Heart failure (HF) represents the culmination of numerous abnormal signaling pathways that impair the ability of the cardiovascular system to meet metabolic demands [1]. Today, HF remains one of the most common disorders in the United States, with an estimated prevalence of 6.2 million and a 5-year mortality rate exceeding 50 percent [2]. Left ventricular assist devices (LVAD) have emerged as an important therapeutic option for advanced HF with reduced ejection fraction (HFrEF) – a subcategory of HF characterized by markedly impaired systolic function. LVADs support cardiac output by transferring blood from the left ventricle into the ascending aorta, resulting in significant improvements in morbidity and mortality [3-5].

Even with LVAD support, however, patients exhibit impaired exercise tolerance and decreased quality of life. The gold standard for quantifying this functional limitation is peak oxygen consumption (VO_2) determined by cardiopulmonary exercise testing (CPET) [6]. During CPET, a patient exercises on a cycle ergometer that gradually increases in pedal resistance until patient exhaustion or objective signs of hemodynamic instability or myocardial ischemia occur. The patient is simultaneously monitored via 12-lead EKG, a metabolic cart assessing pulmonary gas exchange and minute ventilation, and brachial sphygmomanometer [7]. Through this monitoring system, CPET allows us to objectively assess patient effort and differentiate anaerobic from aerobic exercise capacity. Interestingly, while prior studies have shown that LVAD support significantly improves New York Heart Association class and the ability to conduct daily activities, peak VO_2 assessed during maximal exercise remains severely decreased [8-12].

The precise mechanism of this decoupling phenomenon remains incompletely understood in the LVAD patient population. VO_2 reflects the relationship of two factors: 1) cardiac output (CO), which is determined by heart rate and stroke volume; and 2) the difference in arterial and mixed venous oxygen content (ΔAVO_2), which reflects oxygen diffusion into and subsequent utilization by skeletal muscle. These principles are reflected in the Fick Equation, $VO_2 = CO * \Delta AVO_2$ [13].

Prior studies have shown an association between inadequate exercise CO and decreased peak VO_2 in LVAD patients. Specifically, chronotropic incompetence (CI), defined as an impaired heart rate increase to meet increased metabolic demands, has been cited as a major contributor. In HF, the impaired cardiac output results in a high catecholamine state that leads to cardiac catecholamine receptor desensitization and consequent CI [14]. Even after augmenting cardiac output via LVAD, patients continue to demonstrate CI and an associated decrease in peak VO_2 [15,16]. The effect of ongoing CI on peak VO_2 in LVAD patients may be explained by the differential effect of LVAD support on right- and left-sided circulation. The LVAD supports left-sided CO by continuously draining left ventricular blood volume, irrespective of the heart rate. In contrast, right-sided CO is not supported by the LVAD and may be more reliant on an adequate chronotropic response [17]. Therefore, in LVAD patients with CI, diminished right-sided CO may reduce left-sided preload to ultimately manifest as decreased exercise tolerance. Still, a causal relationship between CI and decreased peak VO_2 has not yet been demonstrated.

On the other hand, HF-induced skeletal muscle dysfunction (reflected by decreased peak ΔAVO_2) has also been validated as a contributor to abnormal peak VO_2. Normally, oxygen extraction and utilization by skeletal muscle increases in response to exercise, resulting in an increased ΔAVO_2. The metabolic disturbances associated with HF induce various histological and biochemical changes in peripheral skeletal muscle that impair this physiological response. One of the major changes is a shift toward a predominance of type II glycolytic fibers and a relative decrease in type I oxidative fibers. In concordance with this shift away from oxidative metabolism, decreases in capillary density, oxidative enzyme activity, and the number and size of mitochondria have been shown in HF [18-20]. These alterations manifest as a reduction in peak ΔAVO_2 and peak VO_2 [21-23]. In the setting of LVAD, however, our understanding of skeletal muscle pathology and its effect on exercise tolerance remains limited.

Thus, the precise etiology of exercise intolerance in the LVAD patient population remains unclear. Accordingly, I propose a clinical trial that investigates the impact of central cardiac impairment and peripheral skeletal muscle dysfunction on functional capacity in patients with continuous flow LVAD.

Phase 1: Baseline Measurements

In this first phase, I will enroll 80 patients in order to establish baseline peak VO_2 measurements and to examine their association with chronotropic response and altered skeletal muscle function. This will be accomplished by invasive cardiopulmonary exercise testing (iCPET), a method that has been shown to be both safe and effective. This evaluative tool features the same exercise protocol and monitoring apparatus as non-invasive CPET (described above) with the addition of radial and pulmonary artery catheters for arterial and mixed venous blood sampling, respectively. This additional component allows for direct analysis of hemodynamic changes and peripheral oxygen extraction, an element that has been lacking in prior studies [7].

By measuring heart rate, ΔAVO_2, and peak VO_2, I will assess the effect of chronotropic response and/or peripheral oxygen extraction on functional capacity. Heart rate reserve (HRR) will be used as a measure of chronotropic response. HRR is determined by calculating the chronotropic response between rest and maximal exertion and relating this value to the patient's age-predicted maximal heart rate (APMHR), the latter estimated as (220-age). Therefore, HRR = [(peak HR minus resting HR)/(APMHR minus resting HR) x 100] [14]. On the other hand, peripheral oxygen extraction will be directly measured as ΔAVO_2 determined by iCPET. The spectrum of HRR and ΔAVO_2 measurements will then be plotted against peak VO_2. The strength of using this iCPET approach is that it directly assesses ΔAVO_2 as well as HRR. This information allows comparison of the relative impacts of chronotropic response and peripheral muscle-related abnormalities on impaired peak VO_2. While these relationships would be associational, they would provide novel insights into the mechanisms mediating impaired VO_2 in LVAD patients.

To deepen the dissection of the skeletal muscle processes responsible for expected decreases in ΔAVO_2 and to provide a baseline for the interventions in phase 2 below, we will also obtain percutaneous needle biopsies of the vastus lateralis (a skeletal muscle classically used in these studies) 1 week before iCPET. These will be used for enzyme analysis and histological assessment as described [18]. Protein levels and activities will be assessed for enzymes involved in glycolytic and oxidative metabolism – including phosphofructokinase, pyruvate kinase, lactate dehydrogenase, succinate dehydrogenase, and citrate synthetase. Histological assessment will include capillary density, fiber type, and mitochondrial volume and density. To minimize the risk of bleeding, anticoagulation medications will be temporarily stopped and coagulation tests will be performed before biopsies are obtained.

Phase 2: Intervention and Reassessment

In the second phase, I propose to assess the effect of correcting CI and/or peripheral muscle function on peak VO_2. In order to accomplish this, I will identify three patient groups based on the iCPET measurements in phase 1: those with CI, those with decreased peak ΔAVO_2, and those with both CI and decreased peak ΔAVO_2. Consistent with previous studies, CI will be defined as an age-predicted HRR below 80% [14]. Likewise, based on previously reported estimates, reduced peak ΔAVO_2 will be defined as less than 14 mL O_2/100 mL blood at maximal exertion [24]. Each group will undergo a specific intervention with follow-up iCPET assessment and muscle biopsy. Of the 80 patients from phase 1, I hope to include at least 20 patients in each intervention group – similar to prior studies investigating interventions in LVAD patients [25-28].

Hemodynamic Intervention: Specific guidelines regarding appropriate cardiac pacing settings in the context of HF are currently lacking. Interestingly, HF patients with concomitant CI managed with permanent pacemaker (PPM) have exhibited improved exercise tolerance following rate-responsive pacing (RRP), a mode of cardiac pacing in which the heart rate increases in response to increased metabolic needs during activity [28,29]. Attempts to reproduce this finding with objective CPET measurements in the LVAD patient population, however, have yielded inconclusive results [26]. Accordingly, I will test this hypothesis with the CI cohort. One week after baseline iCPET (phase 1), patients with CI who already have a PPM in

place will be switched to RRP mode for 10 weeks. Devices will be programmed to reach 80% HRR within two minutes of exercise.

Peripheral Muscle Intervention: Studies have shown improved functional capacity following exercise-based rehabilitation programs among HF patients [30]. This improvement has been linked to an increase in oxidative metabolism and a return to native mitochondrial structure and function. While improved peak VO_2 following exercise-based rehabilitation has been shown in the LVAD patient population, the mechanism of this improvement has not been established [25,27]. Using the decreased peak ΔAVO_2 cohort, I will examine the effect of exercise-based rehabilitation on ΔAVO_2 and determine if improvements in ΔAVO_2 correspond with an increase in peak VO_2. These patients will undergo a high-intensity interval training (HIIT) program that has demonstrated improved exercise performance in patients with LVAD. Patients will be supervised during 15 standardized training sessions using a cycle ergometer over the course of 8 weeks (2 sessions per week). In each session, patients will begin with a 3-minute warm-up phase at 50% of the peak power output (PPO) achieved on baseline iCPET (phase 1). This will be followed by six 30-second challenge phases at 100% PPO separated by 4-minute rest phases at 40% PPO [25].

Combined Intervention: The cohort of patients with concurrent CI and decreased peak ΔAVO_2 will undergo both interventions simultaneously.

One week after the conclusion of intervention, study subjects will undergo repeat iCPET. In the hemodynamic cohort, responders to the RRP intervention will be defined as those who achieve HRR greater than 80% at maximal exertion on repeat iCPET. In the peripheral muscle cohort, responders to HIIT intervention will be defined as those who demonstrate $\Delta AVO_2 \geq 14$ mL O_2/100 mL blood at maximal exertion on repeat iCPET. Finally, in the combined cohort, responders will be defined as those who meet criteria for response to either intervention. Among responders, pre- and post-intervention peak VO_2 will be compared to determine the extent to which each intervention arm improved functional capacity. Additionally, repeat biopsies of the vastus lateralis muscle will be obtained from responders in each group for comparison with baseline biopsy results from phase 1. Again, anticoagulants will be held and coagulation studies will be performed before biopsy obtainment to ensure patient safety.

The purpose of phase 2 is to move the associational findings in phase 1 to the level of cause and effect. Moreover, comparison of muscle biopsy findings before and after intervention may provide mechanistic insights into the relationship between CI, peripheral oxygen usage, and the metabolic alterations of skeletal muscle. While this information would be of great value, I recognize that pacing and exercise are themselves very complex interventions with respect to the precise underlying molecular and cellular processes. Therefore, further investigations would be needed to dissect effects at a more fundamental level. That acknowledged, identification of RRP and exercise as therapeutic interventions to improve peak VO_2 in LVAD patients would be of clinical value.

By elucidating the etiology and mechanism of impaired functional capacity among LVAD patients, this project may provide evidence for specific therapeutic targets and have far-reaching implications for long-term treatment strategies. I anticipate that both CI and decreased ΔAVO_2 will be associated with decreased peak VO_2. Accordingly, I expect that correction of each abnormality, via RRP or HIIT, will result in significant improvements in peak VO_2 with the greatest increase in peak VO_2 occurring in the combined cohort. Lastly, I hypothesize that these post-intervention improvements will correlate with evidence of increased oxidative metabolism in muscle biopsies (reflected by increases in oxidative enzyme activity, capillary density, mitochondrial volume/density, and predominance of type I oxidative fibers).

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