University of Alberta

CARDIOVASCULAR FUNCTION AND KINETICS DURING UNILATERAL KNEE EXTENSOR EXERCISE IN HEART TRANSPLANT RECIPIENTS

by

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"Learning is not attained by chance; it must be sought for with ardor and attended to with diligence."

Abigail Adams, (1744-1818)

Dedication

This manuscript and the work necessary to complete it are dedicated to Lindsay Hunt. Without her love and support, this could not have taken place. Deserving equal dedication are my mother and father who listened patiently to my thoughts and feelings and provided support however possible.

Abstract

The aim of this investigation was to determine pulmonary oxygen uptake, cardiovascular and muscle oxygenation reserve and kinetics between heart transplant recipients (HTR) and healthy age- and activity-matched controls during unilateral knee extensor (UKE) exercise. The main findings were: 1) a lower peak and reserve pulmonary oxygen uptake secondary to reduced peak and reserve cardiac output and muscle oxygen extraction; 2) increased oxygen extraction for any sub-maximal value of pulmonary oxygen uptake; 3) impaired pulmonary oxygen uptake kinetics secondary to prolonged cardiac output and muscle de-oxygenation kinetics and; 4) impaired pulmonary oxygen uptake off-kinetics as a result of slower muscle re-oxygenation kinetics in HTR compared to controls. HTR have impaired cardiovascular and skeletal muscle peak, reserve and kinetics compared to controls despite the reduction of active muscle mass during UKE exercise.

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List of Abbreviations

AMP- the difference between the baseline and steady state of the monoexponential increase or decrease

$a\text{-}vO_{2diff}$ - arterio-venous oxygen difference; the oxygen concentration difference between arterial and venous blood

CON - control group

Cr - creatine; end product of PCr phosphorylation, required in ATP production

ECG - electrocardiogram

HHb - deoxygenated hemoglobin + myoglobin concentration gained from near infrared spectroscopy

[Hb+Mb] - hemoglobin + myoglobin concentration

HR - heart rate

HTR - heart transplant recipients

MAP - mean arterial pressure

μM - micromoles

NIRS - near infrared spectroscopy

O_2Hb - oxygenated hemoglobin + myoglobin concentration gained from near infrared spectroscopy

PCr - phosphocreatine; phosphorylated to phosphate and Cr during energy production

PO_2 - capillary oxygen pressure

$\dot{Q}$ - cardiac output

SEM - standard error of measurement

SV- stroke volume

SVR - systemic vascular resistance

τ – tau; effective time constant (representative of a 63% change from baseline)

$t^{1/2}$ - time to achieve an overall 50% change from baseline

TD - time delay from onset of exercise

TOI - tissue oxygenation index; a ratio of $O_2Hb/O_2Hb+HHb$ expressed as a percentage

UKE - unilateral knee extensor

θt - ventilatory threshold, the point at which expired carbon dioxide concentration exceeds expired oxygen concentration

$\dot{V}O_{2p}$ - pulmonary oxygen uptake; the amount of oxygen that an individual can transport from the lungs and utilize by the working muscles during peak exercise

$\dot{V}O_{2m}$ - muscle oxygen uptake, the amount of oxygen used by the muscle during exercise

W - watts

Chapter One: Introduction

1.1 Brief Background

Heart transplantation is an important surgical intervention that improves survival in select individuals with end-stage heart failure. Despite increased survival and improved left ventricular systolic function, heart transplant recipients' (HTR) peak aerobic capacity is 30-50% lower than age-matched healthy individuals[1]. The mechanisms responsible for the abnormal aerobic capacity have been attributed, in part, to abnormalities in cardiovascular and skeletal muscle function that result in a reduced oxygen delivery and utilization by the active muscles[1-3]. A limitation of previous studies examining the acute cardiovascular responses in HTR was the primary focus on aerobic exercise involving a large muscle mass (i.e. two-legged cycling or treadmill exercise)[1-3]. Saltin[4] demonstrated that the heart's capacity to deliver oxygen to the active muscles during large muscle mass aerobic exercise is a major limiting factor. Specifically, a cardiac output ($\dot{Q}$) of 50-60L·min^{-1} would be required to deliver oxygen to the muscles during intense large muscle mass aerobic exercise. In a later investigation, these researchers demonstrated that vascular conductance rates were similar between heart failure patients and healthy individuals during small muscle mass (unilateral knee extensor, UKE) exercise[5]. Thus, the ability of the central circulation to deliver oxygen to the muscle may not be the primary limiting factor provided that the size of the active muscle mass is small. As such, the impaired cardiovascular performance found in HTR may not be the main limiting factor during UKE exercise. A further limitation of previous research is the failure to assess cardiovascular and skeletal

muscle oxygenation kinetics during a step-increase in work rate as well as a step-decrease back to rest during UKE exercise in HTR.

1.2 Purpose

The aim of this study was to examine peak cardiovascular and skeletal muscle function and reserve during maximal UKE exercise in HTR and healthy age- and activity-matched controls (CON). A secondary objective was to examine pulmonary oxygen uptake ($\dot{V}O_{2p}$), $\dot{Q}$ and skeletal muscle de-oxygenation (HHb) on-kinetics during a square-wave onset to moderate intensity UKE exercise. The tertiary objective was to assess the $\dot{V}O_{2p}$, $\dot{Q}$ and skeletal muscle re-oxygenation (O_2Hb) off-kinetics during a step-decrease to rest from moderate intensity UKE exercise.

1.3 Hypothesis

The primary hypothesis was that peak and reserve $\dot{V}O_{2p}$ would be significantly lower due to a lower peak and reserve $\dot{Q}$ and HHb in HTR compared to CON. A secondary hypothesis was that HTR would have prolonged $\dot{V}O_{2p}$ on-kinetics secondary to prolonged $\dot{Q}$ and HHb on-kinetics. Finally, the tertiary hypothesis was that the $\dot{V}O_{2p}$, $\dot{Q}$ and O_2Hb off-kinetics would be impaired in HTR versus CON following UKE exercise.

1.4 Delimitations

1) The sample consisted of 5 male HTR and 5 CON.

2) Testing was performed on a specially designed knee extensor ergometer[6] (Appendix A).

1.5 Limitations

1) The degree to which the HTR participants represented clinically stable HTR in the general population and the degree to which CON represents healthy persons in the general population.

Chapter Two: Review of the Literature

2.1 Introduction

Heart transplantation is an important surgical intervention improving ventricular systolic function and survival in select individuals with end stage heart failure. Despite these improvements, HTR continue to have a marked reduction in peak $\dot{V}O_{2p}$[1]. The reduced peak $\dot{V}O_{2p}$ is due, in part, to: cardiac allograft de-innervation[1,2,7,8], diastolic dysfunction[1-3,9-17], abnormal vascular function[18-21] and skeletal muscle dysfunction[22,23] associated with pre-transplant/post-transplant deconditioning[24-26], the surgical procedure and post-transplant immunosuppressive therapy[27-32]. The following literature review describes the cardiopulmonary, vascular and muscle oxygenation factors which limit exercise capacity during large muscle mass aerobic exercise in HTR.

2.2 Cardiovascular Limitations to Exercise Capacity in Heart Transplant Recipients

In healthy individuals, the removal of parasympathetic tone results in an increase in heart rate (HR) from the basal rate to approximately 100 beats·min^{-1}, thereafter, enhanced sympathetic tone increases the HR response to peak exercise with assistance from catecholamine circulation. In contrast, as a result of de-innervation, HTR have a higher resting HR and the increase in HR during exercise is primarily due to circulating catecholamines resulting in a delayed and blunted chronotropic response. A consequence of this impairment is that a greater reliance on preload is necessary to increase stroke volume (SV) and $\dot{Q}$ during exercise[1-3,9-17]. However, mechanisms responsible for preload increase at rest and exercise are attenuated in HTR compared to their healthy age-matched counterparts. Abnormal diastolic function[1-3,9-17] is attributed to ischemic reperfusion injury,

fibrosis and hypertension[33] associated with immunosuppressive therapy[34,35]. As a result, the abnormal diastolic function reduces left ventricular compliance during exercise resulting in reduced preload and peak SV. Taken together, the blunted HR peak and reserve and blunted peak SV, result in reduced peak and reserve $\dot{Q}$ in HTR during large muscle mass aerobic exercise[1,3].

The regulation of blood flow during exercise is essential for optimal distribution of $\dot{Q}$ to the active muscles and is mediated by mechanical factors, local metabolic intermediates and sympathetic tone[36]. The skeletal muscle pump is responsible for the increase in blood flow when beginning exercise by increasing the arterial-venous pressure gradient[36-38]. Following the initial rise of blood flow local metabolic intermediates (endothelial and non-endothelial regulated) further control vessel size in order to accommodate a greater $\dot{Q}$. Once a steady state is reached blood flow regulation becomes a balance between mechanical and metabolic influences with over-arching sympathetically mediated vasoconstriction.

Heart transplant recipients have increased resting systemic vascular resistance (SVR) as a result of attenuated peripheral vascular endothelial function[39]. Decreased nitric oxide production contributes to difficulties augmenting exercising blood flow[19]. The mechanism underlying the decrease in endothelial vasodilatation may be due to reduced nitric oxide production associated with cyclosporine[21]. Globally, the increased sympathetic tone of the vasculature affects blood volume balance by reducing inhibition of atrial natriuretic peptide secondary to de-innervation[40-42]. Ultimately, increased SVR attributed to poor endothelial function[21] and increased sympathetic tone[40-42] lead to reduced oxygen delivery by reducing vascular conductance during exercise[1].

Oxygen utilization during exercise is diminished in HTR as a result of abnormalities in muscle structure[43] and aerobic oxidative enzymes[44]. Specifically, decreased skeletal muscle capillary density and capillary to fiber ratio are reduced resulting in a decreased oxygen delivery to the muscle[43,45]. Pre-transplant alterations of skeletal muscle morphology are also present post-transplantation, and include a shift in muscle fiber type from type I oxidative fibers to less efficient type II glycolytic muscle fibers[31,45]. This affects oxidative efficiency of the mitochondria, where rate limiting enzymes (pyruvate dehydrogenase, citrate synthase and cytochrome oxidase complex III) are also decreased[45]. As such, a consequence of de-conditioning[24-26] and immunosuppressive therapy[27-32], is the decreased ability to utilize oxygen efficiently at the site of muscular contraction in HTR[22,23], reducing peak $\dot{V}O_{2p}$.

2.3 Unilateral Knee Extensor Exercise and the Relation to Heart Transplant Recipients

Peak $\dot{V}O_{2p}$ is severely reduced post-heart transplant as a result of abnormal cardiovascular and skeletal muscle function. A limitation of prior investigations was the use of large muscle mass exercise. Specifically, intense large muscle mass exercise would require a $\dot{Q}$ of 50-60 $L \cdot min^{-1}$ in healthy humans in order to supply oxygen sufficiently to the active muscles[4]. However, the heart's pumping capability, in healthy populations, is able to sufficiently delivery blood to the active muscle if the amount of muscle mass engaged during exercise is small, such as in UKE exercise[4-6,46,47]. Thus, small muscle mass exercise may provide a model where blood flow exceeds oxygen demand. Currently, UKE exercise has only been examined in healthy males[4,6,46], chronic obstructive pulmonary disease patients[48] and heart failure patients[5]. It remains unknown whether the central circulation of

HTR is able support an adequate blood supply to the working musculature during UKE exercise.

2.4 On-Kinetics during Large Muscle Mass Aerobic Exercise in Heart Transplant Recipients

A second limitation of prior studies involving HTR is the use of peak exercise. However, peak exercise does not reflect the physiological requirements necessary for activities of daily living. Physiological responses during transitions from rest to moderate intensity steady state exercise are reproducible, do not require maximal effort, and have prognostic significance in clinical populations[49]. In comparison to peak exercise data, the kinetic modeling of cardiovascular indices during on-transition to moderate intensity workloads may provide better insight into HTR's adaptation to exercise.

2.4.1 Pulmonary Oxygen Uptake On-Kinetics during Large Muscle Mass Aerobic Exercise in Heart Transplant Recipients

Pulmonary oxygen uptake on-kinetics is controlled by oxygen delivery[50,51] and oxygen utilization[52] and is dependant on the type, duration and intensity of exercise as well as the population under investigation[53]. In healthy individuals, it has been shown that during moderate intensity exercise, the cardiovascular system is sufficient in supplying oxygen to muscles where, consequently oxygen utilization becomes the rate limiting mechanism[54-58].

The adaptation of $\dot{V}O_{2p}$ during on-transient exercise occurs in three distinct phases. Phase I, marked by an abrupt increase in $\dot{V}O_{2p}$ lasting approximately 20 seconds, is a time where latent venous return is delivered into pulmonary circulation. Whereas the phase II (primary component) reflects the return of mixed venous blood from the working muscle at

the lung and has been shown to be similar in time course of actual muscle oxygen uptake ($\dot{V}O_{2m}$)[50]. The phase III (slow component), comprises of the steady state in moderate intensity exercise but with intensities above ventilatory threshold (θt) a slow deviation from steady state occurs which is reflective of the increasing anaerobic contribution to exercise derived mostly from the working muscle[59].

Heart transplant recipients present unique deficiencies to $\dot{V}O_{2p}$ kinetics and may provide new insights into the issue of oxygen delivery and utilization. The progression of phase I $\dot{V}O_{2p}$ kinetics in HTR during cycling exercise is slower due to cardiac de-innervation and diastolic dysfunction[60-63] as compared to CON. Initially, the slowed onset of oxygen delivery was thought to be ameliorated by 'priming' with a precedent bout of exercise[63]. However, Grassi and colleagues[64] demonstrated that the impaired $\dot{Q}$ adaptation to exercise was not solely responsible for reduced $\dot{V}O_{2p}$ kinetics. They concluded that skeletal muscle oxidative respiration is a key rate limiting mechanism to $\dot{V}O_{2p}$ kinetics in HTR increasing the overall time constant (τ, time required for a 63% change from baseline to steady state). This is consistent with evidence of decreased local oxygen delivery and oxygen utilization due to poor vascular function and skeletal muscle abnormalities[43,45]. The deficits observed in $\dot{V}O_{2p}$ kinetics in HTR may be due to the amount of muscle mass engaged in exercise due to the oxygen demand. The use of UKE exercise may provide insight by comparing $\dot{V}O_{2p}$, $\dot{Q}$ and HHb on-kinetics during a condition where delivery is not limiting.

Shoemaker et al.[65] found that $\dot{V}O_{2p}$ kinetics were slowed during double limb knee extension exercise compared to cycling exercise in healthy participants which may reflect a decreased adjustment of $\dot{Q}$ and call for a primary role of local delivery and utilization. In

contrast, Koga et al.[66] found that $\dot{V}O_{2p}$ kinetics were similar between UKE exercise and cycling conditions suggesting potential methodological inaccuracies in past studies. Presently the speed of $\dot{V}O_{2p}$ kinetics during differing protocols is under debate in healthy humans but, within any given exercise modality investigated, are slowed in HTR.

2.4.2 Cardiac Output On-Kinetics during Large Muscle Mass Aerobic Exercise in Heart Transplant Recipients

Heart transplant recipients have reduced $\dot{Q}$ kinetics during large muscle mass exercise due to de-innervation and diastolic dysfunction[60-63]. Cardiac allograft de-innervation will eliminate the parasympathetic withdrawal and sympathetic onset and requires an increased SV to compensate for the blunted HR response. It has been shown that increases in SV are able to compensate for the blunted HR kinetics and thus $\dot{Q}$ kinetics were similar between HTR and controls during a step increase to moderate intensity cycling[61,67,68]. However, Grassi and colleagues[64] found that the predominant mechanism which slowed $\dot{Q}$ kinetics in HTR was secondary to slowed HR kinetics. This was demonstrated by a linear relationship in HR increase rather than a monoexponential increase required for kinetic modeling, where SV was unable to overcome this deficit. However an initial 'priming' bout of exercise, did increase the rate of $\dot{Q}$ for a subsequent on-transient bout of exercise[64], but not substantially to meet the values of controls. It remains to be seen whether the reduced muscle mass of UKE exercise will ameliorate the time constant of $\dot{Q}$ during a primary bout of exercise. Even though a reduced HR reserve and diastolic dysfunction are present in HTR, the $\dot{Q}$ may adjust as quickly in HTR as in controls due to a lesser demand of central circulation when the working muscle mass is reduced.

2.4.3 Muscle De-oxygenation On-Kinetics during Large Muscle Mass Aerobic Exercise in Heart Transplant Recipients

Muscle oxygen uptake kinetics are dependant on the parallel activation of phosphocreatine (PCr) dissociation to creatine (Cr) and phosphate, adenosine diphosphate: adenosine triphosphate ratio and aerobic respiration of the citric acid cycle[69,70]. However, invasive measures which give information on microvascular and $\dot{V}O_{2m}$ kinetics such as the measurement of continuous capillary oxygen pressure (PO_2) by phosphorescence quenching techniques[58], are methodologically and ethically unfeasible for human studies. The use of near infrared spectroscopy (NIRS) has allowed the non-invasive assessment of HHb kinetics with optimal temporal resolution, during the adaptation to exercise. Light in the near-infrared spectrum is primarily absorbed by hemoglobin and myoglobin in the microvasculature and cellular milieu of which approximately 84% of this volume is accounted by capillaries[71]. The presence or absence of bound oxygen to hemoglobin and myoglobin alters light absorption characteristics and provides a measure of oxygenated, deoxygenated and total hemoglobin and myoglobin concentration ([Hb + Mb])[72], with 90% of the signal coming from hemoglobin[73-75]. Current investigators are in agreement that this device reflects the adjustment of muscle oxygen utilization/delivery balance[76-78] under the area of interrogation, as investigators relate this measure to local arterio-venous oxygen difference ($a\text{-}vO_{2diff}$)[76,78-81].

The adaptation of de-oxygenated [Hb + Mb] (HHb) kinetics demonstrates a time course which follows the adaptation of PO_2 in the arterioles/capillaries[77,82,83]. The initial 15-20 second steady PO_2 as shown by Behnke et al.[58] is mirrored by a steady HHb signal of NIRS which than follows a decline in PO_2 or an increase in the HHb signal[57,77,82,83]. A

previous investigation is in agreement that the simultaneous measurement of $\dot{V}O_{2p}$ and HHb kinetics by NIRS will give information on the adaptation of oxygen utilization at the muscle[77]. However, HHb kinetics using NIRS has yet to fully elucidate its true control mechanisms[77].

Lafranconi et al.[81] recently demonstrated that HHb kinetics are similar in HTR and healthy controls during moderate cycling exercise, however peak HHb values were lower in HTR. This finding was attributed to the similar diffusive oxygen exchange between HTR and controls with the deficit lying in the oxidative machinery of the mitochondria. Whether de-conditioning[24-26] and immunosuppressive therapy[27-32] will attenuate HHb kinetics in HTR remains to be fully understood, and may prove to be different during UKE exercise.

2.5 Off-Kinetics during Large Muscle Mass Exercise in Heart Transplant Recipients

Previous studies have shown differences between the time course of on- and off-kinetic adjustment[84-88]. A dynamic asymmetry between on- and off-kinetics in healthy humans has been established as the physiological processes underlying the re-adjustment from exercise to a resting baseline differ[84]. Namely, after the cessation of exercise $\dot{Q}$ remains elevated and the re-adjustment to rest is slowed[89]. This is likely due to a demand for oxygen and other metabolites necessary for recovery of the muscle or simply, due to the re-payment of an oxygen deficit acquired at exercise onset. Moreover, a sluggish re-adjustment of PCr kinetics was discovered by Rossiter et al.[84] showing that skeletal muscle oxygen utilization is slowed upon recovery as well, likely due to more difficulty in re-attaining homeostasis because of increased adenosine diphosphate: adenosine triphosphate ratio and lower pH in the muscle upon completion of exercise. As such, the $\dot{V}O_{2p}$ recovery kinetics of HTR are attenuated when compared to age-matched controls during cycling

exercise[64,90]. The mechanisms responsible for the attenuated adjustment to rest lie within oxygen utilization mechanisms. This seems counter-intuitive as delayed oxygen delivery is a key mechanism responsible for the slowing of the on-kinetics in HTR. However as mentioned above, $\dot{Q}$ remains elevated upon exercise termination in HTR and follows a slowed time course of re-adjustment. This may be beneficial as it would maintain a high PO_2 to aid in recovery after exercise cessation.

The muscle re-oxygenation kinetics (oxygenated [Hb + Mb], O_2Hb) response is similar to PCr readjustment exercise completion and re-adjustment to rest[91]. Its use instead of the HHb signal during exercise off-set is more intuitive as it would represent the concentration of oxygen present to repay the oxygen deficit and continue the re-synthesis of PCr[92].

Therefore, the key variables in determining re-adjustment to rest would be $\dot{V}O_{2p}$ and O_2Hb kinetics. This underscores the fact that skeletal muscle deficiencies are a key limiting factor of the readjustment to rest in HTR, and would give further information regarding skeletal muscle function. It remains to be seen whether UKE exercise will allow for quicker recovery kinetics in HTR as the re-adjustment to homeostasis may be less taxing after small muscle mass exercise.

2.6 Summary

A number of inherent cardiovascular limitations present in HTR result in a reduced peak $\dot{V}O_{2p}$ during large muscle mass aerobic exercise. Theses limitations are due to cardiac de-innervation[1,2,7,8], diastolic dysfunction[1-3,9-17], abnormal vascular function[18-21] and muscle dysfunction[22,23] as a result of pre-transplant/post-transplant de-conditioning[24-26], the surgical procedure and post-transplant immunosuppressive therapy[27-32]. However, these

cardiovascular limitations to peak $\dot{V}O_{2p}$ during large muscle mass exercise may not limit performance during UKE exercise, as shown in other populations[4-6,46]. This raises the issue whether HTR have the ability to perform maximal UKE exercise with similar cardiovascular responses compared to CON. Moreover, the on-kinetic parameters of $\dot{V}O_{2p}$, $\dot{Q}$ and HHb to a moderate intensity workload during UKE exercise may be ameliorated in HTR. Finally the re-adjustment from steady state exercise back to rest of $\dot{V}O_{2p}$, $\dot{Q}$ and O_2Hb also remains to be investigated. Accordingly, the aim of this study is to examine how UKE exercise may provide insight into possible mechanisms of cardiovascular and skeletal muscle function in HTR.

Chapter Three: Methods

3.1 Participants, Inclusion and Exclusion Criteria

The participants included 5 male HTR (Age: 53 ± 3 years, Height: 175 ± 2 cm, Weight: 82 ± 8 kg, Time post-transplant: 6 ± 4 years and 5 CON (Age: 53 ± 3 years, Height: 178 ± 3 cm, Weight: 87 ± 5 kg). Heart transplant recipients were clinically stable and had no clinical or biopsy evidence of rejection. This investigation received approval from the University of Alberta Health Research Ethics Board (Biomedical Panel, Appendix B). Informed consent was obtained prior to study participation.

3.2 Test Day 1: Incremental Unilateral Knee Extensor Test

Testing was conducted at the Exercise Stress Laboratory, Division of Cardiology University of Alberta Hospital. All tests were performed on a specially designed UKE ergometer replicated from a previous investigation[93] (Appendix A), using the participants' dominant leg. Initial practice sessions were performed to allow for protocol familiarity and to ensure that the exercising limb remained passive during the knee flexion phase by allowing the momentum of the flywheel to pull the participant's limb back to the resting position. After a 5 minute rest period, the incremental test proceeded from an initial power output of 0 watts (W) for 1 minute and then increased by 3-5 $W \cdot min^{-1}$ until volitional exhaustion. During the test, expired gases were collected and analyzed with a computerized metabolic mixing chamber system (TrueOne® 2400 Metabolic Measurement System ParvoMedics, Salt Lake City UT). Ventilatory threshold was assessed using the V-slope method[94]. All $\dot{V}O_{2p}$ values were taken as an average of 30 seconds from the last half of each workload.

The impedance cardiograph was used to calculate SV during a10 second sampling period within the last 30 seconds of each workload using Bernstein's equation[95] (Minnesota Impedance Cardiograph, model 304B; Surcom Inc., Minneapolis MN). Heart sounds (S1 and S2) were identified by the phonocardiogram (Hewlett Packard, model 21050A) in conjunction with a 3 lead electrocardiogram (ECG), which were used to landmark B and X points of the dZ/dt waveform by two independent investigators in order to ascertain SV[96]. Heart rate was monitored by 12 lead ECG with values taken within the last 30 seconds of each workload. This ensured close matching of HR to SV for $\dot{Q}$ calculation. Blood pressure was measured within the last 30 seconds of every workload by auscultation of the brachial artery with the use of a sphygmomanometer. Calculations of systemic a-vO_{2diff} ($\dot{V}O_{2p} \div \dot{Q}$), mean arterial blood pressure (MAP, 1/3 x (systolic blood pressure - diastolic blood pressure) + diastolic blood pressure) and SVR (MAP $\div$ $\dot{Q}$ x 80) were calculated from the above measurements.

Oxygenated and de-oxygenated [Hb + Mb] of the dominant limb were determined using a spatially resolved NIRS (Hamamatsu Photonics NIRO 300, Japan) oxygenation monitor. The theory of NIRS is based on the modified Beer-Lambert Law where small [Hb + Mb] absorb different wavelengths of light[97]. Larger conduit vessels have a larger [Hb + Mb] and are said to absorb all of the infrared light, thus NIRS reflects [Hb+Mb] at the microvascular level[72] as approximately 84% of the microvascular volume is accounted by capillaries[71]. The NIRO 300 uses 4 wavelengths of light (775, 805, 850 and 905nm) which enables the detection of more chromophores and thus increases sensitivity of the instrument when compared to other NIRS devices[72,97,98].

The NIRS probes were placed midway between the greater trochanter and lateral epicondyle of the femur on the belly of the vastus lateralis muscle. The probes were fixed in a black probe holder to ensure maintenance of distance between light source and detection probe (5cm), secured to the muscle by tape and then wrapped in a black cloth to ensure minimal intrusion of external light sources.

The intensity of transmitted light was sampled at 2 Hertz continuously and stored as 1 second samples for data analysis. A differential pathlength factor of 3.83 was used[83]. Values of oxygenated O_2Hb and HHb are reported in micromoles (μM) and tissue oxygenation index (TOI; $O_2Hb \div O_2Hb + HHb$) is reported as a percentage of oxygen delivery/utilization; values were taken as an average from the last 30 seconds of each workload.

3.3 Test day 2: Measurement of Pulmonary Oxygen Uptake, Cardiac Output and Muscle Oxygenation On- and Off-Kinetics during Moderate Intensity Unilateral Knee Extensor Exercise

A square wave on-transition to moderate intensity UKE was performed from 0W kicking to 50% of max workload (assessed from Day 1), at the Exercise Stress Laboratory, Division of Cardiology University of Alberta Hospital. The participants performed 4 repetitions (3 minutes 0 W kicking (baseline) + 5 minutes moderate intensity UKE exercise + 5 minutes measured rest with 20 minutes rest between repetitions) of sub-maximal square wave on-transition exercise calculated from the method described by Lamarra et al.[99] (Appendix D). During the tests, continuous $\dot{V}O_{2p}$ was assessed with an expired gas analysis mixing chamber system (ParvoMedics, Salt Lake City, UT). Heart rate was acquired with a CM-5 ECG configuration integrated with the metabolic cart. Stroke volume was

determined continuously by impedance cardiography (Minnesota Impedance Cardiograph, model 304B; Surcom Inc., Minneapolis, MN). Oxygenated and deoxygenated [Hb + Mb] were determined continuously by NIRS (Hamamatsu Photonics NIRO 300, Japan).

3.4 Sample Size

The sample size calculation was based on a 40% predicted difference in $\dot{Q}$ between HTR and CON during peak UKE exercise. The approximate values were 7.0 $L \cdot min^{-1}$ for HTR, 9.8 $L \cdot min^{-1}$ for CON during peak UKE exercise. Based on these values and a standard deviation of 1.6 $L \cdot min^{-1}$, a sample size of 3 was required; thus 5 participants per group were recruited (Appendix D).

3.5 On-Kinetic Data Analysis

Breath-by-breath $\dot{V}O_{2p}$, beat-by-beat HR, SV, $\dot{Q}$, and instantaneous near-infrared spectroscopy derived HHb data were sampled and recorded continuously throughout exercise. Data points were removed if greater than 3 standard deviations from the local mean[99] and interpolated to 1-second intervals. Data from the four square-wave protocols were then time aligned and averaged to yield a single response profile for respective variables. These data were averaged into 5 second time bins to further clarify the response profiles.

The onset of phase II $\dot{V}O_{2p}$ kinetics was carefully determined from the phase I-phase II interface as previously described and modeled up to 180 seconds into exercise. Data were then fit using a monoexponential equation of the form:

$$Y_{(t)} = Y_{(b)} + AMP \cdot [1 - e^{-(t-TD)/\tau}]$$

where Y represent the variable chosen at any time (t); b is the baseline value of Y at the point in time from which the data were fitted; AMP is the amplitude of the increase in Y

above the baseline value; τ is the time constant defined as the duration of time for Y to attain a value which is 63% of AMP, and TD is the time delay.

The same monoexponential model was used, to fit stroke volume and $\dot{Q}$ from the time of load onset. Given that a linear HR increase was observed, in HTR, half times ($t^{1/2}$) were used for group comparison. The NIRS derived HHb data were fit from an increase of one standard deviation above the mean baseline value and fit until 180 seconds into the work load[77,83,100].

3.6 Off-Kinetic Data Analysis

The continuous data was gathered as described above and then further fit for off-kinetic analysis. Pulmonary oxygen uptake, $\dot{Q}$, SV were all fit from the end of exercise (300 seconds) to the end of the recovery period (600 seconds). Heart rate $t^{1/2}$ was used for off-kinetic group comparison. Oxygenated [Hb + Mb] was obtained and fit from an increase of one standard deviation above the mean steady state workload value and fit until end of recovery[101,102].

3.7 Goodness of Fit

The iterative procedure of the computer program (Origin 7.5, Origin Lab Corp., Northampton, MA) employs a Levenberg-Marquardt algorithm whereby the best fit was defined by minimization of the residual sum of squares. The data fitting window was extended iteratively until the exponential fit departed from the measured response profile as determined by 1) visual inspection of the curve for appropriateness of fit, 2) visual inspection of the residuals for clustering and systematic deviations from the x-axis, 3) a sudden increase in τ, and 4) demonstration of a local threshold in the reduced χ^2 value

and minimization of residuals plot. The steps utilized for fit follow the recommendations of Rossiter et al.[84].

3.8 Statistical Analysis

Statistical analysis was performed using SPSS 14.0 (Chicago, IL) software, by independent t-test for between group comparisons of all variables at rest, peak and reserve function of the incremental UKE exercise data. As well, the on- and off-kinetics variables were compared using independent t-test. Relationships between the speed of kinetic onset and offset were determined by Pearson product correlation. All data are presented as mean ± standard error of measurement (SEM). Values were significant at $p \leq 0.05$.

Chapter Four: Results

4.1 Participant Characteristics

Heart transplant recipients were 53 ± 3 years of age, 82 ± 8kg and 175 ± 2cm tall. The amount of time elapsed post-transplantation was 6 ± 4 years. Controls were well matched to the HTR group as they were 53 ± 3 years of age, 87 ± 5kg and 178 ± 3cm tall ($p > 0.05$). Both HTR and CON were deemed to be mildly physically active as all participants reported to participate in at least 30 minutes of activity for no less than 3 days a week. Heart transplant recipients continued all medication throughout testing. Control participants were not receiving any cardiovascular pharmacologic therapy prior to or during the time of testing.

4.2 Resting Hemodynamics

Stroke volumes were significantly lower in HTR compared to CON (Table 1). All other resting variables were similar between groups (Table 1).

4.3 Peak Effort and Exercise Tolerance during Unilateral Knee Extensor Exercise

Both study groups achieved a maximal exercise effort supported by a respiratory exchange ratio above 1.10 (HTR: 1.12 ± 0.05 vs. CON: 1.13 ± 0.02, $p > 0.05$). The ability to complete half or more of the last workload was used as the maximum workload. Pulmonary oxygen uptake and peak power output were significantly lower in HTR versus CON (Table 1).

4.4 Peak Hemodynamic Responses during Unilateral Knee Extensor Exercise

Peak exercise HR, SV, $\dot{Q}$ and a-vO_{2diff} were lower in HTR compared to CON (Table 1). Peak TOI, O_2Hb and HHb were similar between groups (Table 1).

4.5 Cardiovascular and Skeletal Muscle Oxygenation Reserve Function

Reserve $\dot{V}O_{2p}$, Q and a-vO_{2diff} were 40% to 52% lower in HTR compared to CON (Table 1). The reduced $\dot{Q}$ reserve was due to an impaired HR reserve as SV reserve was similar between groups. Reserve SVR was lower in HTR while TOI, O_2Hb and HHb reserve were similar between groups (Table 1). Lastly, HHb was higher for any sub-maximal $\dot{V}O_{2p}$ value in HTR compared to CON (Figure 1).

Table 1. Cardiovascular, skeletal muscle rest, peak and reserve function during unilateral knee extensor exercise

	Rest		Peak		Reserve	
	HTR	**CON**	**HTR**	**CON**	**HTR**	**CON**
PO (Watts)	-	-	36 ± 3	62 ± 5*	-	-
$\dot{V}O_{2p}$ ($mL \cdot min^{-1}$)	309.2 ± 23.7	310.0 ± 25.8	923.4 ± 87.4	1582.1 ± 234.2*	614.2 ± 66.7	1272.1 ± 224.8*
HR ($beats \cdot min^{-1}$)	92 ± 4	78 ± 7	119 ± 4	130 ± 11	27 ± 5	52 ± 12‡
SV ($mL \cdot beat^{-1}$)	60 ± 2	76 ± 4*	78 ± 2	95 ± 5*	18 ± 2	18 ± 3
$\dot{Q}$ ($L \cdot min^{-1}$)	5.5 ± 0.4	5.9 ± 0.4	9.4 ± 0.4	12.2 ± 0.9*	3.8 ± 0.4	6.4 ± 0.9*
MAP (mmHg)	98 ± 5	107 ± 4	124 ± 2	152 ± 6*	26 ± 4	45 ± 3*
a-vO_{2diff} ($ml \cdot 100ml^{-1}$)	5.7 ± 0.7	5.3 ± 0.3	9.8 ± 0.7	12.7 ± 1.0*	4.1 ± 0.3	7.4 ± 1.1*
SVR ($dynes \cdot s \cdot cm^{5}$)	1441 ± 96	1490 ± 140	1070 ± 39	1006 ± 53	-372 ± 100	-485 ± 143
O_2Hb (μM)	0.1 ± 0.4	1.9 ± 1.1	-7.4 ± 1.1	-11.3 ± 2.7	-7.5 ± 1.3	-13.2 ± 2.3
HHb (μM)	-0.4 ± 0.4	0.9 ± 0.6	14.0 ± 3.2	18.6 ± 3.1	14.4 ± 3.2	17.7 ± 3.1
TOI (%)	65.2 ± 1.8	68.2 ± 1.1	47.8 ± 5.1	50.0 ± 2.6	-17.4 ± 3.4	-18.3 ± 2.2

Power output (PO), pulmonary oxygen uptake ($\dot{V}O_{2p}$), heart rate (HR), stroke volume (SV), cardiac output ($\dot{Q}$), mean arterial pressure (MAP), arterio-venous oxygen difference (a-vO_{2diff}), systemic vascular resistance (SVR), oxygenated hemoglobin + myoglobin concentration (O_2Hb), deoxygenated hemoglobin + myoglobin concentration (HHb), tissue oxygenation index (TOI).
*$p \leq 0.05$ vs. HTR; ‡ $p = 0.09$ vs. HTR.

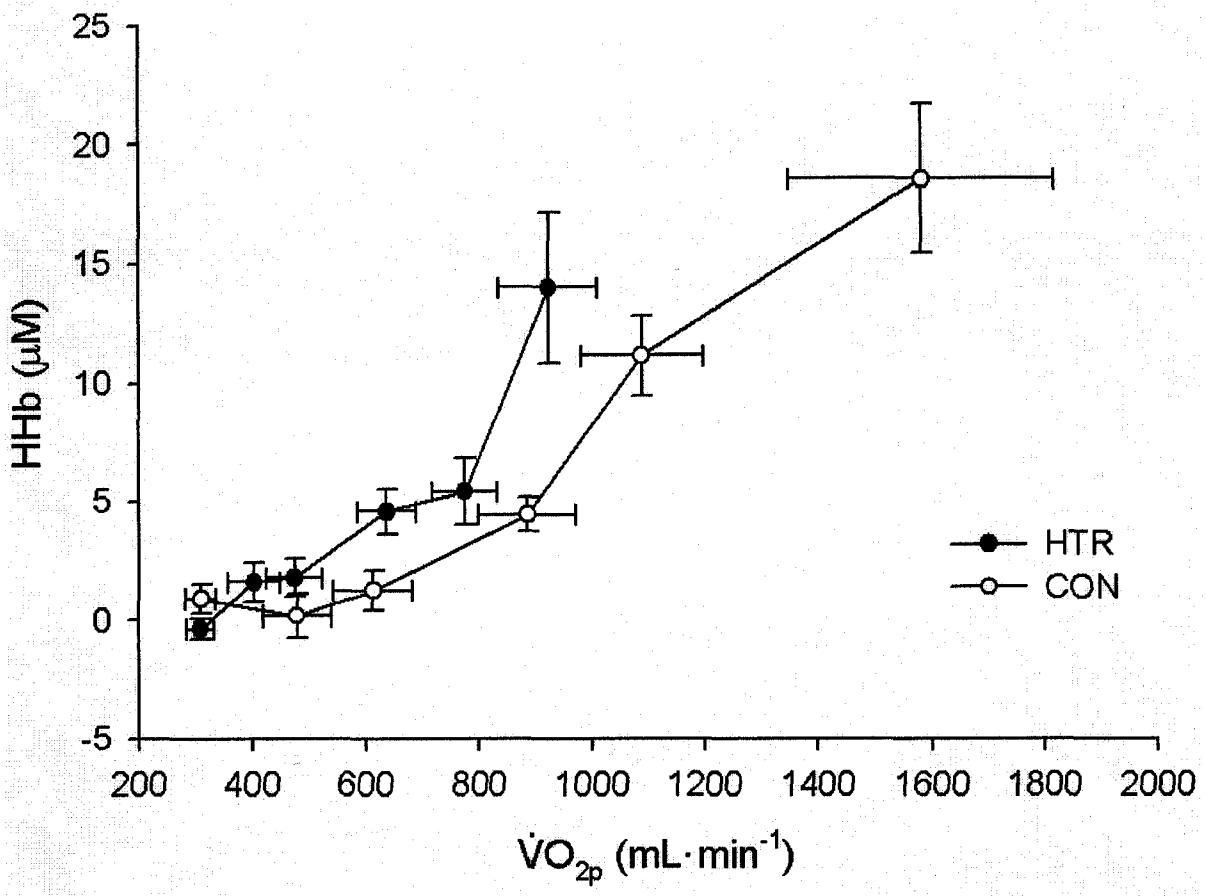

Figure 1. Deoxygenated hemoglobin + myoglobin concentration (HHb) versus pulmonary oxygen uptake ($\dot{V}O_{2p}$) at time points of rest, 0W kicking, 25%, 50%, 75% and peak UKE exercise in HTR versus CON. Values are mean ± SEM.

4.6 Absolute Workload Comparison

Matching of absolute workloads demonstrated no statistical difference between groups for all outcomes except SV and MAP (Table 2).

Table 2. Cardiovascular and skeletal muscle function at matched intensity.

	HTR	CON
PO (Watts)	36 ± 3	40 ± 4
$\dot{V}O_{2p}$ ($mL \cdot min^{-1}$)	923.4 ± 87.4	1051.5 ± 107.9
HR ($beats \cdot min^{-1}$)	119 ± 4	107 ± 8
SV ($mL \cdot beat^{-1}$)	78 ± 2	92 ± 5*
$\dot{Q}$ ($L \cdot min^{-1}$)	9.4 ± 0.4	9.8 ± 0.7
MAP (mmHg)	124 ± 2	139 ± 4*
a-vO_{2diff} ($ml \cdot 100ml^{-1}$)	9.8 ± 0.7	10.8 ± 1.2
SVR ($dynes \cdot s \cdot cm^{5}$)	1070 ± 39	1149 ± 81
O_2Hb (μM)	-7.4 ± 1.1	-9.5 ± 3.0
HHb (μM)	14.0 ± 3.2	7.5 ± 1.3
TOI (%)	47.8 ± 5.1	57.4 ± 2.2

Power output (PO), pulmonary oxygen uptake ($\dot{V}O_{2p}$), heart rate (HR), stroke volume (SV), cardiac output ($\dot{Q}$), mean arterial pressure (MAP), arterio-venous oxygen difference (a-vO_{2diff}), systemic vascular resistance (SVR), oxygenated hemoglobin + myoglobin concentration (O_2Hb), deoxygenated hemoglobin + myoglobin concentration (HHb), tissue oxygenation index (TOI).
*$p \leq 0.05$ vs. HTR.

4.7 Confirmation of Moderate Intensity during Square Wave Onset

To confirm a moderate-intensity workload the difference between the steady state $\dot{V}O_{2p}$ (average of 60 seconds between 180-240 seconds) and end exercise $\dot{V}O_{2p}$ (average of 60 seconds between 240-300 seconds) were calculated for both groups. HTR achieved minimal drift (0.4 ± 8.6 mL·min^{-1}) as did CON (-19.7 ± 9.5 mL·min^{-1}, $p > 0.05$) who gradually began to decrease from 180 seconds to the end of exercise demonstrating a small and insignificant amount of $\dot{V}O_{2p}$ drift. Ventilatory threshold (θt) was lower in HTR (722.8 ± 40.5 mL·min^{-1}) compared to CON (1051.5 ± 107.9 mL·min^{-1}, $p \leq 0.05$). The moderate intensity workload was approximately 90% θt for both HTR and CON. The power output was significantly lower in HTR (18 ± 2 W) than CON (31 ± 3 W, $p \leq 0.05$).

4.8 Pulmonary Oxygen Uptake, Cardiac Output and Muscle De-oxygenation On-Kinetics during Moderate Intensity Unilateral Knee Extensor Exercise

Heart rate during the 0W kicking baseline was significantly higher (HTR: 98 ± 2 beats·min^{-1} versus CON: 81 ± 7 beats.min^{-1}, $p \leq 0.05$) while SV was lower in HTR (61 ± 3 mL·beat^{-1}) versus CON (77 ± 5 mL·beat^{-1}, $p > 0.05$). Baseline $\dot{V}O_{2p}$ (HTR: 518.0 ± 53.9 mL·min^{-1} versus CON: 493.1 ± 34.7 mL·min^{-1}), $\dot{Q}$ (HTR: 6.0 ± 0.3 L·min^{-1} versus CON: 6.2 ± 0.3 L·min^{-1}) and HHb (HTR: 2.7 ± 0.6 versus CON: 0.8 ± 1.1μM) were similar. The $HRt^{1/2}$ was longer in the HTR (113 ± 21 seconds) compared to CON (21 ± 2 seconds, $p \leq 0.05$, Figure 3). The SV kinetics was similar between groups (Table 3). The $\dot{Q}$ kinetics was slower in HTR with a consequent lengthened MRT when compared to CON (Table 3). The HHb kinetics was slower in HTR compared to CON (Table 3). The $\tau\dot{V}O_{2p}$ and the MRT were increased in HTR compared to CON (Table 3) while $\dot{V}O_{2p}$ phase I AMP was lower in HTR (51.7 ± 21.7 mL·min^{-1}) compared to CON (141.0 ± 36.5 mL·min^{-1}, $p >$

0.05). The $\dot{V}O_{2p}$ phase II AMP and TD were lower and slower, respectively, in HTR compared to CON (Table 3). No difference was observed for HR AMP (HTR: 7± 1 versus CON: 10 ± 2 beats·min^{-1}, $p > 0.05$). Steady state values for SV, $\dot{Q}$, HHb and $\dot{V}O_{2p}$ were not different between groups (Table 3). However, HR steady state was higher in HTR (106 ± 2 beats.min^{-1}) versus CON (91 ± 5 beats.min^{-1}, $p \leq 0.05$). The gain ($\Delta\dot{V}O_{2p}/\Delta$Work rate mL·min^{-1}·W^{-1}) was similar in HTR (9.1 ± 0.6) compared to the control group (11.3 ± 1.4, $p > 0.05$).

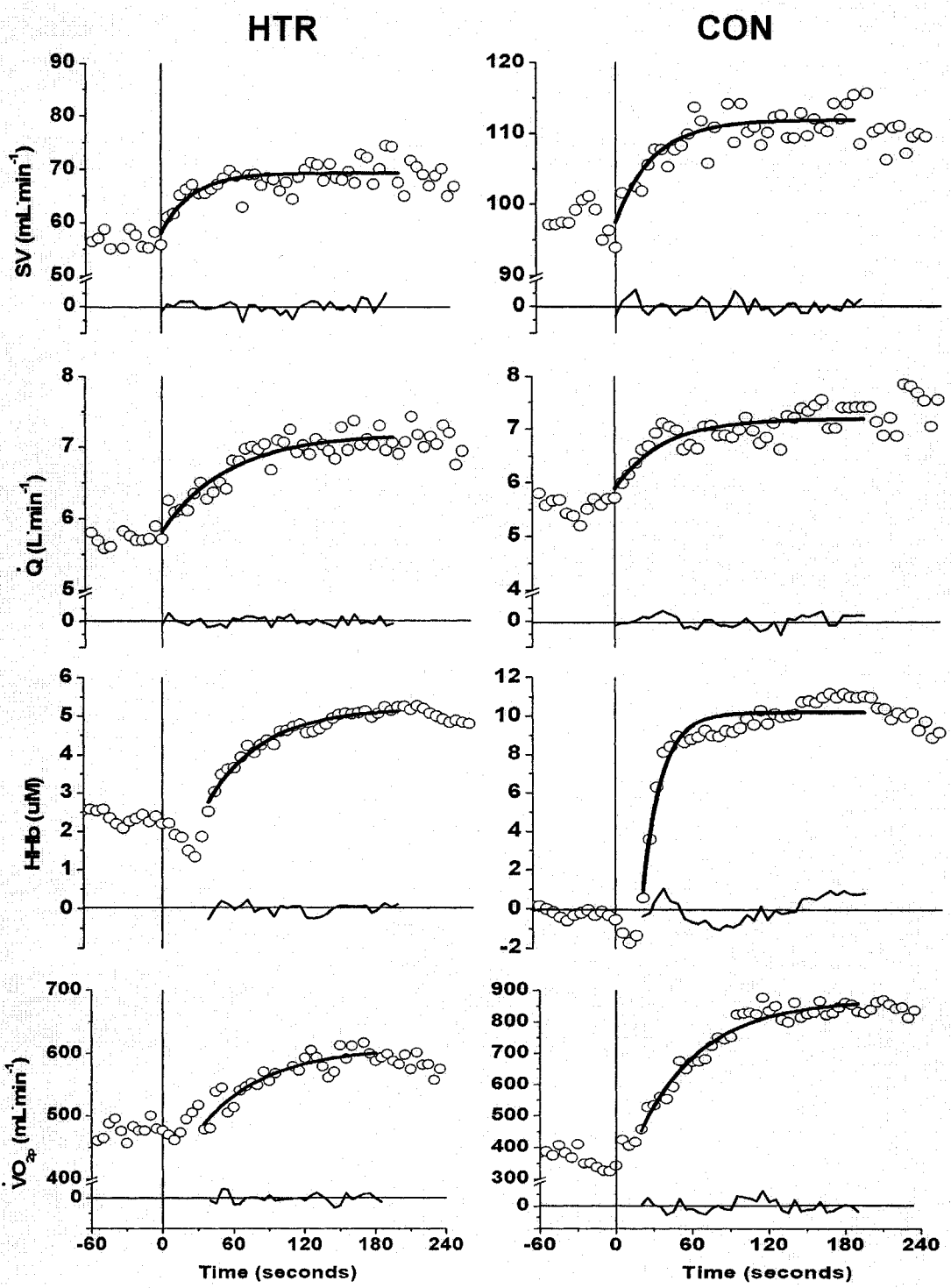

Figure 2. Representative individual on-kinetics for stroke volume (SV), cardiac output ($\dot{Q}$), de-oxygenated hemoglobin + myoglobin concentration (HHb) and pulmonary oxygen uptake ($\dot{V}O_{2p}$) from a 0W kicking baseline to a moderate intensity workload for HTR and CON. Monoexponential fits are presented with residuals plot. Vertical lines (time 0 seconds) represent exercise onset.

Table 3. Pulmonary oxygen uptake, cardiac output and skeletal muscle de-oxygenation on-kinetic responses.

	AMP	TD (seconds)	τ (seconds)	MRT (seconds)	Steady State	95% Confidence Interval (seconds)
SV ($mL \cdot beat^{-1}$)						
HTR	9 ± 2	1.4 ± 0.5	38.7 ± 8.5	40.1 ± 8.1	70 ± 4	14.2 ± 2.0
CON	7 ± 2	0.9 ± 0.3	31.4 ± 6.2	32.3 ± 6.0	84 ± 7	11.1 ± 2.4
$\dot{Q}$ ($L \cdot min^{-1}$)						
HTR	1.3 ± 0.2	0.9 ± 0.3	66.1 ± 7.9	67.1 ± 7.9	7.3 ± 0.4	10.3 ± 1.4
CON	1.4 ± 0.3	1.8 ± 0.8	28.3 ± 3.8*	30.1 ± 4.0*	7.6 ± 0.3	7.9 ± 1.5
HHb (μM)						
HTR	5.2 ± 1.7	20.7 ± 4.1	26.8 ± 4.5	47.5 ± 7.1	7.9 ± 1.3	3.4 ± 0.6
CON	5.4 ± 1.4	21.8 ± 3.0	12.7 ± 2.5*	34.5 ± 4.0	6.2 ± 1.2	3.0 ± 0.5
$\dot{V}O_{2p}$ phase II ($mL \cdot min^{-1}$)						
HTR	152.0 ± 11.3	37.7 ± 1.6	54.2 ± 8.0	91.9 ± 9.5	721.7 ± 64.1	6.0 ± 0.9
CON	226.8 ± 20.4*	25.3 ± 1.2*	30.7 ± 3.3*	56.1 ± 2.2*	860.8 ± 54.4	5.1 ± 1.3

Amplitude, (AMP), time delay (TD), time constant (tau, τ), mean response time (MRT), stroke volume (SV), cardiac output ($\dot{Q}$), deoxygenated hemoglobin + myoglobin concentration (HHb), pulmonary oxygen uptake ($\dot{V}O_{2p}$). *$p \leq 0.05$ vs. HTR.

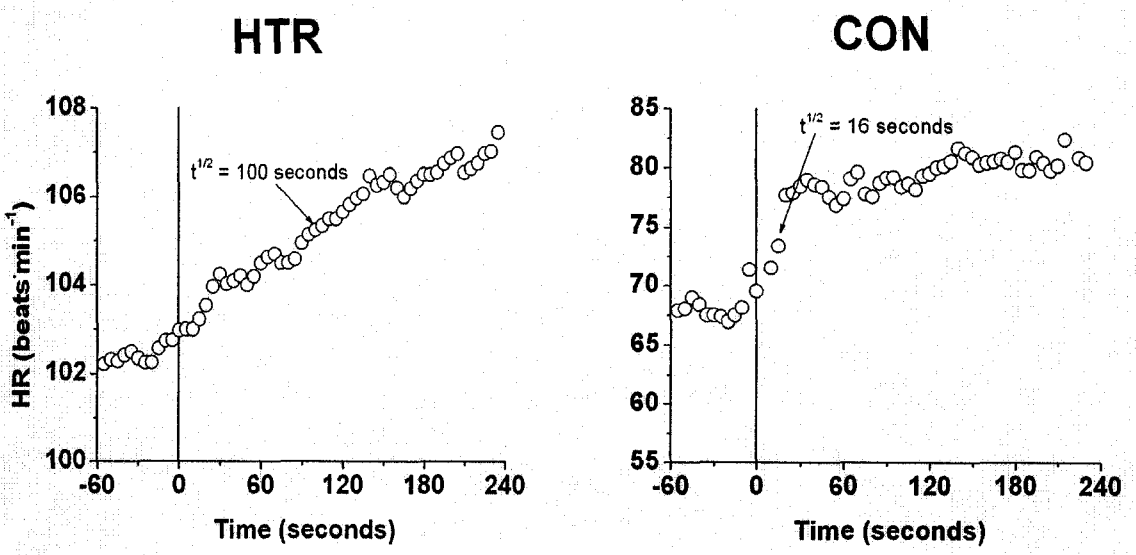

Figure 3. Representative individual heart rate half time on-responses ($HRt^{1/2}$) for HTR and CON.

4.9 Relationships between Pulmonary Oxygen Uptake, Cardiac Output Muscle De-Oxygenation Onset

Cardiac output and HHb time constants were significantly related to $\tau\dot{V}O_{2p}$ ($\tau\dot{Q}$ R = 0.79, $p \leq 0.05$; τHHb R = 0.85, $p \leq 0.05$, Figure 4). The speed of $HRt^{1/2}$ and τSV were not related to the speed of $\tau\dot{V}O_{2p}$.

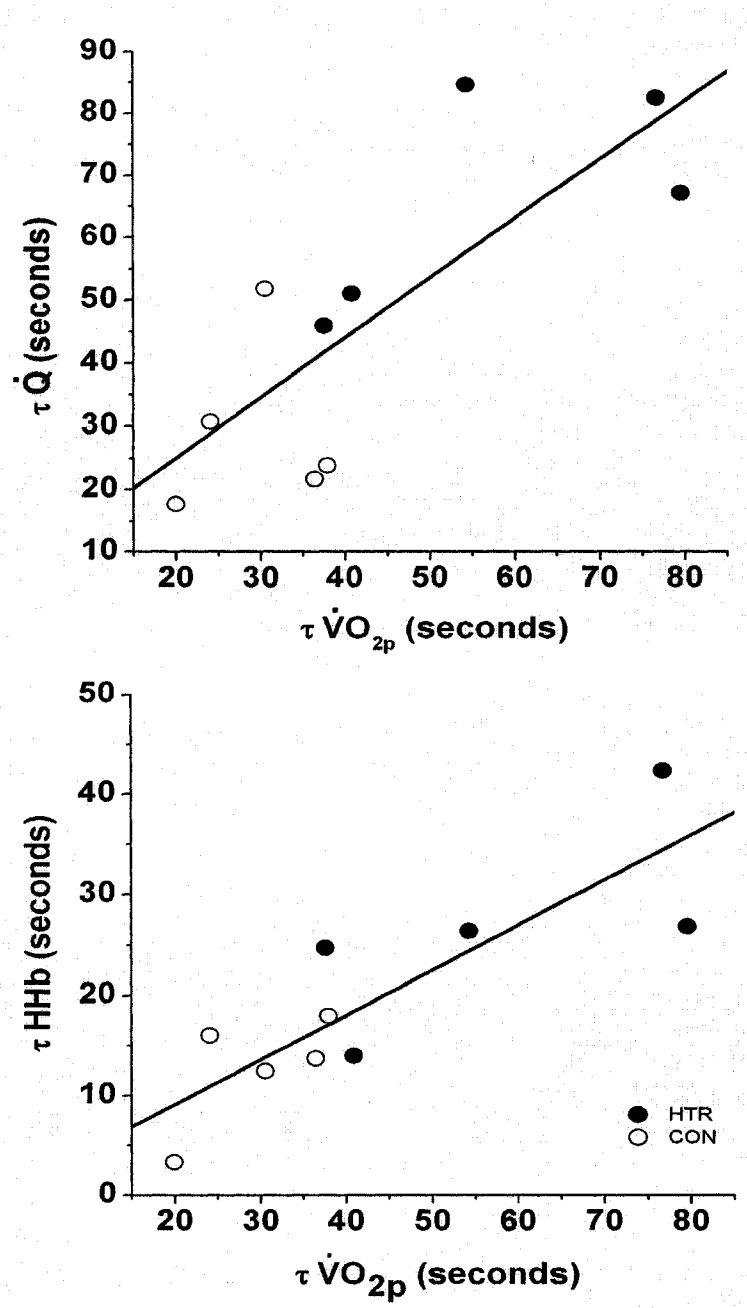

Figure 4. Relationships of on-kinetics between $\tau\dot{Q}$ (cardiac output time constant) vs. $\tau\dot{V}O_{2p}$ (pulmonary oxygen uptake time constant) ($y = 6.09 + 0.95x$, $R = 0.79$, $p \leq 0.05$) and τHHb (muscle de-oxygenation time constant) vs. $\tau\dot{V}O_{2p}$ (pulmonary oxygen uptake time constant) ($y = 0.21 + 0.45x$, $R = 0.85$, $p \leq 0.05$) using grouped data.

4.10 Pulmonary Oxygen Uptake, Cardiac Output and Muscle Re-oxygenation Off-Kinetics following Moderate Intensity Unilateral Knee Extensor Exercise

Steady state HR at the end of exercise was higher in HTR (106 ± 2 beats·min^{-1}) compared to CON (91 ± 5 beats·min^{-1}, $p \leq 0.05$), while steady state SV was lower in HTR (69 ± 3 mL·beat^{-1}) versus CON (84 ± 8 mL·beat^{-1}, $p > 0.05$). End exercise $\dot{Q}$ (HTR: 7.3 ± 0.3 L·min^{-1}versus CON: 7.5 ± 0.4 L·min^{-1}, $p > 0.05$), O_2Hb (HTR: -3.0 ± 1.4 versus CON: -1.2 ± 1.3, $p > 0.05$) and $\dot{V}O_{2p}$ (HTR: 722.1 ± 68.9 versus CON: 841.1 ± 46.0 mL·min^{-1}, $p > 0.05$) were similar between groups. The $HRt^{1/2}$ was longer in the HTR (57 ± 11 seconds) compared to controls (30 ± 4 seconds, Figure 6, $p \leq 0.05$). The HR AMP was lesser in HTR (-6 ± 1 beats·min^{-1}) compared to CON (-14 ± 3 beats·min^{-1}, $p \leq 0.05$). Stroke volume and $\dot{Q}$ kinetics and their respective AMP were not different between groups (Table 4). The respective AMP of O_2Hb and $\dot{V}O_{2p}$ were similar between groups (Table 4). Muscle re-oxygenation kinetics was 52% longer in HTR compared to controls (Table 4, $p \leq 0.05$) contributing to a 24% longer $\tau\dot{V}O_{2p}$ (Table 4, $p > 0.05$), with a trend toward a 33% longer $\dot{V}O_{2p}$ MRT (Table 4, $p = 0.08$). Time delays for all off-kinetic variables were not statistically different (Table 4).

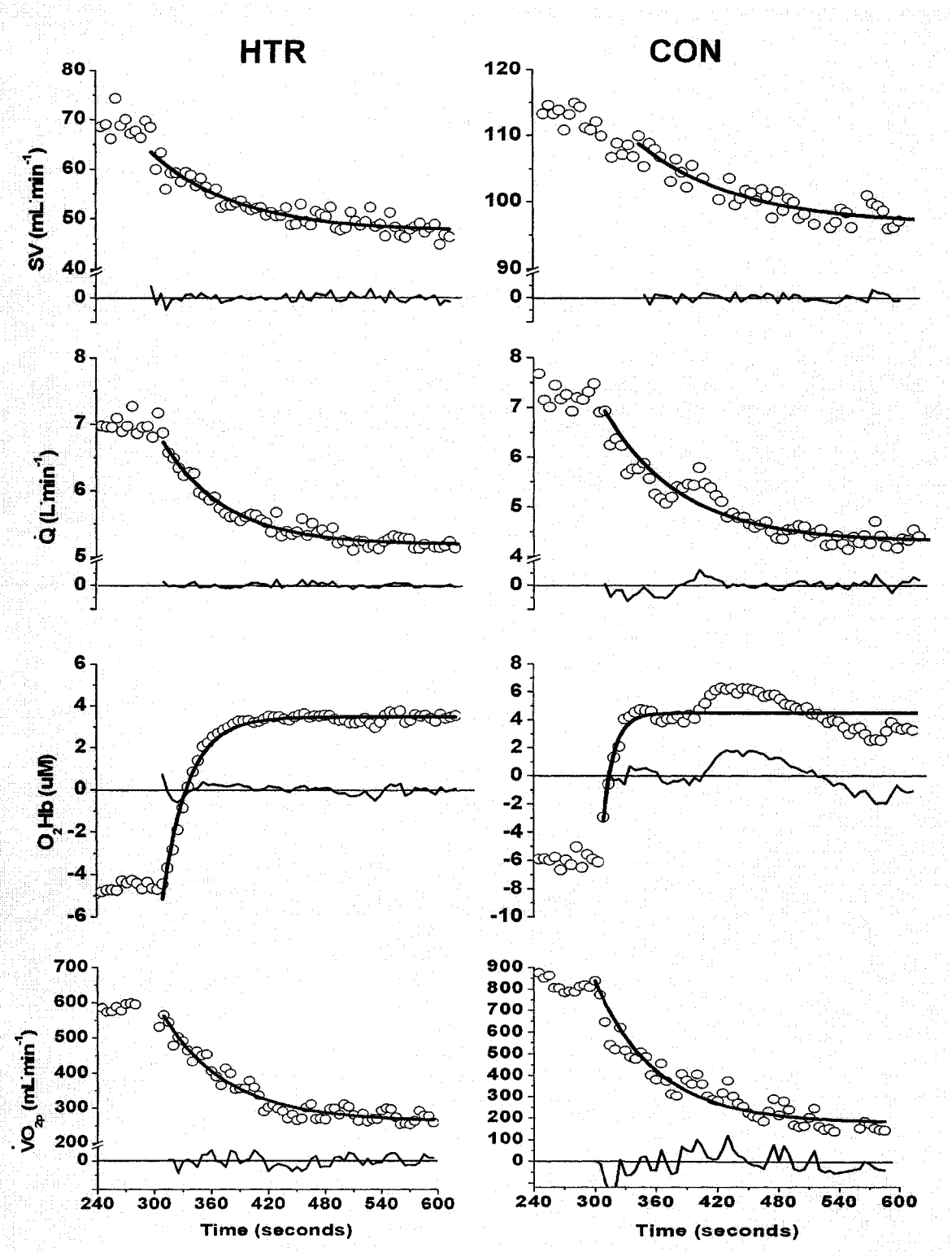

Figure 5. Representative individual off-kinetics for stroke volume (SV), cardiac output ($\dot{Q}$), oxygenated hemoglobin + myoglobin concentration (O_2Hb) and pulmonary oxygen uptake ($\dot{V}O_{2p}$) for HTR and CON. The monoexponential fit for each parameter are included with residuals plot. Time 300 seconds signifies the end of exercise.

Table 4. Pulmonary oxygen uptake, cardiac output and skeletal muscle re-oxygenation off-kinetics responses.

	AMP	TD (seconds)	τ (seconds)	MRT (seconds)	Resting Baseline	95% Confidence Interval (seconds)
SV ($mL \cdot beat^{-1}$)						
HTR	-15 ± 2	4.8 ± 3.3	53.9 ± 12.7	58.7 ± 14.4	54 ± 4	4.4 ± 1.2
CON	-12 ± 2	9.1 ± 6.5	81.6 ± 21.3	90.7 ± 26.4	72 ± 7*	7.4 ± 1.4
$\dot{Q}$ ($L \cdot min^{-1}$)						
HTR	-1.9 ± 0.2	9.0 ± 3.8	64.8 ± 13.1	73.8 ± 12.4	5.4 ± 0.4	3.5 ± 0.8
CON	-2.2 ± 0.4	1.4 ± 1.1	57.1 ± 10.2	58.5 ± 9.9	5.3 ± 0.3	4.0 ± 0.7
O_2Hb (μM)						
HTR	6.1 ± 1.4	9.8 ± 3.7	33.9 ± 6.1	43.7 ± 5.5	3.0 ± 0.2	4.9 ± 2.6
CON	6.1 ± 1.0	13.4 ± 6.4	16.0 ± 3.7*	29.4 ± 4.6	5.0 ± 0.6*	6.0 ± 2.2
$\dot{V}O_{2p}$ ($mL \cdot min^{-1}$)						
HTR	-411.5 ± 56.3	5.7 ± 1.9	77.7 ± 6.9	83.4 ± 6.2	310.6 ± 25.3	2.5 ± 0.5
CON	-557.5 ± 49.6	5.8 ± 2.8	59.2 ± 6.8	65.0 ± 6.6	312.4 ± 39.4	1.8 ± 0.4

Amplitude, (AMP), time delay (TD), time constant (tau, τ), mean response time (MRT), stroke volume (SV), cardiac output ($\dot{Q}$), oxygenated hemoglobin + myoglobin concentration (O_2Hb), pulmonary oxygen uptake ($\dot{V}O_{2p}$). *$p \leq 0.05$ vs. HTR.

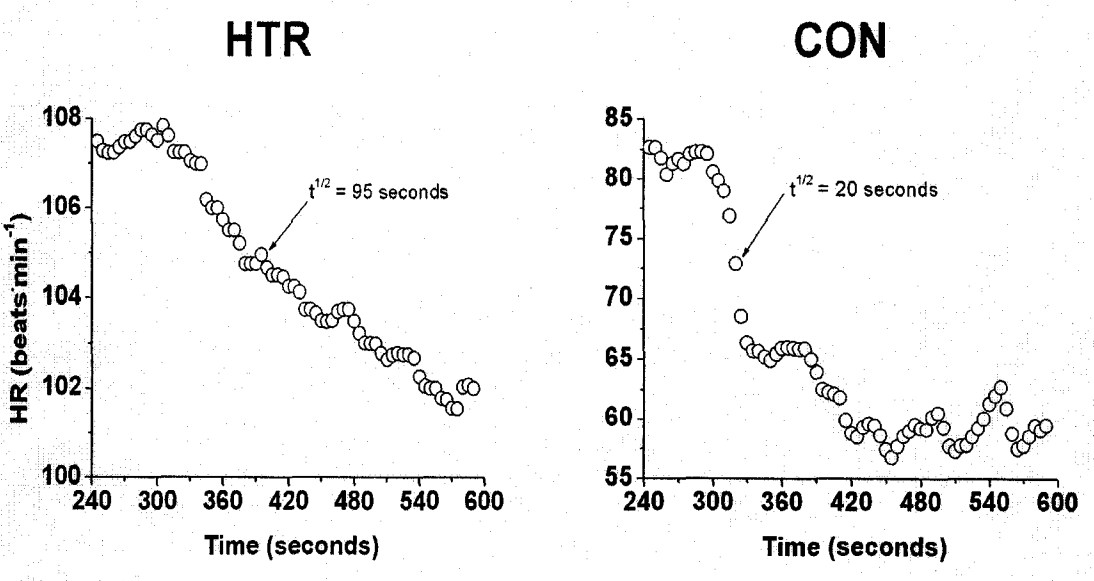

Figure 6. Representative individual heart rate half time off-responses ($HRt^{1/2}$) for HTR and CON.

Chapter Five: Discussion

5.1 Introduction

The key findings of this investigation are as follows: 1) HTR have a lower peak and reserve $\dot{V}O_{2p}$ secondary to the reduced peak and reserve $\dot{Q}$ and a-vO_{2diff}; 2) HTR have an increased oxygen extraction (HHb) for any sub-maximal $\dot{V}O_{2p}$; 3) HTR have impaired $\dot{V}O_{2p}$ kinetics secondary to prolonged $\dot{Q}$ and HHb kinetics and; 4) HTR have impaired $\dot{V}O_{2p}$ off-kinetics as a result of slower O_2Hb kinetics. These findings extend previous studies during peak[1,3,103,104], $\dot{V}O_{2p}$ on-[61,64,68,81,90,105] and off-kinetics[64,90] during large muscle mass (cycling) exercise by demonstrating that $\dot{V}O_{2p}$ peak, reserve, on- and off-kinetics were reduced in HTR during exercise involving a small muscle mass.

5.2 Cardiovascular and Skeletal Muscle Peak and Reserve Function during Unilateral Knee Extensor Exercise

Previous study findings involving large muscle mass aerobic exercise have indicated that limitations to aerobic capacity in HTR occur primarily as a result of a reductions in $\dot{Q}$ and, to a lesser degree, a-vO_{2diff}[1]. In the current investigation, peak and reserve $\dot{V}O_{2p}$ during UKE exercise were 42 and 52% lower in HTR versus CON. The lower peak and reserve $\dot{V}O_{2p}$ were due, in part, to a 23% reduced peak and 40% reduced reserve $\dot{Q}$. Consistent with the findings during large muscle mass aerobic exercise, the blunted $\dot{Q}$ reserve was secondary to a reduced HR reserve as SV reserve was similar between groups. In contrast to studies involving cycling exercise, abnormal oxygen utilization played an equally important limiting role as peak and reserve a-vO_{2diff} were 23% and 45% lower, respectively in HTR versus CON.

The difference between the present study and those conducted previously[1,3,103,104] is the reduction of exercising muscle mass. Andersen and Saltin[6,93] have demonstrated that the central circulation is sufficient to supply oxygen to the skeletal muscles during peak exercise in healthy males when the active muscle mass is small (i.e. 1/3 of total muscle mass). Liguzinski and Korzienewski[106] have modeled the effects of differing muscle mass on oxygen delivery during exercise in silico. They demonstrated that PO_2 is maintained during small muscle mass exercise rather than large muscle mass exercise, as $\dot{Q}$ is sufficient to maintain a suitable PO_2 in the microvasculature during peak exertion. In line with this hypothesis, if HTR peak skeletal muscle perfusion is 250mL·100g·min^{-1}[6] then exercise involving > 5 kg muscle mass will exceed a peak $\dot{Q}$ of 12.5 L·min^{-1}[104], and surpass the ability of the cardiac allograft to maintain blood flow to the working skeletal muscle. Indeed our peak HR (119 beats·min^{-1}), SV (78 mL·beat^{-1}), $\dot{Q}$ (9.4 L·min^{-1}), a-vO_{2diff} (9.8 mL·100mL^{-1}) and $\dot{V}O_{2p}$ (11.5 mL·min·kg^{-1}) values during UKE exercise (involving an estimated 1.2 ± 0.2 kg quadriceps muscle mass) are similar to that found during two-legged cycling (HR:113 beats·min^{-1} ; SV: 82 mL·beat^{-1}; $\dot{Q}$: 9.0 L·min^{-1}; a-vO_{2diff}: 10.42 mL·100mL^{-1} and $\dot{V}O_{2p}$:11.1 mL·min·kg^{-1})[1,3]. Therefore, exercise involving more than this small amount of muscle will exceed the heart's ability to perfuse the exercising muscle mass. Thus, by reducing the active muscle mass, the cardiovascular limitation to exercise is less significant than seen during large muscle mass exercise, revealing an equivalent limitation in the periphery of HTR.

5.3 Cardiovascular and Skeletal Muscle De-oxygenation On-Kinetics during Moderate Intensity Unilateral Knee Extensor Exercise

Prior investigations examining $\dot{V}O_{2p}$ on-kinetics in HTR have reported a delayed adaptation of onset in comparison to healthy age-matched individuals during cycling

exercise[60-64,67,68,107]. This was attributed to a delayed onset of $\dot{Q}$[60,62,63,67] and the slower adaptation of oxidative respiration within the skeletal muscle[61,64,68]. Paterson et al.[63] found that an initial bout of 'priming' exercise increased the adaptation of oxygen delivery sufficiently in order to ameliorate $\dot{V}O_{2p}$ kinetics upon the subsequent square wave transient. Specifically, they demonstrated that an 8% increase in HR ameliorated the $\dot{V}O_{2p}$ on-kinetics in HTR to a rate comparable to their respective healthy controls during the second of two step-increases to moderate intensity cycling exercise. Thus, these investigators concluded that the primary cause of the slowed $\dot{V}O_{2p}$ on-kinetics in HTR was that of a slowed adaptation of $\dot{Q}$ Grassi et al.[64] used varying protocols of square wave onset in order to 'prime' oxygen delivery, and further investigate the controlling mechanisms of $\dot{V}O_{2p}$ kinetics in HTR. They demonstrated that $\dot{Q}$ kinetics was faster upon the second of two on-transients, but was not associated with faster $\dot{V}O_{2p}$ kinetics in HTR. Thus, the delayed $\dot{V}O_{2p}$ kinetics during cycling exercise in HTR was attributed to the slowed onset of oxidative respiration within the working skeletal muscle[64].

Our data extend the findings of these previous investigations, as the slowed $\dot{V}O_{2p}$ kinetics in HTR compared to CON during moderate-intensity UKE exercise was due to a prolonged phase I duration and slower phase II kinetics, which contributed to the overall slower $\dot{V}O_{2p}$ MRT (Table 3 and Figure 2). The prolonged phase I $\dot{V}O_{2p}$ duration was due to the slower $HRt^{1/2}$ (Figure 3) as SV kinetics were similar between the two groups while phase II $\dot{V}O_{2p}$ kinetics were due to slower $\dot{Q}$ and HHb kinetics (Table 3 and Figure 2).

Importantly, $\dot{Q}$ and HHb kinetics were significantly related to the adaptation of phase II $\dot{V}O_{2p}$ kinetics (Figure 4). In the present study a 48% slower $\dot{Q}$ kinetics and 47% slower HHb kinetics slowed phase II $\dot{V}O_{2p}$ kinetics by 43% during UKE exercise. In

comparison, previous research using large muscle mass (cycling) exercise found that τHHb was only 20% slower with an overall 37% attenuation of phase II $\tau\dot{V}O_{2p}$ in HTR compared to controls[81] (however no comparison of $\dot{Q}$ kinetics' contribution to phase II $\dot{V}O_{2p}$ kinetics can be made from this study). This demonstrates that by reducing the active muscle mass to ≤ 5kg places equal importance on the speed of $\dot{Q}$ and HHb kinetics.

5.4 Cardiovascular and Skeletal Muscle Re-oxygenation Off-Kinetics during Moderate Intensity Unilateral Knee Extensor Exercise

Generally, SV, HR and subsequently $\dot{Q}$ remain elevated in order to maintain oxygen delivery, aiding recovery from a prior bout of exercise in healthy individuals[89]. Neither $\dot{V}O_{2p}$ or $\dot{Q}$ off-kinetics were significantly different between groups, however, $\tau\dot{V}O_{2p}$ was 33% reduced in HTR with a trend of a longer MRT in HTR (Table 4 Figure 5). Also, $\tau\dot{Q}$ was 12% longer due to 47% longer $HRt^{1/2}$ in HTR compared to controls.

The use of HHb for off-kinetic analysis has not been previously used as the O_2Hb signal is thought to produce a more meaningful off-kinetics analysis[73,91]. The O_2Hb provides a reflection of microvascular oxygenated [Hb + Mb] concentration at the site of interrogation[72,73,91,98]. McCully et al.[91] demonstrated that upon exercise cessation the O_2Hb signal followed an identical time course to that of PCr off-kinetics measured by nuclear magnetic spectroscopy. In the current study, the slower O_2Hb kinetics in HTR during UKE exercise contributed to the slower $\dot{V}O_{2p}$ off-kinetics. The slowed O_2Hb off-kinetics may be due to decreased capillary density and capillary to fiber ratio in HTR[45] which likely result in decreased oxygen utilization[45] and re-synthesis of PCr upon recovery. Therefore, HTR have longer $\dot{Q}$ off-kinetics compared to CON, which should have allowed for an adequate repayment of the oxygen deficit. However, the 53% slower

O_2Hb kinetics remained as a result of the larger oxygen deficit in HTR compared to controls, supporting the lengthened $\dot{V}O_{2p}$ off-kinetics and MRT.

5.5 Mechanisms of the Impaired Cardiovascular and Skeletal Muscle Function

In the present investigation, abnormal cardiovascular function played an important role in the impairment of $\dot{V}O_{2p}$ peak, reserve, on-and off-kinetics. Specifically, cardiac allograft de-innervation blunted the HR response and attenuated $\dot{Q}$ peak, reserve, and on- and off-kinetics. The blunted HR reserve was the primary factor for the lower peak and reserve $\dot{Q}$ as SV reserve and kinetics were similar between groups. However, diastolic dysfunction and increased myocardial stiffness associated with the cardiac allograft may also reduce $\dot{Q}$ reserve[1,3]. Mettauer et al.[62] correlated phase I $\tau\dot{V}O_{2p}$ to the isovolumic relaxation time of the left ventricle in HTR during exercise onset demonstrating that the slowed $\dot{Q}$ onset was partially due to poor relaxation of the ventricle. Although the present investigation did not measure diastolic function if an absolute change of 8% in ejection fraction is assumed, then the increase in end diastolic volume (+ 15%) would be greater than the decrease in end systolic volume (- 8%) during UKE exercise[1]. This is consistent with a greater utilization of preload reserve as the current findings show similar SV reserve and kinetics (Tables 1, 3 and 4). However, diastolic dysfunction of the cardiac allograft increases chamber stiffness, reducing the effectiveness of preload reserve[12] consistent with the lower peak SV values found during UKE exercise. In combination, a lower peak SV, attenuated reserve HR and $HRt^{1/2}$ are in line with previous studies as the main mechanisms for a blunted $\dot{Q}$ peak, reserve[1,14,16,104] and $\dot{Q}$ on- and off- kinetics in HTR during large muscle mass exercise[64,90].

Pre and post-transplant abnormal vascular function further decreases the available blood flow to the muscle during exercise. Previous studies have shown that HTR have

abnormal endothelial function[18,28,108], as well as a higher sympathetic tone[109], decreasing blood flow and blood flow re-distribution[109]. Furthermore, decreased vascular function has been positively correlated with poor exercise tolerance post heart transplant[18]. Our data support previous research as SVR reserve was lower in HTR compared to CON. In combination, a reduced peak and reserve $\dot{Q}$ and lower reserve SVR will decrease the available blood flow during UKE exercise. The abnormal SVR will also contribute to the impaired O_2Hb off-kinetics in HTR as the oxygen concentration at the site of exchange will decrease upon recovery[91]. Thus the lower $\dot{V}O_{2p}$ peak, reserve and on-and off-kinetics may be secondary to deficiencies in SVR, reducing oxygen delivery to the working muscles.

Persistent skeletal muscle deficiencies serve to limit oxygen utilization post-transplant. Alterations of skeletal muscle morphology are demonstrated by a shift from type I oxidative fibers to less efficient type II glycolytic muscle fibers[31,45] affecting oxidative efficiency. Furthermore, rate limiting enzyme concentrations (pyruvate dehydrogenase, citrate synthase and cytochrome oxidase complex III) are decreased, affecting the overall oxidative capacity[45] and the rate of oxidative adaptation to exercise stress[70,110]. Despite a reduced a-vO_{2diff} at peak HTR demonstrated a higher HHb at the same absolute $\dot{V}O_{2p}$ in HTR compared to CON (Figure 1).When matched for exercise intensity (Table 2), both groups displayed similar hemodynamic values where HTR were unable to attain a higher $\dot{V}O_{2p}$, as they were unable to maintain an adequate rate of oxygen extraction in the face of their lower $\dot{Q}$ at peak[81,111]. It is important to note that the controlling mechanisms of oxygen extraction kinetics are different than the mechanisms responsible during peak exercise[112], suggesting that oxygen extraction was still intact during moderate intensity UKE as shown by similar steady state values of HHb. Only its

speed of onset was attenuated in HTR during UKE exercise. This is easily explained by parallel activation[70,110]. Interestingly, this finding of attenuated HHb on-kinetics in HTR during UKE is in contrast to that of Lafranconi et al.[81]. This investigation showed a slowing of phase II $\dot{V}O_{2p}$ on-kinetics in HTR during cycling exercise, however a speeding of HHb on-kinetics in contrast to their initial hypothesis[81]. Moreover, the HHb steady state values from Lafranconi et al.[81] disagreed with their incremental exercise data, as these investigators demonstrated a similar if not greater oxygen extraction at sub-maximal workloads during an incremental cycling test, and lower AMP of HHb in HTR compared to the control group during the square-wave test. The only explanation available is the difference in exercise modalities and the muscle mass activated.

Finally the lengthened O_2Hb off-kinetics in HTR would be secondary to deficiencies within the re-synthesis of PCr upon exercise cessation. The noted enzymatic deficiencies would serve to slow the use of oxygen and the diffusion of oxygen from the vessels to the cell. Therefore, as a result of de-conditioning[24-26] and immunosuppressive therapy[27-32] peak muscle oxygen extraction and kinetics limit peak exercise and the adaptation to exercise onset and offset equally during UKE exercise in HTR compared to controls.

5.6 Limitations

A limitation of this study was the use of impedance cardiography. However, impedance cardiography has been validated in HTR during large muscle mass exercise[113,114]. It has also been shown to give accurate SV calculation, highly correlated to thermodilution and direct fick methods ($r = 0.9$), when the marking of waveforms was attained through a consensus of two trained individuals as was done in this

investigation[96]. Furthermore, our CON peak $\dot{Q}$ values were similar to that reported by Magnusson et al.[46] for older healthy males during peak UKE exercise.

The absence of limb blood flow measurement is another limitation to this study. However, given the reduced $\dot{Q}$ peak, reserve and kinetics in association with reduced SVR reserve, one would expect HTR to have a reduced limb blood flow in comparison to CON.

The moderate intensity workload HTR did come close to their θt (99.7 ± 5.9%). In fact 2 out of 5 HTR did exercise slightly over their respective θt (~ 105%) during the kinetics protocol. When these 2 HTR are removed the remaining 3 HTR were below θt at 91.7 ± 3.3%, compared to CON at 83.5 ± 5.9%. However, graphical and inter-observer differences are possible in the assessment of θt[94] affecting the percentage of moderate intensity work. Importantly, the $\dot{V}O_{2p}$ kinetics during heavy exercise are only lengthened due to the fact that the MRT is lengthened and not the actual lengthening of the phase II τ value[53]. Therefore, by only fitting to the end of phase II, the present kinetics values represent true responses to moderate intensity exercise. Therefore any contribution of the phase III component that may have come into play was not included in the fitting window. Also, the functional gain during square-wave UKE exercise was within moderate intensity limits (HTR: 9.1 ± 0.6 vs. CON: 11.3 ± 1.4 $mL \cdot min^{-1} \cdot W^{-1}$ $p > 0.05$).

Finally, HTR did not stop their respective vaso-active pharmacologic therapy prior to testing. The ramifications of which may be seen in our resting data as MAP and SVR were lower in HTR compared to CON. This likely had an effect during exercise as SVR and MAP were previously reported to be much higher in HTR compared to age-matched healthy controls[18,28,108,109]. Further, vaso-active medications commonly used in

HTR can have an effect on cardiac (preload, afterload and contractility) and vascular function.

5.7 Future Directions

Based on the key findings and limitations of this study the following recommendations are presented in hope that future investigations will further improve on the techniques used here and add further information to the cardiovascular and skeletal muscle function of HTR:

1) Employ measurements of blood flow, oxygen delivery and oxygen utilization in order to attain a more complete answer of the controlling mechanisms of cardiovascular and skeletal muscle function at peak and during a step-increase to moderate intensity UKE exercise.
2) Perform a UKE training study, having the contralateral leg as an internal control. This would be of interest in order to further give information regarding the mechanisms responsible for exercise training improvements in HTR.
3) Within the suggested training study perform additional pre- and post-testing on a two-legged cycle ergometer in order to provide information whether UKE exercise training may improve cardiovascular function during large muscle mass exercise.

Chapter Six: Conclusion

Peak and reserve $\dot{V}O_{2p}$ remained 40-50% lower in HTR compared to CON during small muscle mass exercise. Consistent with our hypothesis, the decreased $\dot{Q}$ and a-vO_{2diff} played an equal role in limiting $\dot{V}O_{2p}$ during peak UKE. Importantly, the reduced peak and reserve $\dot{Q}$ were mainly due to a reduction in HR reserve as SV reserve was similar between groups. In contrast to our initial hypothesis, a greater HHb for any sub-maximal $\dot{V}O_{2p}$ in HTR indicated the presence of a compensatory oxygen extraction mechanism. Furthermore, the attenuated $\dot{V}O_{2p}$ kinetics were equally reduced by prolonged $\dot{Q}$ and HHb kinetics. Finally, the off-kinetics were lengthened in HTR due to slowed O_2Hb kinetics as the similar $\dot{Q}$ off-kinetics would have been beneficial in repaying the incurred oxygen deficit of HTR compared to CON.

These results support equal arguments for delivery and utilization limitations in HTR during peak UKE exercise and UKE on-set and off-set. Interestingly, in contrast to large muscle mass exercise, greater importance was placed on skeletal muscle function as the mitigation of exercising muscle mass to $\leq$ 5kg showed $\dot{Q}$ and oxygen extraction to equally contribute to exercise intolerance in HTR. Therefore, HTR would stand to gain from small muscle mass exercise as it may maximize a peripheral training effect without taxing central circulation.

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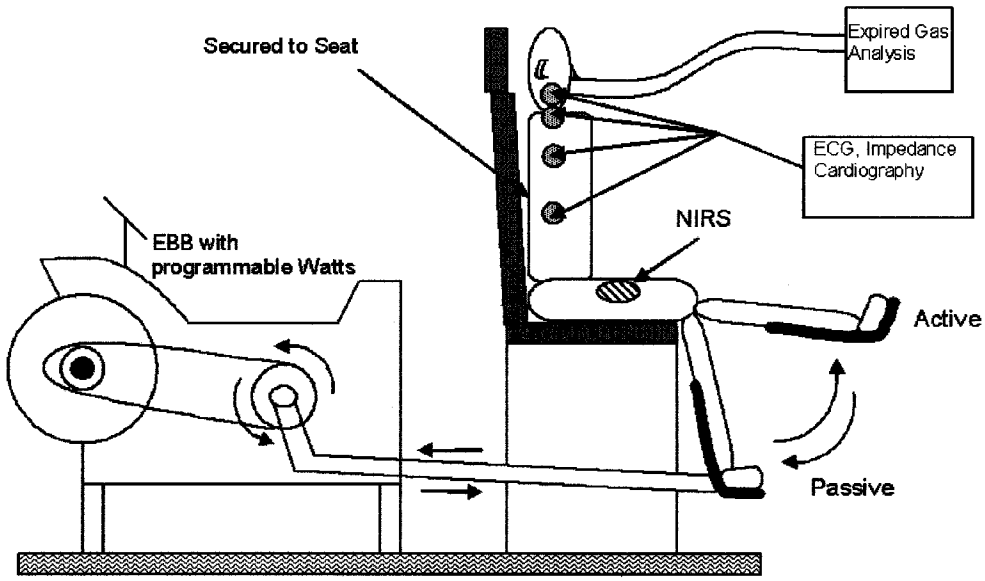
APPENDIX A

UNILATERAL KNEE EXTENSION APPARATUS SET-UP



UKE Ergometer. Participants performed repeated knee extension at 50 repetitions per minute. The concentric knee extension pulled on the crank arm of the electronically braked bicycle (EBB). This allowed the crank arm to conduct a full rotation by counter-weighting the opposite crank arm, enabling a passive eccentric motion and returning the lower leg to the beginning of the kicking motion.

APPENDIX B

ETHICS APPROVAL

Health Research Ethics Board

213 Heritage Medical Research Centre
University of Alberta, Edmonton, Alberta T6G 2S2
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ETHICS APPROVAL FORM

Date: August 2006

Name(s) of Principal Investigator(s): Dr. Mark Haykowsky

Faculty: Rehabilitation Medicine

Title: **Cardiac, limb blood flow and muscle oxygenation kinetics during knee extensor exercise in heart transplant recipients**

The Health Research Ethics Board (Biomedical Panel) has reviewed the protocol involved in this project which has been found to be acceptable within the limitations of human experimentation. The panel also approved the subject information sheet and consent form.

Specific Comments: The Research Ethics Board assessed all matters required by section 50(1)(a) of the Health Information Act. Subject consent for access to identifiable health information is required for the research described in the ethics application, and appropriate procedures for such consent have been approved by the REB Panel..

SEP - 1 2006

D.W. Morrish, MD, PhD
Chairman, Health Research Ethics Board
Biomedical Panel

Date of Approval Release

This approval is valid for one year

Issue #6447

UNIVERSITY OF ALBERTA

Capital Health
Edmonton and area

CARITAS HEALTH GROUP

APPENDIX C

PROTOCOL INFORMATION SHEETS

Date: _____/_____/_____

Participant Code: _____ Participant Initials:_____
Impedance Electrode Distance: _____ (cm)

Height: _____ cm

Weight: _____ kg

Age:_____

Pre-Transplant Etiology:_____ Time since Transplant:_____

Thigh Skinfold NIRS SITE (mm):_____

THIGH VOLUME FORMULA:

$$V = L*(12\pi)^{-1} *\left(O_1^2 + O_2^2 + O_3^2\right) - (S - 0.4)*2^{-1} *L*(O_1 + O_2 + O_3)*3^{-1}$$

Length of thigh **L**
(greater trochanter to lateral epicondyle) **(cm):** _____

Thigh Circumference- VOLUME CALCULATION $\underline{O_1}$
(gluteal furrow)

(cm):_____

Thigh Circumference- VOLUME CALCULATION $\underline{O_2}$
(1/3 subischial height)

(cm): _____

Thigh Circumference- VOLUME CALCULATION $\underline{O_3}$
(minimum circumference above the knee joint)

(cm): _____

Skinfold
Anterior (1/3 subischial height) **(mm):** _____

Skinfold +
Posterior (1/3 subischial height) **(mm):** _____
Sum= **S** **(mm):** _____
Convert to **(cm):** _____

Quadriceps femoris muscle mass (M)=:

$$M = 0.307 * V + 0.353$$

Net Efficiency Formula=Work@Max Load/ (VO_2@Max Load – VO_2@unloaded)

Unilateral Knee Extension: Incremental

Participant Code: _____ **Impedance Electrode Distance:** _____ (cm)
Date: ____/_____/____
Duty Cycle: 50 rpm

Peak VO2:_____
Peak VO2rel:_____
Signal Display:
Pre O_2:_____
Pre CO_2:_____
Post O_2:_____
PostCO_2:_____
Impedance cal file:_____
Impedance file name:_____
NIRS optode distance:_____
Multiplier: 4
End Test Time:_____

- UKE in place
- Calibrate Met Cart **Trial#:**_____
- Calibrate Impedance
- Connect leads
- Connect phonocardiogram
- Calibrate NIRS- probes in place
- Connect CM5 ECG connection
- Portapress/ Pulse oximeter in place
- Set up head gear
- Review exercise instructions (angina, RPE, termination etc
- Connect hose
- Echo Technician ready/ Instructions made clear for technician
- Cardiac Echo Ready?
- Begin test

Time (min)	**Work Rate (W)**	**SaO2%**	**HR bpm**	**BP mmHg**	**Impedance Sample**
0-5	REST				
5-6	0				
6-7	5				
7-8	8				
8-9	11				
9-10	14				
10-11	17				
11-12	20				
12-13	23				
13-14	26				
14-15	29				
15-16	32				
Time	**Work**	**SaO2%**	**HR**	**BP**	**Impedance**

(min)	Rate (W)		bpm	mmHg	Sample
16-17	35				
17-18	38				
18-19	41				
19-20	44				
20-21	47				
21-22	50				
22-23	53				

Unilateral Knee Extension: Sub-Maximal Square Wave On-transition

Participant Code: _____ **Impedance Electrode Distance**: _____ (cm)
Date: _____
Duty Cycle: 50rpm

Steady State VO2:_____
Steady State VO2rel:_____
Signal Display:
Pre O_2:_____
Pre CO_2:_____
Post O_2:_____
PostCO_2:_____
Impedance calfile:_____
Impedance file name:_____
NIRS optode distance:_____
Multiplier: 4

- UKE in place
- Calibrate Met Cart **Trial#:**_____
- Calibrate Impedance
- Connect leads
- Connect phonocardiogram
- Calibrate NIRS- probes in place
- Connect CM5 ECG connection
- Portapress/ Pulse oximeter in place
- Set up head gear
- Review exercise instructions (angina, RPE, termination etc…)
- Connect hose
- Echo Technician ready/ Instructions made clear for technician
- Vascular Echo Ready?
- Cardiac Echo Ready?
- Begin test

Peak $\dot{V}O_{2p}$:_____
Peak WR:_____
% Peak: 60%_____
Expected Steady State $\dot{V}O_{2p}$:_____
WR @ 60% $\dot{V}O_{2p}$:_____

Time (min)	**Work Rate (W)**	**SaO2%**	**Impedance Sample**	**NOTES**
REST 0-2:59	-			
3:00-8:00	60% Peak Watts= _____watts			

APPENDIX D

EFFECT SZE AND SAMPLE SIZE CALCULATIONS

The sample size was based on the main outcome measure of peak $\dot{Q}$. Kappagoda et al.[113] validated the use of impedance cardiography for $\dot{Q}$ measurement in HTR. They found that HTR have a $\dot{Q}$ during peak cycle exercise of 7.5 ± 0.8 SEM $L \cdot min^{-1}$ in cycling exercise. Hosenpud et al.[2] using the direct Fick method found peak $\dot{Q}$ values for HTR of 9.9 ± 1.7 standard deviation ($L \cdot min^{-1}$) in cycle exercise. This shows disparity between measurement techniques, however a new formula for impedance cardiography has yielded statistically similar $\dot{Q}$ values compared to thermodilution; impedance cardiography 6.06 ± 1.48 SD $L \cdot min^{-1}$, thermodilution 5.97 ± 1.41 SD $L \cdot min^{-1}$ $p \leq 0.05$ in cardiac patients[96,113].

We used UKE instead of cycling exercise. Magnusson et al.[5] used UKE and found that heart failure patients have a 58% difference in $\dot{Q}$ compared to the control group. Therefore, we used an approximation of Magnusson et al.[5] results for our sample size calculation.

UKE	$\dot{Q}$ ($L \cdot min^{-1}$)
Heart Failure	6.6 ± 0.5
Controls	10.4 ± 1.0

We hoped to find a 40% difference between HTR and age-matched controls in our UKE condition. Starting with a $\dot{Q}$ value of 7.0 $L \cdot min^{-1}$ in HTR a 40% difference compared to controls would yield a difference of 2.8 $L \cdot min^{-1}$. Using a value of 1.6 for the standard deviation, which is congruent to a standard deviation using impedance cardiography.

$$\Delta = \frac{\bar{x}_1 - \bar{x}_2}{\sigma} \quad = 9.8\text{-}7.0/1.6 = 1.75$$

$$N^{115,116} = \frac{SD^2(Z_\alpha + Z_\beta)^2}{\Delta^2} \quad N = \frac{(1.1)^2(1.96+0.842)^2}{1.75^2} \quad N = \frac{1.21 \times 7.85}{3.0625} \quad N = 3.10155102$$

We required 3 participants. Therefore, we set the sample size at n = 5 for both groups, in order to account for the use of non-invasive measurement techniques. This sample size was feasible and effective in finding powerful statistically significant differences in $\dot{Q}$ between HTR and CON during UKE exercise.

In terms of the kinetics analysis we employed Lamarra's method[99] of repeat trials to minimize inter-variable noise. Where K_n, is a constant based on repeat inter-breath ($\dot{V}O_{2p}$ mL·min^{-1}) fluctuations, $\hat{L}$ represents the confidence interval desired, ΔY_{ss} represents the amplitude ($\dot{V}O_{2p}$ mL·min^{-1}) and s_o represents the standard deviation of inter breath fluctuation of Y ($\dot{V}O_{2p}$ mL·min^{-1})

We desired a 95% confidence interval.

$n = \left[\frac{\hat{L} \cdot s_0}{K_n \cdot \Delta Y_{ss}}\right]^2$ to yield (n) number of repetitions we used the same variables as defined before. Using data from heart failure patients during UKE exercise from Magnusson et al.[5], ($\Delta Y_{ss} = 0.5$; $s_o = 0.10$; $\hat{L} = 47.5$ and $K_n = 5$s), we yield-

$$n = \left[\frac{47.5 \cdot 0.10}{5 \cdot 0.5}\right]^2 = 3.61$$

Therefore we had each participant undergo 4 repeat trials of the sub-maximal square wave step-transition protocol for kinetic modeling.