen during testing. To maintain measure consistency between testing sessions, anthropometric measures and anatomical landmarks were used to ensure consistent electrode placement. The light emitting diodes were secured to the skin surface using adhesive tape. The inter-diode surface distance was consistently 5 cm, as ensured by a NIRO-200 black diode holder. As a general rule, the penetration depth of the NIR light is half that of the inter-diode distance, resulting in a relatively small and superficial volume of skeletal muscle tissue being assessed (15). A black cloth blocked room light from penetrating the

measurement area, and an elastic bandage held everything securely in place. This technique was successfully used in HF patients to assess muscle O_2 delivery at rest, during the progressive transition to steady-state moderate intensity exercise (41), maximal exercise, and during suprasystolic cuff ischemia to the muscle in question (49). Prior to each test, the NIRS system ran for 2-5 minutes before commencing baseline recordings to obtain a steady signal, and all channels were zeroed immediately prior to commencing the 5-minute resting baseline.

TOI and nTHI represent the O_2 saturation of tissue hemoglobin and the relative concentration of total tissue hemoglobin, respectively (35). They are calculated as TOI = $\Delta O_2Hb/\Delta CHb$ and nTHI = $\Delta CHb(t)/\Delta CHb(0)$, where $\Delta CHb(t)$ is the ΔCHb at the timepoint in question and $\Delta CHb(0)$ is the ΔCHb immediately following system zeroing. Thus, nTHI represents the ratio of the current value to the initial value of total hemoglobin.

At exercise onset, we observed a sudden and substantial reduction in both HHb and O_2Hb in all subjects, with a large portion of subjects' HHb remaining below baseline. Similar observations have been reported using a NIRO200 system (7), whose authors opted to use TOI as it was far more reproducible and allowed confidence in interpretation over the HHb signal, which was likely more sensitive to changes in arterial or venous capacitance (38, 43). We therefore opted to use the same variable (TOI) for hemoglobin data analysis.

2.2.13 Peripheral Quantitative Computed Tomography

For demographic purposes, subjects underwent imaging of the right plantar flexors using an XCT 2000 peripheral quantitative computed tomography (pQCT) device (Stratec Medizintechnik GmbH, Pforzheim, Germany). The scanner was factory calibrated against the European Forearm Phantom for a single energy. For the present investigation, the pQCT scanner measured subcutaneous adipose and lean tissue with hydroxyapatite equivalent volumetric densities of 0 mg/cm^3 and 60 mg/cm^3, respectively (14). The same technician performed all scans and visually inspected images for movement artifact, and repeated the scan if required. Cross-sectional scans were taken at the 38% and 66% sites of the tibia (*i.e.,* measured from the medial malleolus of the tibia to the medial condyle). The 66% site represents the preferential site to evaluate plantar flexor lean tissue. However, the scanner can only fit a calf diameter of 40 cm; due to some subjects having larger leg mass, a scan at 38% (naturally smaller diameter) was also taken to ensure each subject had muscle quality and subcutaneous adipose data to report.

2.2.14 Data Acquisition and Processing

Absolute $\dot{V}O_{2p}$ was exported from the collection system as a text file in breath-by-breath format. Heart rate, blood pressure, and TOI were recorded and integrated through a data acquisition hardware system that sampled at 1 kHz/s (Powerlab 16/30, ADInstruments, New South Wales, Australia) and was analyzed offline using compatible software (LabChart 7.0, ADInstruments, New South Wales, Australia). The NIBP signal was calibrated in LabChart to yield SBP, MAP, and DBP using resting brachial blood pressure values (Tango+ Stress Blood Pressure, SunTech Medical Inc., Morrisville, NC, USA). Premature beats were removed from LabChart data for analyses. Heart rate, MAP, and TOI data were exported from LabChart as a text file. Using the exported MAP signal, beat-by-beat stroke $volume_{MF}$ analysis (ModelFlow method (48)) was conducted offline with an emulator program (DOSBox x86, version 0.74, DOSBox Inc.) in order to run the BeatScope® Easy software (FMS, Finapres Medical Systems BV, Arnhem, The Netherlands). The resulting beat-by-beat stroke $volume_{MF}$ was multiplied by the ECG-recorded heart rate to yield cardiac $output_{MF}$.

pQCT data were analyzed with the open source program BoneJ (version 1.3.11) and has been shown to be precise in our laboratory (14) for muscle area, density, and subcutaneous adipose tissue. The program's analysis of soft tissue uses a 7 × 7 median filter to reduce motion artifact and uses tissue density thresholds (selected by user) to binarize the image (14). The thresholds used for muscle (41 to 139 mg/cm^3) and subcutaneous adipose (-40 to 40 mg/cm^3) were consistent with those validated (14). Muscle quality was assessed using muscle density (mg/cm^3, *i.e.,* the denser, the less inter- and intramuscular fat, therefore, the higher the quality). Note that muscle area included intermuscular adipose tissue, and muscle density was calculated as muscle content (mg/cm) divided by muscle area (cm^2) (14). Subcutaneous adipose tissue was determined by the total limb area minus the area of tissues greater than the subcutaneous adipose-muscle boundary (14).

2.2.15 Curve Fitting

A mono-exponential curve fit was used for determining $\dot{V}O_{2p}$ on-kinetics. First, abhorrent breaths were filtered from the breath-by-breath data. All three repetitions were linearly interpolated to 1-s intervals, time aligned, and averaged to 5-s time bins. The repetitions were then superimposed to yield a single averaged signal per subject. This single dataset was curve-fitted

using the analysis software Origin (OriginLab Corporation, Northampton, Massachusetts, USA) with the following equation: $Y\ (t)\ =\ Y(b)\ +\ A\ \bullet\ [1 - e-(t - TD)/\tau]$, where Y (t) was the $\dot{V}O_{2p}$ (ml/kg/min) at time point (t) from exercise onset, Y (b) was the $\dot{V}O_{2p}$ (ml/kg/min) at baseline, A was the change in amplitude (ml/kg/min) of $\dot{V}O_{2p}$ at the end of the time delay to steady-state exercise $\dot{V}O_{2p}$, τ (or tau) was the time constant (s) and represents the rate of $\dot{V}O_{2p}$ increase, and TD was the time delay (s) which represents the cardiodynamic phase: a brief pause between the start of exercise and the start of the exponential increase of $\dot{V}O_{2p}$, believed to be caused by the increase in pulmonary blood flow without a change in arterial or venous O_2 content (36). The speed of the cardiodynamic phase is likely due to the sudden increase in cardiac output, attributed to vagal withdrawal and active muscle pumping (36). For consistency, the window to commence curve-fitting was set at 25 s for each subject. This approach was similar to the optimal method (20s set TD) defined by Benson et al. (3) but was adapted to 25s as the dataset showed a consistently longer TD of at least 25s. The best fit was determined by minimizing the residual sum of squares. The same process was repeated for heart rate and cardiac output on-kinetics - however, curve fitting was commenced at exercise onset (time 0).

The variability and occasional non-exponential response in stroke volume$_{MF}$, TPR, and TOI did not facilitate reliable curve-fitting. Therefore, we opted to analyse the time course changes of these variables as delta values from a baseline (normalized to zero). Upon visual inspection of the data, three time points were chosen to best represent the nature of each physiological response: 15s after baseline (11-15s average) and 30s after baseline (26-30s average) to represent acute adjustments to exercise, and end-exercise (211-240s average) to represent the end-exercise value.

2.2.16 Statistical Analysis

Normality and homogeneity checks determined whether the data were normally distributed and had homogeneity of variance between the three groups. One-way ANOVAs assessed group differences in demographic data, including peak exercise and ventilatory threshold $\dot{V}O_{2p}$, heart rate, and work rate, echocardiographic data, and calf muscle and adipose data.

For $\dot{V}O_{2p}$, cardiac output$_{MF}$, and heart rate on-kinetics data, omnibus group × condition repeated measures ANOVAs (3 × 2) were conducted separately for each on-kinetics parameter (*i.e.,* τ, pre-transition baseline, total amplitude, and steady state). When significant effects were detected, one-way ANOVAs were conducted to assess group differences separately by MOD1 and

MOD2 with Student-Newman Keuls (SNK) post-hoc, while paired *t*-tests assessed changes from MOD1 to MOD2 within a group. Nonparametric equivalent tests (independent samples Kruskal-Wallis one-way ANOVA) were used where the assumption of homogeneity of variances was violated.

Omnibus group × time × condition repeated measures ANOVAs (3 × 4 × 2) were performed for Δstroke volume$_{MF}$, ΔTPR, and ΔTOI. Four timepoints (0, 15s, 30s, and end-exercise) were used within each condition. Where the assumption of sphericity was not met, the Greenhouse-Geisser correction value was used. When significant effects were detected, one-way repeated measures ANOVAs (4 timepoints), split by group assessed time-course changes within a group in either MOD1 or MOD2, one-way ANOVAs with SNK post-hoc were performed to assess group differences at a time point, and paired *t*-tests assessed changes from MOD1 to MOD2 within a group. Where Levene's test was significant, Kruskal-Wallis tests were employed.

The significance level was set a priori at $P<0.05$ for all analyses. Data analyses were conducted using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Data are presented as means ± standard deviation (SD) in tables and text, and means ± standard error of the mean (SEM) in figures.

2.3 Results

2.3.1 Subject Demographics

Groups were of similar age, height, weight, and resting blood pressure (Table 2-1). BMI tended to be greater in the HFpEF group, but was not significantly different (Table 2-1). Due to our control group median split, our high-fit group consisted of all male subjects, while the low-fit and HFpEF groups included 3 and 2 female subjects, respectively. Patient cardiac-related medications are listed in Table 2-1. All echocardiographic parameters passed Levene's test except E, A, and E/e' where Kruskal-Wallis was used, and are detailed in Table 2-1. Further, measurement of A and by extension E/A estimation could not be performed in two patients, and diastolic score could not be determined in a third patient, each due to difficulties with their respective assessments. There were no group differences in muscle density, muscle area, or subcutaneous adipose area at the 38% or 66% site of the leg (Table 2-1). Note that two HFpEF patients could not fit their leg far enough into the scanner to obtain a 66% site scan, so they were excluded from this analysis.

2.3.2 Peak Exercise Testing

Peak exercise and ventilatory threshold data are reported in Table 2-1. Notably, peak $\dot{V}O_{2p}$ was greater in high-fit controls compared to HFpEF and low-fit controls (P<0.001). Peak heart rate was lower in patients with HFpEF compared to both control groups (P=0.003), and HFpEF achieved a lower percent-predicted heart rate compared to both control groups (P=0.002). Ventilatory threshold heart rate was different between HFpEF (lower heart rate) and high-fit (higher heart rate) controls (P=0.032). Work rate at ventilatory threshold was different between all three groups; lowest in HFpEF and highest in high-fit controls (P=0.001).

2.3.3 $\dot{V}O_{2p}$ On-Transient Kinetics

The omnibus ANOVA for $\tau\dot{V}O_{2p}$ yielded significant main effects of priming (P=0.039) and group (P=0.008), but not for the condition × group interaction in phase II $\tau\dot{V}O_{2p}$ (P=0.334; Fig 2-2 and Table 2-2). SNK revealed that high-fit controls (25 ± 6 s) had faster phase II $\tau\dot{V}O_{2p}$ compared to HFpEF (45 ± 15 s) and low-fit controls (50 ± 19 s; P=0.008), pooled across conditions. $\tau\dot{V}O_{2p}$ was faster in MOD2 (37 ± 14 s) compared to MOD1 (47 ± 19 s), pooled across groups.

$\dot{V}O_{2p}$ baseline had a main effect of priming (P=0.001), but no effect of group (P=0.283) or condition × group interaction (P=0.715; Fig 2-2). Pooled across groups, $\dot{V}O_{2p}$ baseline was elevated after priming (0.344 ± 0.066 L/min) compared to MOD1 (0.308 ± 0.059 L/min).

Total $\dot{V}O_{2p}$ amplitude change had a main effect of group (P=0.003) but no effect of priming (P=0.920) or condition × group interaction (P=0.458; Fig 2-2 and Table 2-2). SNK revealed that high-fit controls (0.830 ± 0.199 L/min) had greater amplitude change compared to HFpEF (0.461 ± 0.128 L/min) and low-fit controls (0.575 ± 0.088 L/min; P=0.003), pooled across conditions.

Finally, steady-state $\dot{V}O_{2p}$ had a difference between groups (P=0.014), but no effect of priming (P=0.060), or condition × group interaction (P=0.396; Fig 2-2 and Table 2-2). SNK indicated that high-fit controls (1.181 ± 0.237 L/min) had an elevated steady-state $\dot{V}O_{2p}$ compared to HFpEF (0.797 ± 0.183 L/min) and low-fit controls (0.866 ± 0.123 L/min; P=0.014), pooled across conditions. To illustrate the trend toward priming (P=0.060), MOD2 steady-state (0.925 ± 0.242 L/min) was greater than MOD1 (0.890 ± 0.236 L/min) pooled across groups, although not statistically significant.

2.3.4 Cardiac Output$_{MF}$ On-Transient Kinetics

The omnibus ANOVAs for cardiac output$_{MF}$ τ, baseline, amplitude change, and steady-state yielded no group × condition interactions (all P>0.05), main effects of group (all P>0.05), or main effects of priming (all P>0.05; Fig 2-3).

2.3.5 Heart Rate On-Transient Kinetics

The omnibus ANOVA for heart rate τ yielded a main effect of priming (P=0.001), but not a condition × group interaction (P=0.195) or effect of group (P=0.192; Fig 2-4). MOD2 heart rate τ (44 ± 27 s) was slower than MOD1 τ (34 ± 21 s), pooled across groups.

Heart rate baseline had no group × condition interaction (P=0.975), main effect of group (P=0.626), or priming (P=0.755). MOD1 heart rate baseline was 73 ± 14 beats/min in HFpEF, 78 ± 12 beats/min in low-fit controls, and 79 ± 15 beats/min in high-fit controls. MOD2 heart rate baseline was 72 ± 14 beats/min in HFpEF, 78 ± 9 beats/min in low-fit controls, and 79 ± 12 beats/min in high-fit controls.

Heart rate amplitude change had no group × condition interaction (P=0.116), main effect of group (P=0.280), or priming (P=0.340; Fig 2-4).

Heart rate steady-state had a group × condition interaction (P=0.043), but no effect of group (P=0.283), or priming (P=0.487; Fig 2-4). To investigate the interaction, one-way ANOVAs and paired t-tests were conducted. One-way ANOVAs revealed no group effect in MOD1 (P=0.507) or MOD2 (P=0.132). Paired t-tests also revealed no effect of priming in HFpEF (P=0.129), low-fit (P=0.130), or high-fit controls (P=0.912).

2.3.6 Stroke volume$_{MF}$ On-Transient Time Course Changes

Mauchly's Test of Sphericity was significant, so degrees of freedom values were adjusted with a Greenhouse-Geisser epsilon correction factor. The omnibus ANOVA revealed a time × condition interaction (P=0.012), but no group × time × condition interaction (P=0.579), group × time interaction (P=0.288), group × condition interaction (P=0.412), or main effect of group (P=0.372; Fig 2-5).

A follow-up two-way (4 × 2) repeated measures ANOVA revealed a time × condition interaction (P=0.003). In MOD1, stroke volume$_{MF}$ was significantly increased from baseline at

15s, 30s, and end-exercise (all $P<0.001$), pooled across groups. In MOD2, stroke volume$_{MF}$ was similarly increased from baseline at 15s ($P=0.002$), 30s ($P=0.016$), and end-exercise ($P=0.021$), pooled across groups. Using paired *t*-tests pooled across groups, Δstroke volume$_{MF}$ at 15s ($P=0.005$), 30s ($P=0.008$), and end-exercise ($P=0.004$) were all reduced following priming compared to MOD1 (Fig 2-5).

2.3.7 TPR On-Transient Time Course Changes

Mauchly's Test of Sphericity was significant, so degrees of freedom values were adjusted with a Greenhouse-Geisser epsilon correction factor. The omnibus ANOVA for ΔTPR revealed a group × time interaction ($P=0.004$), but no group × time × condition interaction ($P=0.554$), group × condition interaction ($P=0.734$), time × condition interaction ($P=0.287$), or main effect of priming ($P=0.279$; Fig 2-6).

One-way ANOVAs at 15s, 30s, and end-exercise pooled across conditions were conducted with SNK post-hoc. Levene's test was significant at 15s and 30s, so Kruskal-Wallis one-way ANOVAs were conducted for 15s and 30s. At 15s, high-fit controls had greater negative ΔTPR compared to patients with HFpEF (Kruskal-Wallis $P=0.038$). At 30s, high-fit controls had greater negative ΔTPR compared to patients with HFpEF and low-fit controls (Kruskal-Wallis $P=0.032$). At end-exercise, high-fit controls had greater negative ΔTPR compared to patients with HFpEF and low-fit controls ($P=0.013$).

One-way repeated measures ANOVAs, split by group, were conducted to evaluate each groups' time course changes in TPR from baseline, pooled across conditions. There was an effect of time in patients with HFpEF ($P<0.001$), where ΔTPR at 15s ($P<0.001$), 30s ($P=0.003$), and end-exercise ($P=0.001$) was significantly lower than the baseline reference. A time effect in low-fit controls ($P=0.016$) revealed that TPR was significantly reduced from baseline at 15s ($P=0.023$) and 30s ($P=0.048$) but not at end-exercise ($P=0.208$). As well, ΔTPR was more negative at 15s compared to 30s in low-fit controls ($P=0.042$). A time effect in high-fit controls ($P=0.019$) consisted of end-exercise having greater negative ΔTPR compared to 15s ($P=0.029$).

2.3.8 TOI On-Transient Time Course Changes

Mauchly's Test of Sphericity was significant, so degrees of freedom values were adjusted with a Greenhouse-Geisser epsilon correction factor. The omnibus ANOVA for ΔTOI revealed a group × time × condition interaction (P=0.045; Fig 2-7).

One-way repeated measures ANOVAs, split by group in MOD1 revealed a main effect of time effect in HFpEF (P=0.045) and low-fit controls (P=0.001), but not high-fit controls (P=0.078). However, MOD1 pairwise comparisons revealed no inter-timepoint differences in HFpEF (all P>0.05) or low-fit controls (all P>0.05). MOD2 repeated measures ANOVA revealed a main effect of time in HFpEF (P=0.034) and low-fit controls (P<0.001), but not high-fit controls (P=0.076). MOD2 pairwise comparisons revealed no inter-timepoint differences in HFpEF (all P>0.05), but a difference between baseline and 15s ΔTOI in low-fit controls (P=0.050; Fig 2-7).

Paired t-tests assessed within-group condition effects. In HFpEF, there was no difference in ΔTOI between MOD1 and MOD2 at 15s (P=0.065) and end-exercise (P=0.053), but a greater negative ΔTOI at 30s was present after priming (P=0.033). Neither 15s (P=0.557), 30s (P=0.566), nor end-exercise (P=0.060) presented any priming effects in low-fit controls. High-fit controls exhibited priming effects at all three timepoints; ΔTOI at 15s, 30s, and end-exercise all went from positive change to negative change (P=0.030, P=0.031, and P=0.023, respectively; Fig 2-7).

One-way ANOVAs between groups were significant in MOD1 at 15s (P<0.001) and 30s (P=0.024) but not end-exercise (P=0.086). SNK revealed that high-fit controls had greater (and positive) ΔTOI compared to both groups at 15s and compared to low-fit controls only at 30s. There were no between-group effects in MOD2 at 15s (P=0.051), 30s (P=0.354), or end-exercise (P=0.724; Fig 2-7).

2.4 Discussion

2.4.1 Summary of Results

This study examined 1) $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF compared to their high-fit and low-fit non-diseased counterparts, and 2) the effects of priming exercise on $\dot{V}O_{2p}$ on-kinetics during moderate-intensity exercise in patients with HFpEF compared to their high-fit and low-fit non-diseased counterparts. The primary findings of this study were 1) high-fit controls had faster $\tau\dot{V}O_{2p}$ compared to patients with HFpEF and low-fit controls pooled across conditions, and 2) priming exercise improved phase II $\tau\dot{V}O_{2p}$ pooled across groups.

The finding of faster $\dot{V}O_{2p}$ on-kinetics in high-fit controls compared to patients and low-fit controls is supported by a greater change in amplitude and a greater steady-state $\dot{V}O_{2p}$ in high-fit controls compared to patients with HFpEF and low-fit controls. Our measures of peripheral physiology also support this finding. Specifically, high-fit controls demonstrated a greater reduction in TPR at early onset of exercise (15-30s) and at steady-state exercise compared to patients with HFpEF, and compared to low-fit controls at 30s and at steady-state exercise. Further, TPR in HFpEF decreased at exercise onset and plateaued at 15s, without further significant reduction. High-fit controls had a continual decrease in TPR until steady-state exercise. TPR in low-fit controls was lowest at 15s following exercise onset and was not different from baseline at steady-state exercise, indicating a subsequent rise in TPR after 15s. High-fit controls had a greater and positive change in TOI (*i.e.,* increase) at early exercise onset compared to patients with HFpEF and low-fit controls before priming. However, there was no difference between groups at steady-state exercise before priming.

The priming effect of speeding $\dot{V}O_{2p}$ on-kinetics coincides with an increased baseline $\dot{V}O_{2p}$ following priming in our subjects, but was not supported by cardiac data in any group: heart rate τ was slower following priming; priming exercise decreased Δstroke volume$_{MF}$ at early onset of exercise (15-30s) and end-exercise (final 30s of steady-state exercise); and no difference between groups in cardiac output$_{MF}$ kinetics was detected. Our TOI data demonstrate a group difference in muscle O_2 delivery despite no group difference in $\tau\dot{V}O_{2p}$ after priming exercise. TOI was further reduced following priming in patients with HFpEF at 30s only. While low-fit controls exhibited no effects of priming in TOI, we observed a shift from positive change from baseline TOI to negative change from baseline TOI at 15s, 30s, and steady-state exercise in high-fit controls. Despite this, there were no group differences following priming exercise.

Cumulatively, our findings suggest that peripheral factors (TPR and TOI) may have played a larger role than cardiac factors (cardiac output, heart rate, stroke volume) in limiting phase II $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF compared to high-fit controls. Further, the faster phase II $\dot{V}O_{2p}$ on-kinetics following priming may be explained by improved O_2 extraction (*i.e.,* O_2 diffusion) early in exercise in patients with HFpEF and increased O_2 extraction throughout the exercise bout in high-fit controls, with no priming effect in low-fit controls. The latter effects of priming exercise suggest an impairment of microvascular diffusive O_2 delivery in patients with

HFpEF and high-fit controls, and a potential limitation of O_2 utilization in low-fit controls. Thus, our primary hypothesis was partially confirmed as phase II $\dot{V}O_{2p}$ on-kinetics were slower in patients with HFpEF and low-fit controls compared to high-fit controls. Our secondary hypothesis was not confirmed as there was no difference between groups in the priming effects on phase II $\dot{V}O_{2p}$ on-kinetics, suggesting all study groups demonstrated speeding of phase II $\dot{V}O_{2p}$ on-kinetics. The observed speeding in $\dot{V}O_{2p}$ kinetics, coupled with our priming effects on TOI data, suggest there was an improvement in diffusive O_2 delivery in patients with HFpEF following priming exercise. The latter would suggest a rate-limitation of O_2 delivery rather than utilization may be key in the control of phase II $\dot{V}O_{2p}$ on-kinetics in this patient group.

2.4.2 $\dot{V}O_{2p}$ On-Kinetics in HFpEF

We characterized, for the first time, phase II $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF during large muscle mass exercise, thus furthering our understanding of key limitations to exercise intolerance in the HFpEF population. In previous reports concerning patients with HFrEF, $\dot{V}O_{2p}$ on-kinetics were 42-62% slower than controls matched for age (8, 25, 42), sex, physical activity level (42), and BMI (25). Slower $\dot{V}O_{2p}$ on-kinetics in HFrEF has previously been attributed to slow cardiac output on-kinetics (8, 25, 27, 29, 39, 42, 45), reduced left ventricular ejection fraction (44), and reduced muscle microvascular O_2 delivery during exercise (7, 42). Our patients with HFpEF had 80% slower $\tau\dot{V}O_{2p}$ compared to high-fit controls, which is consistent with the reduced peak $\dot{V}O_{2p}$ that we observed and the extensively reported muscle dysfunction in this population (5, 17, 18, 20, 21, 26). Interestingly, HFpEF and low-fit controls showed similar $\dot{V}O_{2p}$ on-kinetics, suggesting a powerful role of fitness compared to disease on $\dot{V}O_{2p}$ on-kinetics. Indeed, Mettauer et al. (31) also observed strikingly similar muscle mitochondrial oxidative capacity and regulation between patients with HFrEF and sedentary matched controls, but both groups were markedly reduced compared to active controls. Our high-fit controls had faster $\dot{V}O_{2p}$ on-kinetics (25 ± 6 s) and our low-fit controls were similar (50 ± 19 s) compared to previously reported values in a similar age group (58 ± 21 s) (12). However, those subjects had an active pre-transition baseline while our older adults started from rest, which may account for the faster $\dot{V}O_{2p}$ on-kinetics in our high-fit controls (23).

2.4.3 Effect of Priming Exercise on $\dot{V}O_{2p}$ On-Kinetics in HFpEF

Our second novel finding was the speeding of $\tau\dot{V}O_{2p}$ with priming exercise in our three study groups. Speeding of $\dot{V}O_{2p}$ on-kinetics following priming exercise has been previously shown in older sedentary adults with similar age and peak $\dot{V}O_{2p}$ to our high-fit controls (37). Further, priming of $\dot{V}O_{2p}$ was, in part, attributed to improved diffusive O_2 delivery (via muscle NIRS data) (37). Although $\dot{V}O_{2p}$ kinetics in patients with HFpEF have not been studied, HFrEF patients exhibited positive effects of "priming" (moderate intensity exercise) on subsequent $\dot{V}O_{2p}$ on-kinetics (7). That prior study also showed an attenuated "overshoot" in TOI levels following priming exercise, suggesting better matching between O_2 delivery and utilization and supporting the hypothesis that O_2 delivery may play a significant part in the control of $\dot{V}O_{2p}$ on-kinetics in older adults and patients with HFrEF (7). Our TOI data suggest that HFpEF and HFrEF may be similar in this regard, as O_2 extraction may have been increased during early exercise following priming in our patients with HFpEF, and coupled with the faster $\tau\dot{V}O_{2p}$, our data suggest that the faster $\dot{V}O_{2p}$ on-kinetics in our HFpEF group may have been secondary to increased microvascular diffusive O_2 delivery, rather that increased O_2 utilization ability.

$\dot{V}O_{2p}$ amplitude change represents the magnitude of O_2 requirement for a given work rate. High-fit controls had greater amplitude change in $\dot{V}O_{2p}$ than low-fit controls and patients with HFpEF. In contrast to other reports (36), there was no observed amplitude modulation from priming. Steady-state $\dot{V}O_{2p}$ was greater in high-fit controls compared to both groups. The above findings support the faster $\tau\dot{V}O_{2p}$ in high-fit controls over the remaining two groups.

In healthy adults, phase II $\dot{V}O_{2p}$ on-kinetics are typically independent of O_2 delivery and are thus under the primary control of O_2 utilization ability (36). However, age and disease may shift this relationship into a "O_2 delivery dependent zone" (36). Therefore, if $\tau\dot{V}O_{2p}$ were to improve following priming exercise, the source of improvement would likely be in the delivery of O_2 in our study groups. The mechanism accounting for improved O_2 delivery may be identified as related to cardiac, vascular, and/or microvascular changes.

2.4.4 Do Cardiac Factors Modulate $\dot{V}O_{2p}$ On-Kinetics in HFpEF?

Kemps et al. (25) reported cardiac output on-kinetics in patients with HFrEF (62 ± 25 s) and by comparison appear to be markedly slower than our observation of cardiac output on-kinetics in patients with HFpEF (46 ± 49 s). In the current study, cardiac $output_{MF}$ on-kinetics data did not support any indication of functional differences between HFpEF and high-fit and low-fit

control groups. That cardiac output$_{MF}$ was not observed to be affected by priming exercise is consistent with previous findings (37). In HFpEF, cardiac output on-kinetics have not been previously reported, but cardiac output$_{MF}$ reserve may not be reduced in well-compensated patients with HFpEF (5), and is consistent with our cardiac output on-kinetics data showing no difference between HFpEF and healthy high-fit or low-fit controls. That cardiac output did not speed following heavy exercise in patients with HFpEF also suggests that cardiac output may not have been a likely key source of increased O_2 delivery (*i.e.,* convective delivery) that underpinned the effect of priming in $\tau\dot{V}O_{2p}$ across the study groups. Therefore, it seems unlikely that cardiac factors modulated $\dot{V}O_{2p}$ On-Kinetics in our patients with HFpEF.

2.4.5 Cardiac Responses to Square-Wave and Priming Exercise in HFpEF

Heart rate τ was slower following priming exercise in our groups and a similar response to priming exercise has been reported in young adults (9). There was no difference in baseline heart rate in our patients with HFpEF following priming, nor in amplitude change, and therefore the slowing of heart rate τ in HFpEF patients cannot be explained by these parameters. Our patients with HFpEF (37 ± 27 s) and low-fit controls (39 ± 15 s) had similar heart rate τ compared to older adults reported by DeLorey et al. (47 ± 24 and 42 ± 9 s (9, 10)). Heart rate kinetics in our high-fit controls (18 ± 6 s) tended to be faster than previously reported values in young healthy adults (23 ± 12 and 26 ± 7 s) (9, 10). The lack of statistical difference between our high-fit control group and the remaining groups may be due to elevated variance in heart rate responses in our low-fit control and HFpEF groups. Older adults revealed no statistical effect on heart rate τ from a control condition to priming (47 ± 24 vs. 51 ± 31) (9), which contrasts our finding of slowed heart rate τ following priming (Fig 2-4). We suggest that in our subjects, priming exercise may have increased stroke volume$_{MF}$ and cardiac output was maintained, thus facilitating slower heart rate adaptation to square-wave exercise. Alternatively, an elevated pre-transition heart rate following priming exercise (16, 24), although not detected, may have facilitated the slower heart rate on-kinetics (23) because of a shift in the autonomic control of heart rate (34).

Coupling the lack of cardiac output inter-group difference with the effect of priming on heart rate τ in all groups, stroke volume$_{MF}$ on-kinetics may be assumed to be similar between groups. As the variability in the stroke volume$_{MF}$ signal did not lend itself to reliable curve-fitting, time course changes were assessed using delta values, and supported our assumption by not

yielding any group differences. Our lack of group differences contrasts with Borlaug et al. (6) who demonstrated impaired contractility reserve during peak exercise in patients with HFpEF compared to hypertensive and normal controls, and with Kitzman et al. (26) who reported reduced stroke volume reserve in patients with HFpEF. Our lower level of exercise intensity compared to the peak exercise performed in Borlaug et al. (6) and Kitzman et al. (26) may account for the discrepancy between our inter-group findings.

Interestingly, all three groups demonstrated a lower Δstroke volume$_{MF}$ following prior heavy exercise. Thus, the increase in stroke volume$_{MF}$ at exercise onset was attenuated following priming. This may be due to elevated pre-transition baseline stroke volume$_{MF}$ as a result of prior heavy exercise, thus reducing the overall stroke volume$_{MF}$ response. Coupled with our cardiac output and heart rate on-kinetics data, we suggest our HFpEF patients had increased baseline stroke volume and decreased heart rate following priming exercise, thereby maintaining cardiac output. This may be due to a salutary decrease in TPR following priming exercise thus increasing venous return and ventricular preload. This would explain the discrepancy between our data and previously reported impaired stroke volume reserve (6, 26), as the previously reported patients with HFpEF performed maximal exercise testing; perhaps the priming exercise, followed by a brief recovery period, allowed time for peripheral vasodilation. The similar stroke volume$_{MF}$ between our groups may also be due to the recruited HFpEF phenotype, as Bhella et al. (5) reported no difference in peak stroke volume, stroke work, or cardiac output between HFpEF patients and healthy controls. Cumulatively, we suggest that cardiac output control was altered following priming exercise such that baseline stroke volume was elevated, thus allowing heart rate kinetics to slow to maintain cardiac output.

2.4.6 TPR Responses to Square-Wave and Priming Exercise in HFpEF

ΔTPR was markedly less in patients with HFpEF compared to high-fit controls throughout the entire exercise bout. A smaller ΔTPR indicates a greater absolute TPR at the respectively time point, suggesting vascular dysfunction when responding to exercise, and consistent with previous reports in HFpEF (6, 11, 21). Like our patients with HFpEF, TPR in our low-fit controls was also reduced compared to high-fit controls at 30s and end-exercise. Potential mechanisms for the impaired vascular response in HFpEF may be related to impaired endothelial function (6), or exaggerated muscle sympathetic nerve activity in patients with HFpEF (46, 47). No priming effects

were observed with TPR, but extrapolating from the change in group differences from MOD1 to MOD2 at 15s, both HFpEF (-433 ± 176 vs. -353 ± 112 dyn·s/cm^5) and high-fit controls (-901 ± 403 vs. -728 ± 352 dyn·s/cm^5) may have exhibited less change in TPR following priming exercise. The latter suggests a reduced pre-transition baseline TPR following priming in these groups. The suspected improvements in TPR following priming, particularly in high-fit controls, may be associated with the apparent priming of $\tau\dot{V}O_{2p}$. In particular, priming may have improved the adaptation of TPR in patients with HFpEF, thus facilitating an increase in convective O_2 delivery and subsequently $\tau\dot{V}O_{2p}$. An alternative explanation for the differences in ΔTPR is the differences in absolute work rate during the transition to moderate-intensity exercise. A lower absolute work rate would result in a smaller amplitude in TPR response, and as the HFpEF group had a significantly lower absolute work rate compared to high-fit controls, this may explain the significant difference in ΔTPR. However, patients with HFpEF had lower absolute work rate but were exercising at similar relative exercise intensities, and as patients with HFpEF typically present with exaggerated muscle sympathetic nerve activity and/or endothelial dysfunction, the alternative explanation seems less likely.

2.4.7 Role of Muscle Oxygenation on $\dot{V}O_{2p}$ On-Kinetics in HFpEF

Muscle oxygenation (TOI) during moderate intensity exercise (without priming) in patients with HFrEF tends to demonstrate a substantial "overshoot" (*i.e.,* a rapid transient decrease in muscle TOI below steady-state at the onset of exercise, and instead of plateauing and remaining depressed and suggesting adequate muscle oxygenation, a transient increase in TOI back toward baseline levels occurs), indicating a slower rate of muscle oxygenation relative to $\dot{V}O_{2p}$ on-kinetics (7). In our patients with HFpEF (and low-fit controls), we observed no such overshoot before priming (*i.e.,* TOI decreased and plateaued with no subsequent increase until after exercise), indicating sufficient muscle diffusive O_2 delivery and thus a potential source of limitation in the utilization ability of O_2 that adversely affects $\dot{V}O_{2p}$ on-kinetics (1, 4, 41). However, our patients with HFpEF exhibited an effect of priming at 30s into exercise where TOI was further reduced following priming exercise, suggesting greater O_2 extraction ability by the exercising muscle. This finding may coincide with the priming effect on τVO_{2p}, suggesting the increased O_2 extraction in the HFpEF group facilitated the speeding of $\tau\dot{V}O_{2p}$. Similarly, patients with HFrEF demonstrate an attenuated TOI overshoot following priming exercise (indicating increased diffusive O_2

delivery and greater matching between O_2 delivery and utilization) (7), and this interpretation of the TOI profile has been described in previous reports (1, 4, 33). Although not described elsewhere, we observed an increase in TOI above baseline at exercise onset in high-fit controls, and this may indicate a mismatch between O_2 delivery and utilization; delivery is rapidly and efficiently elevated while $\dot{V}O_{2p}$ is slower to adapt to square-wave exercise. This hypothesis is similar to what has been observed in young adults, where the rate-limiting step in $\dot{V}O_{2p}$ on-kinetics is likely not secondary to O_2 delivery, but rather regulated by intracellular O_2 transport or metabolic function (9). However, following priming exercise, high-fit controls demonstrated a transient decrease in TOI at the onset of exercise, with a slight increase toward baseline by end-exercise. The latter suggests that high-fit older adults may have sufficient convective O_2 delivery but limited O_2 extraction, and following priming exercise, O_2 extraction at the muscle may be improved. This observation may be mediated by fitness as no priming response was observed in low-fit controls; indeed, low-fit controls exhibited a TOI profile indicative of impaired O_2 delivery (33) in both MOD1 and MOD2 conditions. Following priming in our patients with HFpEF, the initial (30s) decrease in TOI was greater, suggesting a more rapid TOI response, indicative that the limitation of $\dot{V}O_{2p}$ on-kinetics may shift towards diffusive O_2 delivery. However, there was no difference in TOI at end-exercise (*i.e.,* no transient increase toward baseline) in HFpEF from MOD1 to MOD2, suggesting the potential source of slowing in $\dot{V}O_{2p}$ on-kinetics shifts to O_2 utilization ability once O_2 delivery is normalized to the demand. Taken with the priming effect on $\tau\dot{V}O_{2p}$, it is likely that diffusive O_2 delivery may be a preliminary limitation (early exercise) to $\dot{V}O_{2p}$ on-kinetics in these patients, however with time and warm-up, O_2 delivery may improve and thus transfer the control of $\dot{V}O_{2p}$ to O_2 utilization ability in patients with HFpEF. The latter hypothesis is supported by prior work reporting muscle dysfunction in HFpEF (18–21, 32). Specifically, aerobic enzymes (32), mitochondrial content and quality (32), muscle quality (20), capillary density (20, 21), and slow-to-fast twitch fibre ratio (20, 21) have all been shown to be reduced in HFpEF. These markers suggest lower oxidative function and capacity in HFpEF skeletal muscles, consistent with our interpretation of the TOI data, suggesting O_2 utilization may be a key rate-limiting factor in $\dot{V}O_{2p}$ on-kinetics once O_2 diffusion reaches homeostasis.

2.4.8 Subject Demographics

Patients with HFpEF demonstrated lower peak $\dot{V}O_{2p}$ compared to high-fit but not low-fit controls, suggesting that lack of disease does not preclude significant deconditioning. Peak heart rate, percent-predicted heart rate, and ventilatory threshold heart rate were lower in HFpEF patients. HFpEF patients also exhibited signs of concentric hypertrophy and increased ventricular filling pressures at rest, and diastolic dysfunction as indicated by diastolic score (Table 2-1, echocardiographic data) – observations that are typical for patients with HFpEF. The muscle and adipose data from the right calf are in support with previously reported skeletal muscle quality measured on the thigh using magnetic resonance imaging (20); however, increased intermuscular adipose area and percent intermuscular fat in HFpEF compared to controls was also reported (20), but not confirmed for our pQCT measure. As such, we could not detect muscle quality differences between groups with our measure.

2.5 Limitations

There are a few limitations to this study. First, although healthy controls were initially sex-matched to our patient group, our subsequent high-fit/low-fit split caused a discrepancy in sex between the two control groups, and therefore HFpEF and high-fit groups as well. Fortunately, the primary outcome, $\dot{V}O_{2p}$ on-kinetics, has been demonstrated to be independent of sex (13). However, as many other variables are affected by sex (*e.g.,* peak $\dot{V}O_{2p}$, skeletal muscle and adiposity), we cannot preclude this inter-group sex difference as a limitation. Second, our patient group was heterogeneous in fitness and disease severity, ranging from NYHA class I-III. This substantially increased the variance in the patient group (usually markedly greater than both controls groups), thus the frequent use of Kruskal-Wallis *P*-values to minimize type I error. The inflated variance increased our risk of type II error, and may have obscured some intergroup differences. Finally, we did not account for subcutaneous adipose for the NIRS measurement. Although no threshold is recognized, Grassi and Quaresima (15) suggest that a skin and adiposity layer > 2 cm would render the signal meaningless if the goal is to investigate skeletal muscle. We recognize that this is a limitation to our TOI data, however, from careful inspection of our data and additional pilot testing, we believe our data is representative of skeletal muscle oxygenation levels.

2.6 Conclusion

Cumulatively, our data suggest that O_2 delivery, particularly microvascular delivery and O_2 extraction, may be a key rate-limiting factor for $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF. Our

data suggest that peripheral limitations (vascular function and O_2 extraction) play a leading role in determining exercise intolerance in patients with HFpEF, similar to patients with HFrEF whose major limitation seems to be bulk and/or microvascular O_2 delivery to the exercising muscle.

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2.8 List of Abbreviations

A - left ventricular late diastolic filling measured by late mitral inflow velocity

AVO_2Diff – arterial-venous oxygen content difference

cHb – total concentration of hemoglobin; measured by near infrared spectroscopy

E - left ventricular early diastolic filling measured by early mitral inflow velocity

e' - myocardial diastolic motion velocity

E/e' - estimated left ventricular filling pressure

E/A - index of diastolic filling and ventricle recoil

EF – ejection fraction

EDV – end-diastolic volume

ESV – end-systolic volume

HF – heart failure

HFpEF – heart failure with preserved ejection fraction

HFrEF – heart failure with reduced ejection fraction

HHb – concentration of deoxygenated hemoglobin and myoglobin; measured by near infrared spectroscopy

MOD1 – exercise protocol 1: moderate intensity cycling

MOD2 – exercise protocol 2: heavy intensity & moderate intensity cycling

nTHI – normalized tissue hemoglobin index; percentage change in the amount of initial hemoglobin; measured by near infrared spectroscopy

O_2 - oxygen

O_2Hb – concentration of oxygenated hemoglobin and myoglobin; measured by near infrared spectroscopy

RER – respiratory exchange ratio

τ – tau; time constant; time to reach 63% of the overall amplitude increase from rest to steady-state exercise

TOI – tissue oxygenation index; O_2 saturation level; measure by near infrared spectroscopy

$\dot{V}CO_{2p}$ – carbon dioxide production; measured by breath-by-breath CO_2 expired at the mouth

$\dot{V}O_{2p}$ – pulmonary O_2 uptake; the sum of all the O_2 used by the body cells, as measured by breath-by-breath gas exchange at the mouth

WR – work rate

Table 2-1. Subject Characteristics

		HFpEF	Low-Fit Control	High-Fit Control	*P*-Value
Sex (m, f)		6, 2	2, 3	4, 0	
Age (years)		68 ± 10	68 ± 5	68 ± 10	0.996
Height (cm)		169 ± 12	171 ± 11	175 ± 6	0.692
Weight (kg)		90 ± 18	74 ± 4	84 ± 13	0.142
BMI (kg/m^2)		31.5 ± 5.1	25.5 ± 3.0	27.4 ± 3.5	0.064
Resting SBP (mmHg)		121 ± 17	121 ± 9	124 ± 5	0.936
Resting DBP (mmHg)		70 ± 9	75 ± 11	81 ± 6	0.155
Peak	VO_{2p} (mL/kg/min)	15.8 ± 5.9	19.9 ± 1.4	30.5 ± 5.6*	**0.001**
	Heart Rate (beats/min)	117 ± 16*	139 ± 8	151 ± 16	**0.001**
	Work Rate (Watts)	88 ± 41	115 ± 17	207 ± 50*	**0.003**
	% Predicted VO_{2p} †	68 ± 23	86 ± 9	135 ± 12*	**0.008**[KW]
	% Predicted Heart Rate †	72 ± 7*	84 ± 5	91 ± 10	**0.002**
	RER	1.11 ± 0.12	1.23 ± 0.07	1.19 ± 0.08	**0.127**
Ventilatory Threshold	VO_{2p} (mL/kg/min)	9.1 ± 2.2*	12.9 ± 2.0 ¥	16.3 ± 2.6	**<0.001**
	Heart Rate (beats/min)	90 ± 10*	104 ± 6 ¥	109 ± 17	**0.001**
	Work Rate (Watts)	38 ± 21 ¥	66 ± 12	92 ± 21	**0.032**
Moderate WR (W)		24 ± 16 ¥	39 ± 11	58 ± 14	**0.007**
Heavy WR (W)		63 ± 32	89 ± 44	148 ± 32*	**0.001**
38% Site	Muscle Area (cm^3)	44.0 ± 14.5	33.5 ± 4.4	45.5 ± 8.2	0.211
	Muscle Density (mg/cm^3)	68.5 ± 6.2	71.8 ± 2.9	72.2 ± 3.8	0.565[KW]
	Subcutaneous Adipose Area (cm^3)	18.6 ± 15.7	19.5 ± 7.9	12.2 ± 4.6	0.624
66% Site	Muscle Area (cm^3)	71.4 ± 10.9	68.7 ± 3.4	76.1 ± 12.3	0.519
	Muscle Density (mg/cm^3)	68.3 ± 4.5	71.4 ± 2.3	69.2 ± 3.4	0.388

	Subcutaneous Adipose Area (cm^3)	27.7 ± 18.9	26.7 ± 11.0	16.9 ± 7.1	0.479
Echocardiography	EF (%)	61 ± 9	65 ± 4	68 ± 4	0.278
	End-Diastolic Volume (mL)	110 ± 32	85 ± 17	122 ± 16	0.119
	End-Systolic Volume (mL)	42 ± 12	29 ± 5	42 ± 8	0.09
	Stroke Volume (mL)	68 ± 24	56 ± 13	80 ± 9	0.204
	Left Atrial Volume Index (mL/m^2)	46 ± 20	24 ± 3	31 ± 3	**0.040**ǂ
	Left Atrial Diameter (cm)	4.55 ± 0.72*	3.56 ± 0.38	3.67 ± 0.12	**0.012**
	E (cm/s)	94.6 ± 40.1	69.8 ± 16.1	57.9 ± 7.2	0.287^{KW}
	A (cm/s)	73.9 ± 31.5	80.7 ± 10.8	71.2 ± 14.7	0.654^{KW}
	E/A	1.19 ± 0.44	0.89 ± 0.35	0.84 ± 0.25	0.303
	e' (cm/s)	6.95 ± 1.12	7.54 ± 1.38	7.83 ± 2.12	0.588
	E/e'	13.9 ± 6.3	9.3 ± 1.6	7.7 ± 1.3	0.119^{KW}
	Left Ventricular Mass (index; g/m^2)	103 ± 13*	71 ± 9	84 ± 10	**0.001**
	Posterior Wall Thickness (cm)	1.15 ± 0.16*	0.86 ± 0.11	0.92 ± 0.13	**0.006**
	Diastolic Score (Grade I/II/III)	2/3/0	1/0/0	0/0/0	
Medications (# patients)	Anti-Arrhythmia	2			
	Anti-Coagulant	4			
	Anti-Hypertensives	1			
	ACE-Inhibitor	5			
	ARB	2			
	Aspirin	5			
	Beta Blocker	7			

Calcium Channel Blocker	3
Digoxin	1
Diuretic	3
Loop-Diuretic	3
Statin	7
Thyroid	3

Values are means ± standard deviations. Bolded *P*-values are significant. *****Significantly different from both groups. ¥Significantly different from high-fit controls. ^{KW}Indicates a Kruskal-Wallis *P*-value. †Percent of the peak value obtained to the predicted peak value based on demographics. ǂANOVA *P*-value, however SNK post-hoc testing revealed no difference between groups. A, left ventricular late diastolic filling; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; E, left ventricular early diastolic filling; e', myocardial diastolic motion (*i.e.,* relaxation velocity); E/e', estimate of left ventricular filling pressure; EF, ejection fraction; HFpEF, heart failure with preserved ejection fraction group; MOD1, moderate exercise transition without prior heavy exercise; MOD2, moderate exercise transition following prior heavy exercise; RER, respiratory exchange ratio; VO_{2p}, pulmonary oxygen uptake; WR, work rate.

Table 2-2. $\dot{V}O_{2p}$ On-Kinetics

	HFpEF	Low-Fit Control	High-Fit Control	*P*-Value
Tau (s)	45 ± 15	50 ± 19	25 ± 6*	**0.008**
Amplitude (L/min)	0.461 ± 0.128	0.575 ± 0.877	0.830 ± 0.199*	**0.003**
Steady-State (L/min)	0.797 ± 0.183	0.866 ± 0.123	1.181 ± 0.237*	**0.014**

Values are means ± standard deviations. Bolded *P*-values are significant. *Significantly different from both groups, pooled across conditions. HFpEF, heart failure with preserved ejection fraction group; MOD1, moderate exercise transition without prior heavy exercise; MOD2, moderate exercise transition following prior heavy exercise; $\dot{V}O_{2p}$, pulmonary oxygen uptake.

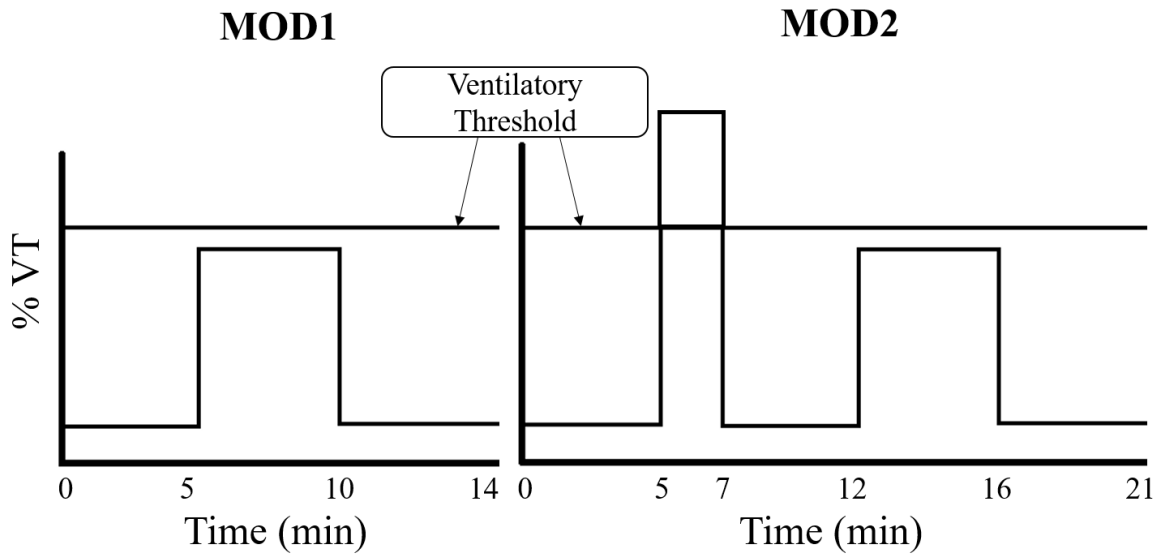

Fig 2-1. MOD1 and MOD2 Exercise Protocols

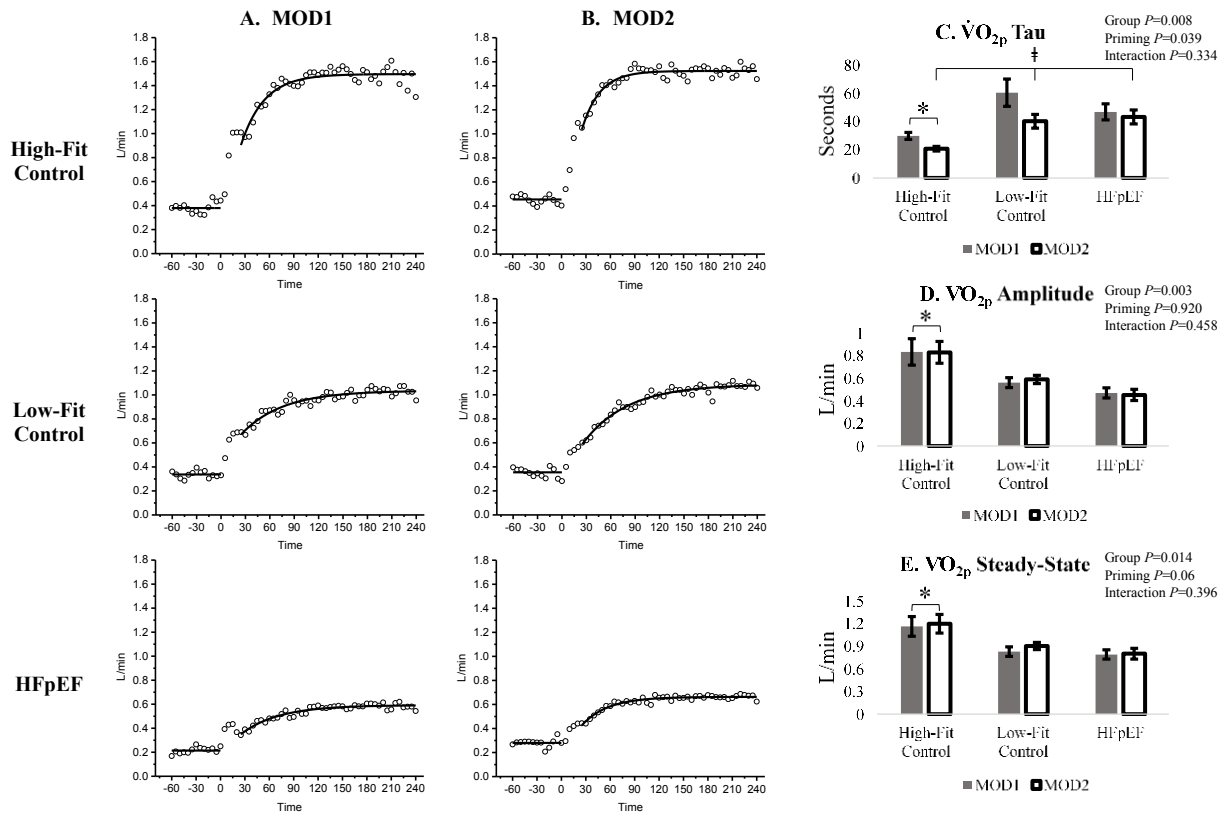

Fig 2-2. $\dot{V}O_{2p}$ On-Kinetics. *A*. MOD1 example curve-fits from representative subjects within each group. *B*. MOD2 example curve-fits from representative subjects within each group. *C*. $\dot{V}O_{2p}$ tau (time constant) during MOD1 and MOD2 in each group. *D*. $\dot{V}O_{2p}$ total amplitude change during MOD1 and MOD2 in each group. *E*. $\dot{V}O_{2p}$ steady-state during MOD1 and MOD2 in each group. Panels *C-E* values are means ± SEM. HFpEF, heart failure with preserved ejection fraction; MOD, moderate exercise transition without prior heavy exercise; MOD2, moderate exercise transition following prior heavy exercise; $\dot{V}O_{2p}$, pulmonary oxygen uptake. *Significantly different from remaining groups, pooled across conditions (P<0.05). ‡Significantly different from MOD1, pooled across groups (P<0.05).

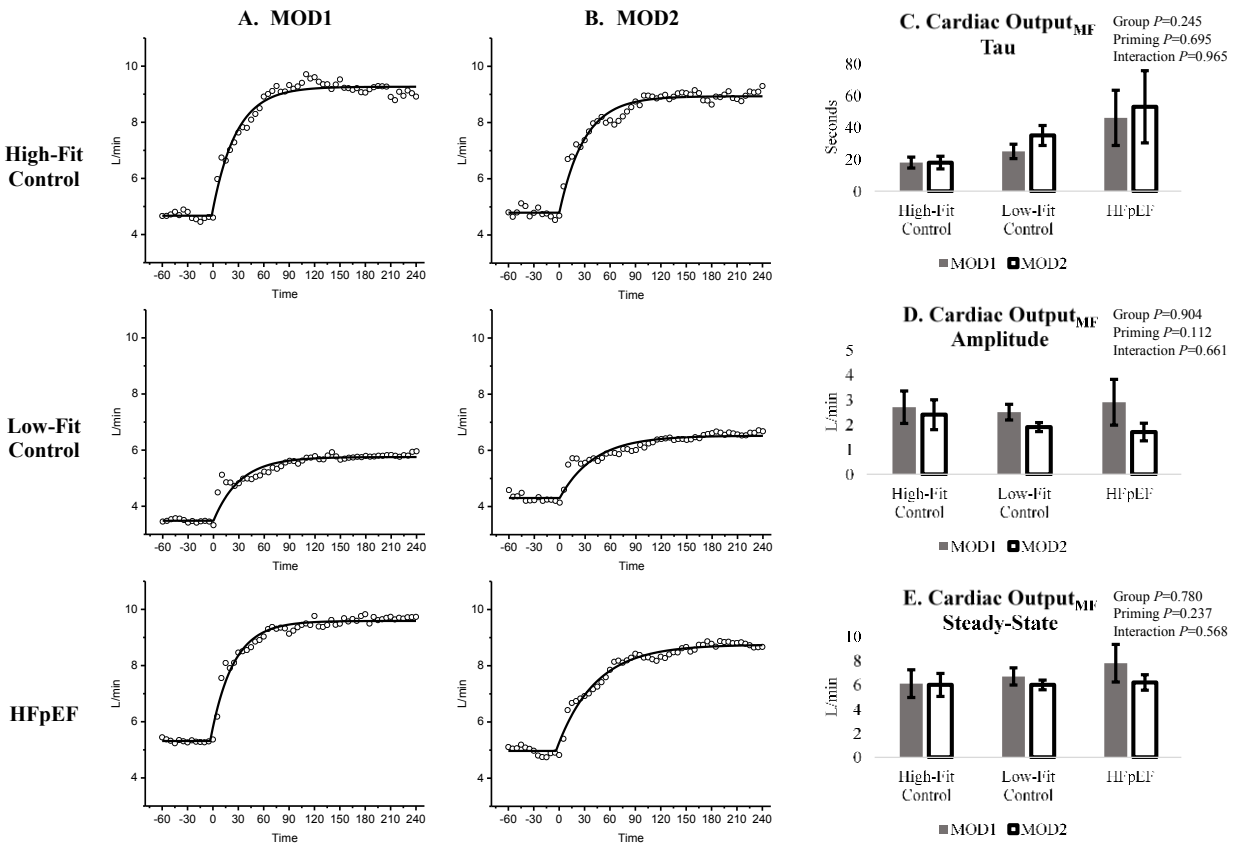

Fig 2-3. Cardiac Output$_{MF}$ On-Kinetics. *A*. MOD1 example curve-fits from representative subjects within each group. *B*. MOD2 example curve-fits from representative subjects within each group. *C*. Cardiac output$_{MF}$ tau (time constant) during MOD1 and MOD2 in each group. *D*. Cardiac output$_{MF}$ total amplitude change during MOD1 and MOD2 in each group. *E*. Cardiac output$_{MF}$ steady-state during MOD1 and MOD2 in each group. Panels *C-E* values are means ± SEM. HFpEF, heart failure with preserved ejection fraction group; MOD1, moderate exercise transition without prior heavy exercise; MOD2, moderate exercise transition following prior heavy exercise.

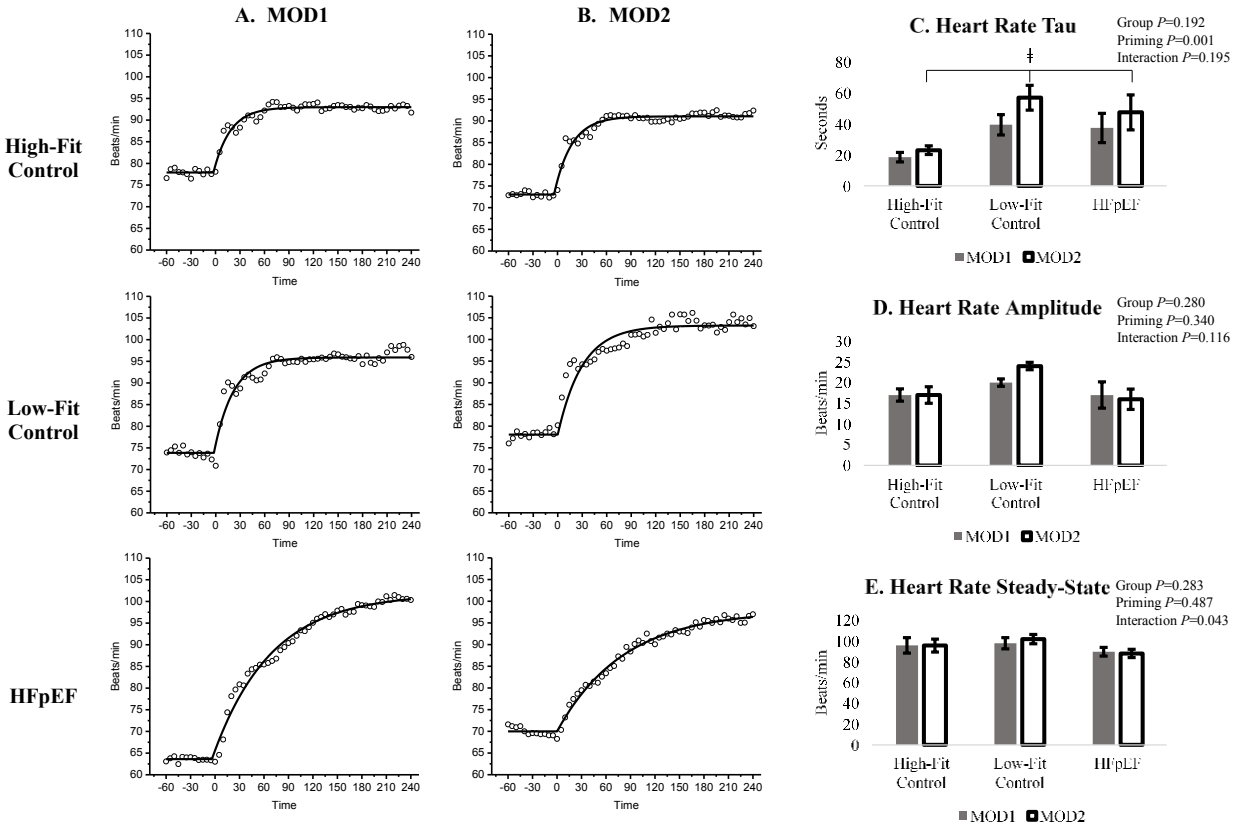

Fig 2-4. Heart Rate On-Kinetics. *A*. MOD1 example curve-fits from representative subjects within each group. *B*. MOD2 example curve-fits from representative subjects within each group. *C*. Heart rate tau (time constant) during MOD1 and MOD2 in each group. *D*. Heart rate total amplitude change during MOD1 and MOD2 in each group. *E*. Heart rate steady-state during MOD1 and MOD2 in each group. Panels *C*-*E* values are means ± SEM. HFpEF, heart failure with preserved ejection fraction; MOD, moderate exercise transition without prior heavy exercise; MOD2, moderate exercise transition following prior heavy exercise. ‡Significantly different from MOD1, pooled across groups (P<0.05).

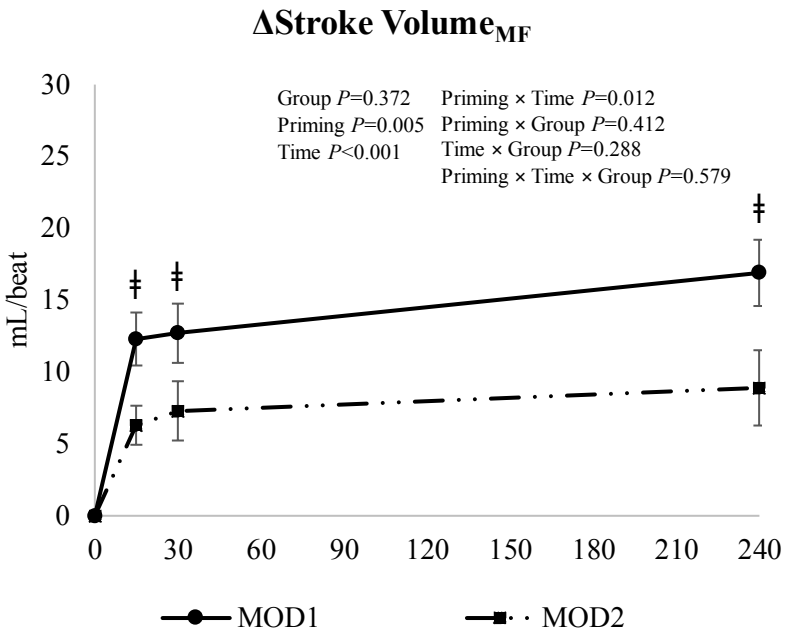

Fig 2-5. ΔStroke Volume$_{MF}$ Time Course Changes. ΔStroke volume$_{MF}$ (from baseline) at 15s, 30s, and end-exercise in MOD1 and MOD2, pooled across groups. Values are means ± SEM. MOD1, moderate exercise transition without prior heavy exercise; MOD2, moderate exercise transition following prior heavy exercise. ǂMOD2 significantly less than MOD1 (P<0.05). All time-points were significantly different from baseline (all P<0.05).

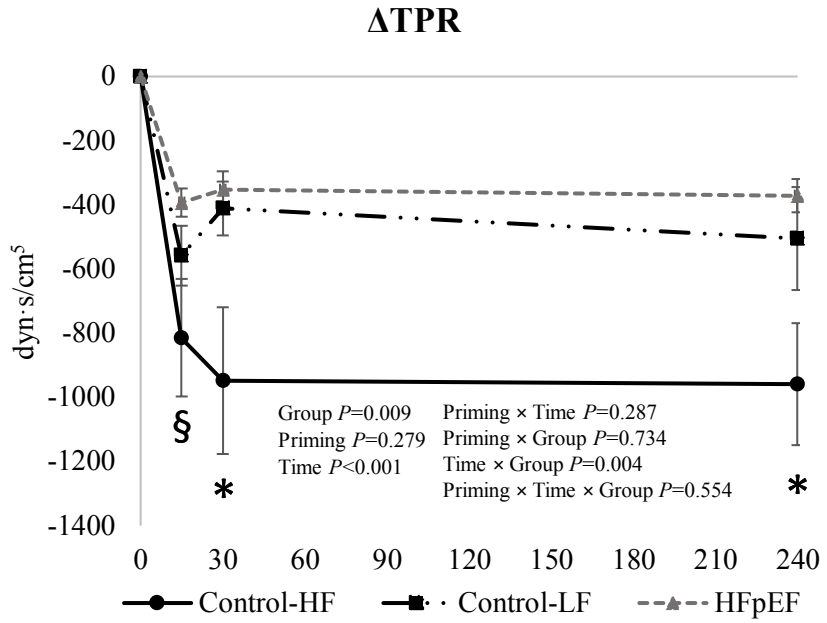

Fig 2-6. ΔTPR Time Course Changes. ΔTPR (from baseline) at 15s, 30s, and end-exercise between groups, pooled across conditions. Values are means ± SEM. Control-HF, high-fit control group; Control-LF, low-fit control group; HFpEF, heart failure with preserved ejection fraction group; TPR, total peripheral resistance. *Control-HF had a significantly greater change than remaining groups at this time point (P<0.05). §Control-HF had a significantly greater change than HFpEF group at this time point (P<0.05).

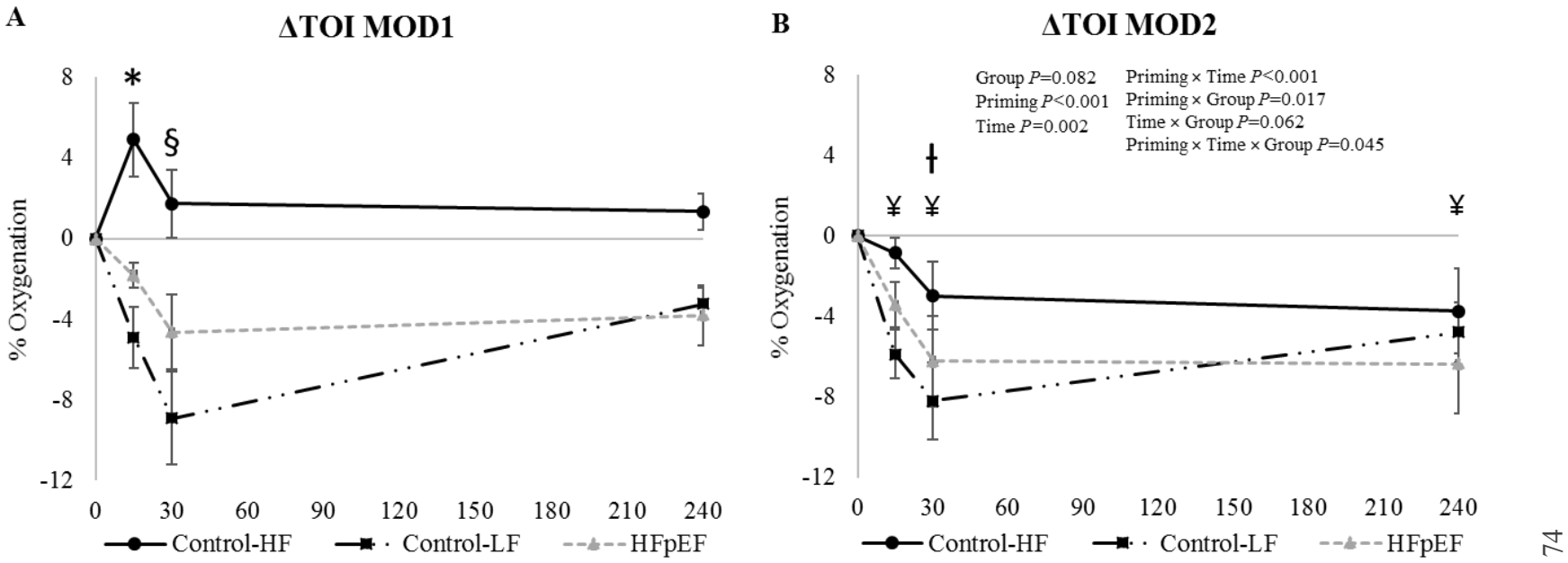

Fig 2-7. ΔTOI Time Course Changes. *A*. MOD1 ΔTOI (from baseline) at 15s, 30s, and end-exercise between groups. *B*. MOD2 ΔTOI (from baseline) at 15s, 30s, and end-exercise between groups. Values are means ± SEM. Control-HF, high-fit control group; Control-LF, low-fit control group; HFpEF, heart failure with preserved ejection fraction group; MOD1, moderate exercise transition without prior heavy exercise; MOD2, moderate exercise transition following prior heavy exercise; TOI, tissue oxygenation index. *Control-HF had a significantly greater change than remaining groups at this time point (P<0.05). §Control-HF had a significantly greater change than control-LF group at this time point (P<0.05). ¥Control-HF group had a significantly different change than MOD1 at this time point (P<0.05). †HFpEF group had a significantly greater change than MOD1 at this time point (P<0.05).

CHAPTER THREE: DISCUSSION

3.1 Main Findings

We characterized, for the first time, phase II $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF compared to healthy high-fit and low-fit control subjects, and $\dot{V}O_{2p}$ on-kinetics following a bout of prior heavy exercise. We found that phase II $\dot{V}O_{2p}$ on-kinetics were slower in patients with HFpEF and low-fit controls compared to high-fit controls, pooled across conditions. This novel finding is not supported with any difference between groups in cardiac factors (cardiac output, heart rate, stroke volume), and did coincide with decreased exercise onset and steady-state TPR in high-fit controls compared to patients with HFpEF and low-fit controls when pooled across conditions. HFpEF patients and low-fit controls had decreased TOI at early onset of exercise and HFpEF patients maintained the minimum TOI value through until end-exercise, while TOI in low-fit controls increased toward near-baseline values. Conversely, TOI increased in high-fit controls at the onset of exercise and decreased toward near-baseline by end-exercise.

While no group difference was observed, all groups demonstrated speeding of phase II $\tau\dot{V}O_{2p}$ following priming exercise. This pooled effect was partnered with a concomitantly slower τ heart rate following priming exercise (also pooled across groups), and a reduction in Δstroke volume within the first 15-30 s of exercise and at steady-state exercise in all three groups. Priming had no effect on ΔTPR. TOI exhibited priming effects in patients with HFpEF and high-fit controls at early and end-exercise with a greater reduction in TOI. The greater reduction in TOI may indicate increased O_2 extraction at the muscle, however, TOI in patients with HFpEF did not display an "overshoot", suggesting a limitation in O_2 utilization towards the end of exercise.

Cumulatively, our findings suggest that peripheral factors (TPR and TOI) may have played a more significant role than cardiac factors (cardiac output, heart rate, stroke volume) in limiting phase II $\dot{V}O_{2p}$ on-kinetics in HFpEF when compared to high-fit controls. Additionally, increased microvascular muscle O_2 delivery following priming exercise (*i.e.,* TOI condition effects in high-fit controls and HFpEF patients) may represent a key factor in the speeding of phase II $\tau\dot{V}O_{2p}$ on-kinetics. Our data thus suggest a rate-limitation of local muscle O_2 perfusion on phase II $\dot{V}O_{2p}$ in all groups. Therefore, our primary hypothesis was partially confirmed as patients with HFpEF did indeed have slower phase II $\dot{V}O_{2p}$ on-kinetics compared to high-fit controls. However, our secondary hypothesis was not supported as priming exercise improved phase II $\dot{V}O_{2p}$ on-kinetics

in all groups, suggesting that patients with HFpEF may present with a significant deficit in local muscle O_2 delivery that limits $\dot{V}O_{2p}$ on-kinetics. Our measures of the integrative physiologic responses (cardiac kinetics, vascular, and muscle oxygenation time-course changes) to square-wave exercise indicate that vascular function and muscle oxygenation may limit $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF, and that muscle oxygenation may improve with priming exercise, thus coinciding with our observation of a speeding in $\dot{V}O_{2p}$ on-kinetics following priming exercise.

3.2 HFpEF Study Group Characteristics

3.2.1 Exercise $\dot{V}O_{2p}$

Patients with HFpEF had lower peak $\dot{V}O_{2p}$ compared to the high-fit controls but not low-fit controls. This is similar to findings reported by Mettauer et al. (17) where the muscle oxidative capacity (measured via muscle biopsy) was the same between patients with HFrEF and sedentary matched controls, but reduced compared to active controls. Our data combined with Mettauer's study suggests that lack of disease alone does not preclude significant deconditioning. By design, peak $\dot{V}O_{2p}$ was higher in high-fit compared to low-fit controls. Percent predicted peak $\dot{V}O_{2p}$ was not different between patients with HFpEF and low-fit controls. Conversely, at submaximal exercise (ventilatory threshold), $\dot{V}O_{2p}$ was not only greatest in high-fit controls, but also different between patients with HFpEF and low-fit controls, suggesting greater aerobic fitness in the low-fit controls compared to HFpEF.

3.2.2 Exercise Heart Rate

Heart rate was lower at maximal exercise in patients with HFpEF compared to all controls, and patients were additionally unable to achieve their percent-predicted maximum heart rate. At submaximal exercise, patients with HFpEF reached a lower heart rate compared only to high-fit controls, continuing a trend of surprising similarity between the low-fit controls and HFpEF patients. The globally observed dysfunction in heart rate may be explained by medications (7/8 patients using beta-blockers) as all medications were taken as normal on testing days. However, heart rate during submaximal (moderate) exercise in HFpEF was inconsistent; while ventilatory threshold heart rate was markedly reduced in patients with HFpEF compared to both control groups, neither MOD1 nor MOD2 yielded a difference between groups in steady-state heart rate. This may be due to the same heart rate being achieved at ventilatory threshold and MOD1 steady-

state (90 ±10 vs. 90 ± 12 bpm, respectively) in patients with HFpEF, while both high-fit and low-fit control groups achieved an average of 88% and 94% of their ventilatory threshold heart rate during MOD1, respectively.

3.2.3 Resting Cardiac Morphology and Function

Regarding cardiac structure, patients with HFpEF had greater left atrial diameter, left ventricular mass (indexed to body surface area), and thicker posterior walls compared to both control groups, similar to previously reported (10); these findings are consistent with concentric hypertrophy (increased mass and wall thickness) and increased left ventricular filling pressure (dilated left atrial diameter as a result of blood pooling in this chamber from reduced pressure difference between the left atrium and ventricle). Similar left ventricular mass (although not indexed) and left atrial diameter have been reported in HFpEF (11), although left ventricular mass index in the current HFpEF study group was greater than commonly reported (21). End-diastolic volume in our patients with HFpEF was also greater compared to a large sample of HFpEF patients (110 ± 32 vs. ~81-85 mL) (21) and other reports (110 ± 32 vs. ~75 mL) (10). However, end-diastolic volume in HFpEF was not different from either control group, whereas a difference in end-diastolic volume between patients with HFpEF and controls was previously reported (~75 ml vs. 87 mL). End-systolic volume was similar between our patients with HFpEF and a previously reported sample (42 ± 12 vs. ~31-36 mL) (10, 21), as well as similar to our control subjects; however, a difference between HFpEF and control end-systolic volume has also been observed (~32 vs. 43 mL). An explanation for the heterogeneous data on cardiac morphology across the literature plays favour with the theory of HFpEF being a heterogeneous "umbrella-type" syndrome, with many sub-phenotypes (depending on clinical presentation and comorbidities) and therefore varying treatment requirements.

Besides an elevated diastolic dysfunction score in patients with HFpEF compared to both control groups, cardiac function (stroke volume, EF, E (left ventricular early diastolic filling), A (left ventricular late diastolic filling), and E/A (index of diastolic filling and ventricle recoil)) displayed no differences between groups as detected using resting echocardiography. This is not unexpected as many resting values have previously been normal in patients with HFpEF (10). Resting stroke volume was less in our patient group compared to commonly reported values (68 ± 24 vs. ~80-90 mL) (21), and greater in our HFpEF group compared to another report (68 ± 24 vs.

~43 mL at rest) (10), though similar compared to controls as also previously shown (1). Resting EF in our patients with HFpEF was directly comparable to that commonly observed in a large sample (61 ± 9 vs. 61 ± 7 %) (21), as well as others (10, 15, 16), with no difference from healthy controls (1, 11). While E was on the low end of previously reported values (94.6 ± 40.1 vs. ~93-118 cm/s), A was markedly lower (73.9 ± 31.5 vs. ~81-93 cm/s) (21). HFpEF E/A has previously been shown as similar to hypertensive controls (1.1 ± 0.7 vs. 0.8 ± 0.3) (16), with values comparable to ours (16, 21). Cumulatively, cardiac morphology and function in our HFpEF study group were comparable to several other HFpEF studies (1, 10, 11, 15, 16, 21).

3.2.4 Skeletal Muscle Quality and Adiposity

Our muscle and adipose data from the right calf are consistent with previously reported skeletal muscle quality variables similar to those we report, but measured on the thigh using magnetic resonance imaging (11); however, the latter study still reported skeletal muscle abnormalities in the form of increased intermuscular adipose area and percent intermuscular fat in HFpEF patients compared to controls (11). Further, area of and percent intermuscular adipose were found to be independent predictors of peak $\dot{V}O_{2p}$ in HFpEF, suggesting that abnormal skeletal muscle adiposity/composition plays a significant role in reducing exercise capacity in HFpEF (11). Intermuscular and intramuscular adipose were not reported in the current study as these variables have not been validated using pQCT. We were therefore unable to detect differences in muscle quality between groups as these key variables that have previously been assessed (11) are not were not measurable with our pQCT scanner.

3.3 Expanding our Knowledge about HFpEF Pathophysiology

3.3.1 $\dot{V}O_{2p}$ On-Kinetics and Muscle Oxygenation

To our knowledge, neither $\dot{V}O_{2p}$ on-kinetics nor muscle oxygenation via TOI (*i.e.,* TOI on-kinetics) have been assessed in patients with HFpEF, which has thus far contributed, in part, to our limited understanding of the rate-limiting physiological processes accounting for the exercise intolerance that is hallmark in this population. Studies in patients with HFrEF have more reporting with muscle TOI measurements (6, 19). As previously described, a rapid transient decrease in muscle TOI below steady-state at the onset of exercise followed by a transient increase in TOI back toward baseline levels (TOI "overshoot"; similar to observed MOD1 low-fit controls) reflects

a limitation in muscle diffusive O_2 delivery, while a slow decrease in TOI below steady-state with no subsequent rise in TOI (similar to the TOI trend we observed in our HFpEF patient group) indicates a limitation in $\dot{V}O_{2p}$ on-kinetics that may be related to muscle O_2 utilization (3, 4, 6, 19). Our combined TOI and $\dot{V}O_{2p}$ data in patients with HFpEF suggest that muscle O_2 delivery may be a preliminary limitation to $\dot{V}O_{2p}$ on-kinetics during the early exercise transient, but that O_2 utilization (*i.e.,* what occurs to the O_2 once it arrives at the exercising muscle fiber) may become the rate-limiting step in $\dot{V}O_{2p}$ on-kinetics towards steady-state exercise. Following priming in our patients with HFpEF, the initial (30s) decrease in TOI was greater, suggesting a more rapid TOI response, indicative that muscle O_2 diffusion may have increased. However, the sustained plateau below baseline levels was present before and after priming, suggesting a limitation of O_2 utilization once O_2 delivery is normalized to demand. Taken with priming of $\tau\dot{V}O_{2p}$, it seems possible that improved O_2 delivery can speed $\tau\dot{V}O_{2p}$ during the on-transient to exercise, but O_2 utilization may become the rate-limiting factor in steady-state $\dot{V}O_{2p}$ in these patients.

The high-fit control group TOI profile during MOD1 that demonstrated an initial increase in TOI at exercise onset is a fairly unique finding; Niemeijer et al. (19) reported two HFrEF patients with similar TOI profiles, but attributed it to excess subcutaneous adiposity and excluded those patients from analyses. Excess adiposity was not evident in our sample, especially our high-fit control group. Therefore, an alternative explanation could be that the interrogated tissue in the 4 subjects was being perfused but was not highly active at exercise onset. However, this is unlikely as the group was homogeneous in fitness and in TOI response profiles. The most likely explanation it that high-fit controls had O_2 delivery in excess of O_2 demand in exercising muscles during the onset of exercise.

Raw baseline TOI values were not analyzed in the present study; however, these values may give an indication behind any priming effects on TOI time-course change and potentially $\tau\dot{V}O_{2p}$ (4). Indeed, Benson et al. (4) concluded that lower pre-exercise blood flow distribution would exaggerate the fall in TOI during exercise onset for any given muscle $\dot{V}O_2$. Therefore, if priming exercise elevated baseline TOI levels in our subjects, this may have contributed to speeding of $\tau\dot{V}O_{2p}$ if ΔTOI remained unchanged (as the case in low-fit controls) or facilitated a smaller TOI "overshoot", which was not observed in our high-fit control and HFpEF groups. It may then be possible that faster $\tau\dot{V}O_{2p}$ in high-fit controls and patients with HFpEF was not wholly

attributed to better muscle oxygenation following priming exercise. Even so, multiple factors affect TOI responses (muscle $\dot{V}O_2$, muscle O_2 delivery, PO_2 gradient from the capillary to inside the muscle fiber, and muscle O_2 diffusion capacity as determined by the capillary-to-fiber ratio), and as such, interpreting TOI signals in the context of the O_2 delivery or O_2 utilization argument is speculative at best.

3.3.2 O_2 Delivery on $\dot{V}O_{2p}$: Cardiac Output On-Kinetics, Heart Rate On-Kinetics, and TPR

That cardiac output and heart rate on-kinetics during moderate intensity exercise in patients with HFpEF were comparable to healthy high-fit and low-fit controls should not be surprising. Indeed, resting cardiac function is generally observed to be similar between patients with HFpEF and healthy controls (10), depending on disease severity.

Conversely, TPR has previously been shown to be increased in patients with HFpEF both at rest and during maximal exercise (5), even compared to hypertensive controls (5). This dysfunction is generally related to the microvasculature (5) and not conduit arteries (14) in patients with HFpEF. We demonstrated this detrimental (or would-be detriment in healthy subjects) vascular response to exercise in our patients with HFpEF. However, priming exercise did not improve TPR in any group and thus may not be associated with the overall faster $\dot{V}O_{2p}$ on-kinetics in our study groups, suggesting that increased O_2 delivery at the diffusion rather than perfusion level (20) (as partially supported by early-exercise TOI data following priming) better accounts for the priming effects on $\dot{V}O_{2p}$ on-kinetics. Taken together, the present study provides critical evidence to support the hypothesis that muscle dysfunction (*i.e.,* O_2 diffusion from capillary to muscle fiber) may limit $\dot{V}O_{2p}$ on-kinetics and thus exercise tolerance in patients with HFpEF. Our interpretation is further supported by increasing evidence of muscle dysfunction in patients with HFpEF (*e.g.,* reduced aerobic enzymes (18), mitochondrial content and quality (18), muscle quality (11), capillary density (11, 12), and slow-to-fast twitch fibre ratio (11, 12)). We extend these prior findings by demonstrating that prior heavy exercise may speed $\dot{V}O_{2p}$ on-kinetics in patients with HFpEF, suggesting impaired O_2 diffusion at the muscle bed that limits the ability to increase aerobic metabolism in the muscle.

3.4 Clinical Relevance

3.4.1 Impact of the Study Findings for Clinicians and Patients

$\dot{V}O_{2p}$ on-kinetics has been increasingly studied in the HFrEF population (6, 22, 23) and, as patients generally don't complete daily activities at their peak $\dot{V}O_{2p}$, assessment of the efficiency of cardiovascular dynamics during submaximal exercise may be more clinically meaningful (20). Our data support the clinical use of $\dot{V}O_{2p}$ on-kinetics testing in patients with HFpEF as our patients did exhibit an abnormal $\dot{V}O_{2p}$ on-kinetics response. However, we understand that proper measurement of $\dot{V}O_{2p}$ on-kinetics requires multiple integrative physiology measurements, testing repetitions, and exhaustive post-processing. Therefore, despite the physiologically meaningful data we reported that may be useful for treatment development for patients with HFpEF, we also argue that our type of study design may be restrictive for "real-world" clinical use.

Muscle tissue oxygenation physiology has been proposed for use as characterization of impairments in O_2 delivery and/or utilization in patients with HFrEF (19). The current study demonstrates that NIRS can be used in patients with HFpEF (should adiposity allow) with confidence. TOI on-kinetics in patients with HFrEF have been proven reproducible with test-retest reliability (19). It would be interesting to advance along this path and observe how TOI responds to interventions and changes over time. In conjunction with other cardiopulmonary dynamics and $\dot{V}O_{2p}$ on-kinetics, TOI on-kinetics may provide evidence for peripheral dysfunction in patients with HF.

3.4.2 Cardiac Rehabilitation

As clinicians are becoming more aware of HFpEF as a distinct diagnosis, several exercise training studies have reported both functional (peak $\dot{V}O_{2p,}$ 6-min walk test distance), mental/emotional (perceived quality of life), and physiological improvements following 12+ weeks of regular exercise training (2, 7–9, 13, 15). Exercise training in patients with HFrEF has been shown to improve both microvascular O_2 delivery and O_2 utilization (13). We propose that both $\dot{V}O_{2p}$ and TOI on-kinetics can be monitored in patients with HFpEF undergoing exercise cardiac rehabilitation to objectively quantify the balance between O_2 delivery and utilization in these patients.

3.5 Future Directions

Based on the results of the current study, future research should pursue two avenues:

1. Continue to quantify peripheral limitations in patients with HFpEF using $\dot{V}O_{2p}$ on-kinetics. For example, measure $\dot{V}O_{2p}$ on-kinetics during small muscle mass, single leg exercise (*e.g.,* seated single-leg knee extension) that would have a relatively minimal impact on cardiac on-kinetics (*i.e.,* "unload" the heart) to truly assess the limitations in the periphery on $\dot{V}O_{2p}$ on-kinetics.
2. Incorporate specific cardiac rehabilitation programs for patients with HFpEF that target muscle dysfunction and improve muscle O_2 delivery and utilization. For example, design a cardiac rehabilitation (*i.e.,* exercise training) study with $\dot{V}O_{2p}$ on-kinetics as the primary outcome, accompanied by cardiac output on-kinetics, stroke volume on-kinetics, heart rate on-kinetics, TPR on-kinetics, and TOI on-kinetics to characterize the $\dot{V}O_{2p}$ kinetic data.

The first avenue of study may benefit the HFpEF population by contributing to the aggregated literature on HFpEF pathophysiology and the primary locations of physiological deficits that impair $\dot{V}O_{2p}$ on-kinetics. By quantifying and understanding the central and peripheral limitations on $\dot{V}O_{2p}$ on-kinetics, therapies targeting these limitations may be developed and trialed to improve morbidity and mortality rates, and quality of life. Specifically, exercise training programs have already been shown to be beneficial for physical function, mental well-being, and physiological factors in patients with HFpEF. These programs can be optimized if carefully planned experimental studies reveal key rate-limiting factors to exercise tolerance.

3.6 Conclusion

We conclude that patients with HFpEF may have a rate-limiting detriment in diffusive O_2 delivery in the exercising muscle bed that limits $\dot{V}O_{2p}$ on-kinetics during moderate intensity exercise. Further, this limitation may be attenuated with acute priming exercise in patients with HFpEF, a finding that is consistent with $\dot{V}O_{2p}$ on-kinetics speeding following priming exercise in patients with HFrEF. Therefore, treatment for patients with HFpEF should investigate methods to improve diffusive O_2 delivery. Similarly, we argue that fit healthy control subjects have a primary limitation of O_2 delivery that may be acutely improved through prior heavy exercise.

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