11-10-2020 2:30 PM

Quantifying The Inter-Relationships Amongst Muscle Blood Flow, O2 Uptake And Muscle Deoxygenation Kinetics From Elevated Baseline Metabolic Rates And Increasing Work Rate Amplitudes

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Kinesiology

Abstract

Upon an instantaneous increase in work rate (WR), pulmonary O_2 uptake ($\dot{V}O_{2p}$) increases in an exponential-like manner towards a new steady-state. To support this increase in $\dot{V}O_{2p}$ there is an increase in blood flow (BF; O_2 delivery) to the muscle. Previous research has shown that the rate of this increase in $\dot{V}O_{2p}$ (which defines $\dot{V}O_{2p}$ kinetics) becomes progressively slower with increasing baseline WR (WR_{bl}). Given the paucity of research on blood flow dynamics and underlying muscle metabolic rate, this thesis examined the effect of metabolic rate on the dynamics and relationships amongst $\dot{V}O_{2p}$, BF, and muscle deoxygenation (deoxy[Hb + Mb]). Using mass spectrometry and volume turbinometry, Doppler ultrasound, and near-infrared spectroscopy (NIRS), the response of $\dot{V}O_{2p}$, BF, and deoxy[Hb + Mb] were measured, respectively, in response to step transitions in alternate-leg, knee-extension exercise from: i) a common WR_{bl} (3 W) to increasing ΔWR (21, 30, 42, 51, 63 W); and ii) increasing WR_{bl} (3, 12, 24, 33, 45 W) with a common ΔWR (21 W). As the BF signal is distorted by fluctuations in arterial pressure (from the heart rate (HR) cycle) and intramuscular pressure (from the contraction-relaxation (CR) cycle), it was necessary to first determine an acceptable technique for integrating the BF signal. From integrating BF by the CR or HR cycle over 1, 2, or 5 cycles using either 'binning' or 'rolling' averages, it was found that averaging the signal over a single CR cycle (CR1) was preferred due to its low variability. In analyzing the kinetic response to the various exercise transitions, it was found that: i) BF kinetics were similar across different ΔWR's, but became progressively slower with increasing WR_{bl} before significantly speeding at the highest WR_{bl}; ii) $\dot{V}O_{2p}$ kinetics became progressively slower with both increasing ΔWR and WR_{bl}; iii) the increase in BF relative to the increase in metabolic demand ($\Delta BF/\Delta\dot{V}O_{2p}$) became progressively smaller with both increasing ΔWR and increasing WR_{bl}; iv) deoxy[Hb + Mb] kinetics became progressively faster with increasing ΔWR, but became progressively slower with increasing WR_{bl}. These findings suggest that with an increasing ΔWR, the muscle has an attenuated increase in bulk BF and a larger reliance on O_2 extraction to meet the O_2 requirements of the muscle, but with an increasing WR_{bl} the muscle relies less on O_2 extraction and more on BF redistribution within the muscle microvasculature.

Keywords: pulmonary O_2 uptake, bulk blood flow, microvascular blood flow, kinetics, near-infrared spectroscopy, muscle deoxygenation, blood flow fitting, knee-extension exercise, baseline work rate, work rate amplitude, blood pressure, vascular conductance, heart rate.

Summary for Lay Audience

Whenever you instantaneously increase your activity level (work rate (WR)), your body must also instantly increase energy production to continue working at the higher WR. However, your body's aerobic (oxidative) system, which requires O_2 to make energy, does not immediately meet all of these energy needs, but instead gradually increases towards the higher energy requirements until its contribution to energy production is maximized. The rate of increase in aerobic energy production becomes progressively slower as the starting, baseline, intensity (WR_{bl}) becomes higher. Given that increases in muscle BF and O_2 delivery are required to support the higher muscle O_2 requirements, this thesis investigated the role that blood flow (BF) and O_2 delivery to exercising muscle plays in the response of the aerobic system by measuring the rate of adjustment of O_2 uptake ($\dot{V}O_2$) and BF. A mass spectrometer and volume turbine were used to measure $\dot{V}O_2$, Doppler ultrasound was used to measure BF, and near-infrared spectroscopy was used to infer muscle O_2 extraction from the blood. Subjects performed an instantaneous increase in WR on a knee-extension ergometer from: i) a WR_{bl} of 3 W to 24, 33, 45, 54, or 66 W; or ii) a WR increase (ΔWR) of 21 W from a WR_{bl} of 3, 12, 24, 33, or 45 W. Due to fluctuations in the BF signal caused by the heart pumping, as well as the muscle contracting and relaxing, it was necessary to determine an appropriate technique to analyze BF. By averaging BF over either a single, or 2 or 5 'binned' or 'rolling' averages of a heart contraction cycle (HR), or a muscle contraction cycle (CR), it was determined that a single CR cycle was the best method for analyzing BF. When looking at how quickly $\dot{V}O_2$ and BF increased to the new WR for each trial, it was found that: i) BF increases at a similar speed with different ΔWR's, but the increase becomes slower as WR_{bl} increases, except for the highest WR_{bl} in which BF increased much faster; ii) $\dot{V}O_2$ increases at a slower speed with larger ΔWRs and higher WR_{bl}s; iii) the increase in BF relative to the increase in $\dot{V}O_2$ becomes less as ΔWR and WR_{bl} become greater; iv) O_2 extraction by the muscle increased faster when ΔWR was greater, but increased slower at higher WR_{bl}s. These findings suggest that to meet the requirements for O_2 and energy, the muscle relies less on bulk BF and more on O_2 extraction at larger ΔWRs, but at higher WR_{bl}s it relies less on O_2 extraction and more on BF in the microvasculature to meet its $\dot{V}O_2$ needs.

Co-Authorship Statement

This thesis includes versions of the following manuscripts that are in preparation to be submitted for publication:

1. Love, L.K., Hodgson, M.D., Keir, D.A. and Kowalchuk, J.M. Data analysis technique influences blood flow kinetics parameter estimates for moderate- and heavy-intensity exercise transitions.

2. Love, L.K., Hodgson, M.D., Keir, D.A. and Kowalchuk, J.M. The effect of increasing work rate amplitudes from a common baseline on the kinetic response of $\dot{V}O_{2p}$, blood flow, and muscle deoxygenation.

3. Love, L.K., Hodgson, M.D., Keir, D.A. and Kowalchuk, J.M. The effect of increasing work rate baseline with a common amplitude on the kinetic response of $\dot{V}O_{2p}$, blood flow, and muscle deoxygenation.

These studies were designed by L.K. Love, D.A. Keir, and J.M. Kowalchuk, with helpful input from J.K. Shoemaker. Data were collected and analyzed by L.K. Love with significant assistance in collection provided by M.D. Hodgson. The original manuscripts comprising this thesis were written by L.K. Love with feedback provided by the co-authors.

Acknowledgments

To Dr. John Kowalchuk, thank you for taking me on as a doctoral student. The decades of hard work that you put into your pursuit of knowledge, and using that to guide me through the research process to learn more about our fascinating bodies has been greatly appreciated! It was always enjoyable to chat with you about physiology. Thank you for your guidance! (Proverbs 16:16)

I extend a huge amount of gratitude to Mike Hodgson for the incredible amount of work you did to aid me with data collection, equipment set up and clean up, as well as your amazing patience and resilience throughout all these trials. I could not have accomplished any of this work without you! (Ecclesiastes 4:9-10)

To Dr. Daniel Keir, I *begrudgingly* (haha) admit that you were a great source of knowledge in this field by providing me with ideas and feedback on my work. I also extend my thanks to you for your akin sense of humour and your false sense of hope in me that I would get through this degree, lol. This will be the only time I ever say anything positive about you, so 'enjoy' this singular moment. For all the other positives things I have to say about you, I refer you to a very comprehensive article by Dennis Upper (1974). (Proverbs 13:20)

To my labmates (who doubled as subjects) in the Cardiorespiratory research lab (as well as the Neuromuscular research lab) who have come and gone over my years here, thank you for your friendship and assistance! And to Brad Hansen and Dr. Glen Belfry, thank you for your assistance in keeping things running in the lab and for good conversations! (Proverbs 15:22)

I also extend a huge thank you to all of the subjects in my studies. These were all very involved and long studies and I cannot thank you enough for your amazing patience and resilience throughout all the trials to allow me to get here. I can't believe you actually performed all those trials… suckers! hahaha… (Luke 6:31)

To my colleagues at Redeemer University, thank you for the amazing blessing of the opportunity to pursue my goal of teaching (and to do so in such a wonderful atmosphere), both throughout my doctoral studies and now into my career. (Colossians 3:17)

To all my friends and church family, thank you for providing me with an immediate community as soon as I arrived in London, and for all the wonderful times of fellowship and prayer. What a blessing you have been in my life! However, if it weren't for all of your distractions, I probably would have finished this degree three years earlier… haha. (Proverbs 17:17)

To Mom, Dad, Shayne, and Marcia, it gives me great assurance to have you as a family. Knowing that I could always find stability in you gave me the confidence to make it through challenging times. I love each of you! (Proverbs 4:1-4)

This has been a long journey, and how ironic that the longest chapter in the Bible should end where mine began, with 'tau'! I surely hope that all this time has not been chasing the wind in vain, lol… (Ecclesiastes 1:12-18; 12:12). May I continue to trust and rely on Christ throughout this journey! (Jeremiah 9:23-24)

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List of Abbreviations

a.u.	arbitrary units
a-vO_{2diff}	arterio-venous oxygen difference
amp	amplitude
ADP	adenosine diphosphate
ATP	adenosine triphosphate
ANOVA	analysis of variance
BP	blood pressure
bpm	beats per minute
bl	baseline
CI_{95}	95% confidence interval
cm	centimeters
CO_2	carbon dioxide
cpm	contractions per minute
CR	contraction-relaxation
CV	coefficient of variation
DBP	diastolic blood pressure
Deoxy [Hb + Mb]	deoxygenated haemoglobin + myoglobin
dL	deciliter
ETC	electron transport chain
$FADH_2$	flavin adenine dinucleotide
G_p	primary gain
G_T	total gain
H^+	hydrogen ion
H_2O	water
Hb	haemoglobin
HR	heart rate
HVY	heavy-intensity
Hz	hertz
K^+	potassium ion
KE	knee-extension

kg	kilograms
L	liters
LT	lactate threshold
MAP	mean arterial pressure
Mb	myoglobin
MBV	mean blood velocity
MHz	megahertz
min	minutes
mL	milliliters
mM	millimolar
mmHg	millimeters of mercury
MOD	moderate-intensity
MRT	mean response time
NAD^+	oxidized nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NIRS	near-infrared spectroscopy
nm	nanometers
O_2	oxygen
Oxy[Hb + Mb]	oxygenated haemoglobin + myoglobin
P	pressure
p	probability value
PCO_2	partial pressure of carbon dioxide
PCr	phosphocreatine
$P_{ET}CO_2$	pressure of end-tidal carbon dioxide
$P_{ET}O_2$	pressure of end-tidal oxygen
pH	potential of hydrogen
P_i	inorganic phosphate
PO	power output
PO_2	partial pressure of oxygen
PSNS	parasympathetic nervous system
P/O	phosphorylation-to-oxidation ratio
P/W	phosphorylation-to-work ratio

$\dot{Q}_T$	cardiac output
$\dot{Q}_{cap}$	microvascular/capillary blood flow
$\dot{Q}O_2$	oxygen delivery rate
r	radius
r^2	correlation coefficient of regression
R-R	QRS complex-to-QRS complex of heart electrical activity
RCP	respiratory compensation point
rpm	revolutions per minute
s	seconds
SBP	systolic blood pressure
SD	standard deviation
SMC	smooth muscle cell
SNS	sympathetic nervous system
t	time
TD	time delay
TOI	tissue oxygenation index
$_{TOT}$[Hb + Mb]	total haemoglobin + myoglobin
TPR	total peripheral resistance
VC	vascular conductance
$\dot{V}CO_2$	carbon dioxide output rate
VH	very heavy-intensity
$\dot{V}O_{2m}$	muscle oxygen uptake rate
$\dot{V}O_{2max}$	maximal oxygen uptake rate
$\dot{V}O_{2p}$	pulmonary oxygen uptake rate
$\dot{V}O_{2peak}$	peak oxygen uptake rate
W	watts
WR	work rate
WR_{bl}	baseline work rate
WR_{max}	maximal work rate
yrs	years
α_1	alpha-1
α_2	alpha-2

η	viscosity
π	pi
l	length
$^\circ$	degrees
Δ	amplitude of variable
β-oxidation	beta-oxidation
θ_L	estimated lactate threshold
τ	tau (time constant) of variable
μM	micromolar
χ^2	chi-squared

CHAPTER I: Introduction

Energy Production

The capacity for the human body to perform work is accomplished by the force generated from muscular contractions, which requires the constant breakdown of energy (in the form of adenosine triphosphate (ATP)) by the muscle. However, the muscle has very limited stores of ATP, and therefore, the body requires the constant regeneration of ATP in order to continue working. This regeneration of ATP is accomplished by the breakdown of carbohydrate and fat (and to a minor extent, protein) from the food we eat through the use of our body's three energy systems. The two anaerobic systems, phosphocreatine (PCr) and glycolysis, rely on substrate-level phosphorylation to resynthesize ATP from adenosine diphosphate (ADP). The third energy system, oxidative phosphorylation, is the preferred system for generating ATP as it produces the largest amount of ATP, and does so very efficiently (breaking down the macronutrient fully, and producing no unwanted by-products (e.g., lactic acid)). The oxidative phosphorylation system is aerobic in nature and relies on oxygen (O_2) as the final electron acceptor at the end of the electron transport chain (ETC), after having used the energy from the electrons to pump protons across the inner mitochondrial membrane to create an electrochemical gradient. As the protons re-enter the mitochondrial matrix through proton channels their kinetic energy is 'coupled' with ATP synthase which resynthesizes ATP from ADP and inorganic phosphate (P_i). The electrons used in the ETC are donated by reducing equivalents, nicotinamide adenine dinucleotide (NADH) (or flavin adenine dinucleotide, $FADH_2$), which are generated from the metabolic pathways of glycolysis, β-oxidation, and the tricarboxylic acid cycle. Therefore, the overall process of oxidative phosphorylation can be summarized as:

$$5\ ADP + 5\ P_i + 2\ NADH + 2\ H^+ + O_2 \rightarrow 5\ ATP + 2\ NAD^+ + 2\ H_2O$$

Based on this equation, a lack in any of the above substrates would serve to limit the rate of oxidative phosphorylation.

As the name implies, oxidative phosphorylation is dependent upon O_2, and therefore the body requires an efficient method of transporting O_2 from the atmosphere to the level of the mitochondria within the muscle cell. In this regard, the pulmonary system, cardiovascular system, and metabolic system all work together to distribute O_2 to where it is needed within the body.

Oxygen Transport

When O_2 enters the lungs during inhalation, it proceeds down its pressure gradient by crossing several very thin membranes (~0.5 µm thick in total) from the alveoli into the blood. Once in the blood, O_2 binds to haemoglobin (Hb) in the erythrocyte with a very small amount of O_2 (~0.3 mL/dL blood) dissolved in the plasma. The blood, now carrying O_2 (and other nutrients), is transported throughout the body via the vascular system to satisfy the O_2 and nutrient requirements of the tissues. Blood flow (BF) to an area is determined by several factors that are represented in Poiseuille's law:

$$BF = \pi P r^4 \cdot (8\eta l)^{-1}$$

where P is the pressure gradient between the two ends of the vasculature, r is the radius of the vessel, η is the viscosity of the fluid (blood), and l is the length of the vessel. In the closed system of the body, the length of the vessels do not change, and while blood viscosity can change by a small amount (Hitosugi et al., 2004) and mean arterial pressure (MAP – equivocal to 'pressure' in Poiseuille's law) can change greatly, these changes are comparative between the different areas of the body when measured at the same level of the arteriolar tree (Fronek & Zweifach, 1975). Therefore, with the factors of viscosity, length, and pressure being unchanged between the different parts of the body, vessel radius becomes the principal factor determining BF to an area in the body, with its role amplified due to its exponential effect (to the power of four). Through changes in vessel radius there is a decrease in BF with vasoconstriction, while vasodilation causes an increase in BF to the area supplied by the effected artery/arteriole. This O_2-rich blood perfuses all of the capillaries supplied by its terminal arteriole, creating an O_2 pressure gradient between the microvasculature in the capillary bed and the muscle cells adjacent to those capillaries. This pressure gradient causes O_2 to dissociate from Hb and diffuse into the myocyte. Inside the cell, O_2 will continue down

its pressure gradient towards the mitochondria, specifically towards complex IV of the ETC in the inner mitochondrial membrane, via either 'free' diffusion through the cytoplasm or via "facilitated" diffusion bound to myoglobin (Mb).

During oxidative phosphorylation, the metabolic processes of the muscle also produce carbon dioxide (CO_2) which diffuses from the mitochondria into the blood where it is transported back to the lungs in one of three ways: dissolved as CO_2 in the plasma (~5-10%); bound to the erythrocyte as carbamino haemoglobin (~20%); or by being converted to bicarbonate (~70-75%) through a reversible reaction with water (H_2O) by carbonic anhydrase.

Exercise Blood Flow

With the increased metabolic demands of exercise (increases that can be more than 20x resting requirements) there is an increased need for O_2 within the active muscle tissue, which is met by a proportional increase in BF (Andersen & Saltin 1985). Blood flow to an area is determined by the vasoconstriction and vasodilation of the arteries/arterioles supplying that area, which is accomplished by the contraction and relaxation, respectively, of smooth muscle cells (SMC) surrounding the vessels. However, due to the large mass of recruited muscle and the vast ability of its vasculature to vasodilate during exercise, there is the potential for BF requirements of the active muscle to far exceed the heart's ability to produce the necessary cardiac output ($\dot{Q}_T$) to maintain MAP, a phenomenon referred to as the "sleeping giant hypothesis" (Rowell, 1997). As MAP is the product of both $\dot{Q}_T$ and total peripheral resistance (TPR – from the friction of blood against the vessel walls; MAP = $\dot{Q}_T$ · TPR), the body must closely regulate the diameter of the vasculature in order to maintain MAP since MAP is regulated less by $\dot{Q}_T$ and more by TPR when HR is elevated (Ogoh et al., 2003). Many factors contribute to vessel tone and BF distribution through their control on the contractile state of the SMC. In particular, there is an increase in output from the sympathetic nervous system (SNS) during exercise that is in proportion to the exercise intensity, causing an increase in $\dot{Q}_T$ (via increased heart rate (HR) and cardiocyte contractility causing a larger stroke volume) and increased TPR (via SMC contraction (vasoconstriction) throughout the body), which acts to increase perfusion pressure (i.e., MAP) in the body.

While vasoconstriction caused by the SNS assists in maintaining adequate MAP during exercise, it is necessary to redirect BF from less active regions within the body towards more active regions within the muscle to help meet the increased metabolic demands required of the exercise (an increase in BF to an area that can be as much as ~100x resting (Laughlin et al., 1996)). To assist with this increase in BF, it has been suggested that there is a blunting of the vasoconstriction signal from the SNS to the vasculature in the contracting muscle, a process known as 'sympatholysis' (Remensnyder et al., 1962). The cause of this sympatholysis may be attributed to the release of various metabolites from the skeletal muscle during exercise and their effect of either inhibiting the release of neurotransmitters from the SNS or by inhibiting the postjunctional receptors on the vasculature (Verhaeghe & Shepherd, 1976, Verhaeghe et al., 1977). However, at very high exercise intensities, when muscle BF demand increases above what $\dot{Q}_T$ is able to provide while still maintaining an adequate MAP (~100 mmHg during HVY whole-body exercise; Clausen, 1976), the response of sympatholysis (i.e., vasodilation in the active musculature) is overridden by the SNS such that BF is attenuated in those areas despite the metabolic need (Calbet et al., 2004, Dela et al., 2003, Laughlin et al., 1996, Mortensen et al., 2008, Secher & Volianitis, 2006).

The redistribution of BF to the active musculature requires an increase in the vessel radius of the arteries/arterioles that are supplying those muscles. However, there is much discussion as to which mechanism(s) cause(s) vasodilation, with potential mediators needing to possess the ability to cause SMC relaxation, as well as respond in proportion to exercise intensity (i.e., metabolic demand), and in a timely manner. While some studies have suggested that vasodilation can be mediated by a neural component (Eklund & Kaijser 1978, 1976, Eklund et al., 1974, Rusch et al., 1981, Sanders et al., 1989), several others have found that blocking the neural signal with atropine had no effect on BF (Armstrong & Laughlin, 1986, Brock et al., 1998, Buckwalter et al., 1997, Shoemaker et al., 1997), demonstrating that there is little to no neural involvement in vasodilation. It has previously been suggested that acetylcholine spillover from motor neurons at the neuromuscular junction could be implicated in the vasodilatory response owing to involvement in muscle activation and proximity to resistance vessels, concurrent timing with muscle contraction, and proportionality with exercise intensity (Welsh & Segal 1997). However, similarly to the direct innervation of neural activity on vasodilation mentioned previously, blocking of neural activity by atropine would

also prevent acetylcholine release, and as this blockade has been shown to have no effect on BF, acetylcholine spillover is also unlikely to be involved in regulating BF during exercise (Dyke et al., 1998; Joyner & Halliwill, 2000). Although $lactate^-$ has been posited as a possible cause for vasodilation, its contribution has been largely dispelled with the observation of no vasodilation following exogenous infusion of $lactate^-$ (Keller et al., 1930). At present, one of the most prevalent substances thought to play a role in vasodilation is K^+ (Bohr & Goulet, 1961, Armstrong et al., 2007, Edwards et al., 1998). The release of K^+ from the myocyte in response to contraction has been shown to occur rapidly (Hnik et al., 1976), allowing for it to play an immediate role in exercise hyperemia. Additionally, the release of K^+ from the muscle cell has been found to be proportional to both the intensity of contraction (Hudlicka & el Khelly, 1985) and the increase in BF (Hilton et al., 1978, Hudlicka & el Khelly, 1985, Mohrman & Sparks, 1974b), suggesting that this "metabolite" would allow for effective coordination of BF with metabolic demand. The role of adenosine as a vasodilator also has received a great deal of interest because of its buildup and release from the muscle cell, particularly during times of metabolic stress when increased BF is needed (Costa et al., 2000, Langberg et al., 2002, Duncker et al., 1993, Laxson et al., 1993), which would allow its vasodilating capacity to be suitably linked with metabolic demand (Marshall, 2007). Similarly, the analogs of adenosine, ATP and ADP, have also been recognized to induce vasodilation (Forrester et al., 1979), and with their concentration increasing with exercise intensity (Forrester, 1972, Forrester & Lind, 1969, Hellsten et al., 1998), they also would provide a direct link between BF and metabolic demand. In addition to its release from the skeletal muscle, studies are also showing that ATP is released from the erythrocyte when it is deoxygenated or mechanically deformed (Ellsworth et al., 2009, Ellsworth et al., 1995). An increase in metabolic activity will lead to increased off-loading of O_2 from the erythrocyte (deoxygenation) and, in combination with more erythrocytes traversing the microcirculation where capillary diameters are often smaller than erythrocytes – causing physical deformation of the erythrocyte (Kaniewski et al., 1994) – more ATP will be released from the erythrocyte to stimulate vasodilation, thus creating a potential link of BF, tissue O_2 utilization and metabolic demand. Although each of the substances discussed above is capable of causing vasodilation, many of these substances appear to work through one of the most prominent vasodilators, nitric oxide. Produced by the endothelium (and synaptic

terminal of the neuron) from O_2 and L-arginine, nitric oxide diffuses from the endothelium (or nearby neuron) to the SMC causing relaxation and, thereby, vasodilation (see Laughlin et al., 2012 for review). Lastly, CO_2 has been shown to be a potent vasodilator in the cerebral circulation (Kety & Schmidt 1948, Reivich 1964), and while it is tempting to speculate that CO_2 could act in a similar manner in the systemic circulation, as an increase in metabolic activity will produce more CO_2 for greater vasodilation, this has not been shown to date.

While the above mentioned substances have the ability to cause vasodilation of the microvasculature that is within the metabolically active muscle, those arterioles/arteries that are upstream (and outside of the active musculature) must rely on other means for vasodilation. To help meet the BF needs of the more distal vessels, BF through the upstream vessels is increased in part by 'conducted vasodilation' in which a hyperpolarization signal is passed proximally up the vasculature via gap junctions in the endothelium (and, less-so, through SMCs themselves (Smith et al., 2008, Tran et al., 2009) causing the SMCs to relax (Beach et al., 1998, Berg et al., 1997, Collins et al., 1998, Song & Tyml, 1993). In addition to the transmission of the electrical signal up the vasculature through 'conducted vasodilation', the vasodilatory response is also spread upstream via flow-mediated dilation. This process is initiated with the vasodilation of the microvasculature within the active muscle, which will increase the capacity for blood, leading to an increase of BF to perfuse the "opened" vasculature of the area. In turn, this will cause blood in the arterioles/arteries that are upstream to flow into the vessels downstream, producing friction along the endothelium from the blood, an effect known as shear stress. This increased shear stress stimulates the endothelium to release NO, which diffuses to the adjacent SMCs causing them to relax, and thus, causing vasodilation. This newly dilated segment, in turn, will generate more shear stress further upstream by the rush of blood to fill the newly expanded area, causing vasodilation to continually progress upstream, and allowing BF to increase to the active muscle. This process of flow-mediated dilation is aided by the vasculature of the larger arterioles (where shear stress will have a greater influence) being more responsive to shear stress than the smaller vessels of the arteriolar tree (Kuo et al., 1995). Furthermore, there are differences in the type of α-adrenergic receptors that are present along the arteriolar tree, with the distal (and smaller) arterioles containing predominantly α_2 receptors, which show an inhibited response to vasoconstriction during muscle contraction, while the more

proximal (and larger) arterioles/arteries have predominantly α_1 receptors, which are still capable of vasoconstriction during muscle contraction (Anderson & Faber, 1991, Faber, 1988, Faber & Meininger, 1990, Nishigaki et al., 1991, Ohyanagi et al., 1991). In line with this, Thijssen et al. (2008) found that artery size impacted a vessel's vasoactivity, with larger arteries having a blunted vasodilatory response compared to smaller arteries. These differences would be expected to have an impact on the overall outcome of sympatholysis, as the smaller arterioles (with α_2 receptors) would be able to dilate in response to muscle contraction to redistribute BF to the areas within the muscle that it is needed, while a maintenance in constriction of the larger arterioles (with α_1 receptors) would help prevent a decrease in MAP.

Although the various substances discussed previously are able to induce vasodilation, many studies have shown that removing or inhibiting the pathway of one of these vasodilators does not affect the vasodilatory response. This is likely attributable to a redundancy in the vasodilatory response, which confounds the ability to ascribe the vasodilatory response to any single vasodilator. Furthermore, several of these vasodilators can be influenced by pH (Stowe et al., 1975), which further confounds the ability of studies to determine the effect of a particular vasodilator in the exercise BF response, as a particular vasodilator's influence on BF could be dependent upon the metabolic state of the tissue. The vasodilatory response is also complicated by some vasodilators exhibiting a 'summing effect' in which they increase the effect of other vasodilators (see Laughlin et al., 2012 for review).

In addition to the metabolic contributions on the BF response to exercise, there are also mechanical contributions from the contracting muscle which cause repetitive changes in intramuscular pressure (Radegran & Saltin, 1998). These changes in intramuscular pressure cause the vasculature within the muscle to compress and expand which forces blood out of and draws blood into the vasculature, respectively, thereby increasing muscle BF via a 'pumping' action (i.e., a 'muscle pump'). In addition to the contracting musculature creating a 'pumping' action to increase BF, there is potentially also a myogenic contribution of the active muscle on exercise hyperemia. By this mechanism, mechanical compression of the vasculature (a decrease in the transmural pressure – e.g., muscle contraction) has been shown to cause vasodilation, while a decrease in mechanical compression (an increase in transmural

pressure – e.g., muscle relaxation) has been shown to cause vasoconstriction (Mohrman & Sparks, 1974a, Clifford et al., 2006).

It is through the effect of these many redundant, synergistic, and competing exercise-related responses on the vasculature of the whole body that MAP is maintained during exercise. Furthermore, this balance between vasoconstriction and vasodilation results in a 'fine-tuning' of O_2 (and nutrient) delivery to meet the precise metabolic demands of the muscle.

Oxygen Uptake Kinetics

A step-increase in external work rate (WR) necessitates an instantaneous increase in ATP regeneration to satisfy the greater rate of ATP utilization consequent to the increased work performed by the involved muscles. While oxidative phosphorylation is the most efficient ATP-regenerating pathway due to its ability to maximize ATP regeneration by fully oxidizing macronutrients, the ability of oxidative phosphorylation to immediately meet the muscle's energy needs upon a change in WR is delayed. This delay in oxidative phosphorylation causes the muscle to rely on PCr breakdown and glycolysis to support any deficits in ATP regeneration in response to immediate increases in energy requirement at exercise onset. This period of delayed activation of oxidative phosphorylation is termed the O_2 deficit, and as a result of the increased reliance on anaerobic energy sources, there is an increased production and accumulation of fatigue-inducing metabolites (e.g., $lactate^-$, H^+, P_i^{x-}, reactive oxygen species, and ADP) which are associated with exercise intolerance (Allen et al., 2008). Therefore, it is beneficial to exercise performance to keep the O_2 deficit as small as possible. A substantial amount of research has focused on identifying the reason for this delay in increasing the activity of oxidative phosphorylation, with research suggesting that 'sluggishness' in the metabolic activation of oxidative regulatory (rate-limiting) enzymes and substrate delivery (besides O_2), or in convective or diffusive O_2 delivery (i.e., BF) to the muscle, alone or in combination, might be responsible for delays in attaining "steady-state" conditions (see Poole & Jones 2012, Rossiter 2011 for review).

Due to the invasive nature of measuring O_2 uptake ($\dot{V}O_2$) and utilization at the level of the muscle ($\dot{V}O_{2m}$), studies utilize pulmonary $\dot{V}O_2$ ($\dot{V}O_{2p}$), which, after correcting for the muscle-to-lung transit time of deoxygenated blood, has been shown to closely reflect $\dot{V}O_{2m}$

(Barstow et al., 1990, Grassi et al., 1996, Rossiter et al., 1999, Krustrup et al., 2009). After the time delay (TD, phase I – discussed below), $\dot{V}O_{2p}$ increases in an exponential-like manner, with the rate of this change being quantified by the time constant (τ). It is the characterization of this response that defines $\dot{V}O_2$ kinetics, with those individuals that have fast $\dot{V}O_{2p}$ kinetics (a small τ) having a smaller O_2 deficit, and thereby less accumulation of fatigue-inducing metabolites and thus greater metabolic stability (Grassi et al., 2011), than those individuals that have slow $\dot{V}O_{2p}$ kinetics (a larger τ).

The $\dot{V}O_{2p}$ response profile differs in its response to exercise transitions of different intensities. For those WRs within the moderate- (MOD, below lactate threshold (LT)) and heavy-intensity domains (HVY, above LT, but below critical power), the contribution of oxidative phosphorylation to energy production continues to increase throughout the O_2 deficit phase and eventually reaches a 'steady-state') in which oxidative phosphorylation is providing 'all' of the ATP regeneration in the muscle, and muscle O_2 consumption ($\dot{V}O_{2m}$) is unchanging (i.e., "steady"). For all transitions there is an initial rapid increase in $\dot{V}O_{2p}$ (phase I), which is caused by a rapid increase in the influx of deoxygenated blood through the pulmonary circulation (caused by an increase in $\dot{Q}_T$). Following this 'cardiodynamic' phase, for those transitions to a WR in the MOD, $\dot{V}O_{2p}$ increases in a monoexponential manner (primary component (phase II)) to its new steady-state (phase III). For transitions into HVY, following the TD and primary component, there is a delay in the attainment of steady-state due to the manifestation of a gradual increase in $\dot{V}O_{2p}$ termed the 'slow component' (phase III). This slow component creates a $\dot{V}O_2$ response that is greater than predicted from the sub-LT, linear, $\dot{V}O_2$ – WR relationship and comprises a larger proportion of the overall $\dot{V}O_{2p}$ response the higher the WR is into the HVY domain (see Figure 1.1). It was formerly theorized that the slow component could be the result of the increased work (and energy expenditure) being performed by accessory muscles (lungs, heart, etc.), or the accumulation of various metabolites or catecholamines and other factors and their effect on the metabolic rate (see Gaesser & Poole 1996, Poole & Jones 2012). However, Poole et al. (1988, 1991) revealed that ~85% of the response is attributable to the working muscle, and may be the result of the recruitment of additional muscle fibres (Krustrup et al., 2004), although this is contested (Zoladz et al., 2008, Scheuermann et al., 2001). This 'excess' $\dot{V}O_{2m}$, therefore, represents an inefficiency in the muscle of either the ATP-to-WR ratio

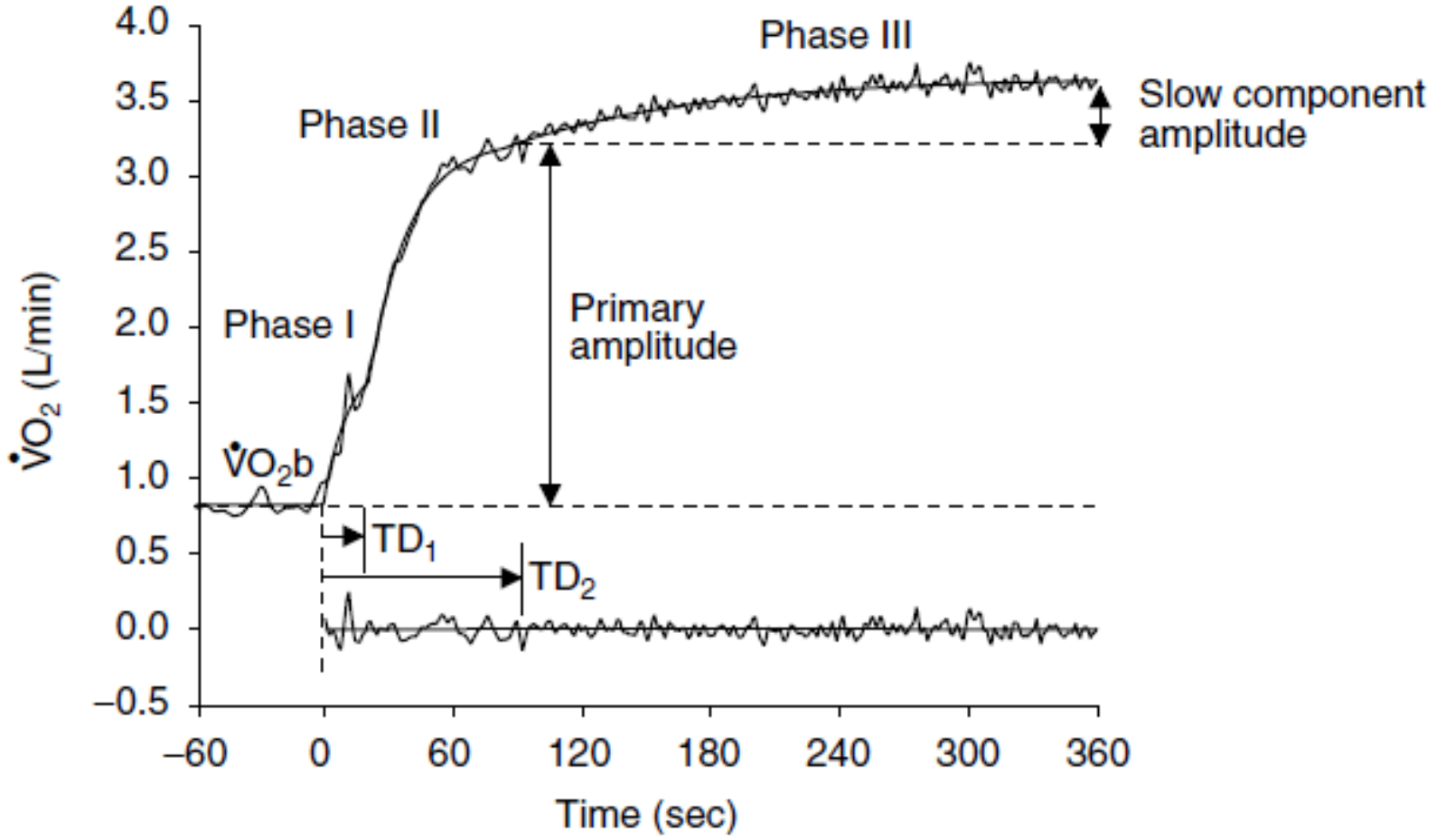

Figure 1.1. $\dot{V}O_{2p}$ response profile for heavy-intensity (HVY) exercise with the cardiodynamic component (phase I), primary component (phase II), and slow component (phase III) shown. TD, time delay; b, baseline. Reprinted with permission from Jones et al., 2003.

(P/W; i.e., an increased cost of ATP for muscle contraction) or the ATP-to-O_2 ratio (P/O; i.e., an increased cost of O_2 to resynthesize ATP). While a $\dot{V}O_{2p}$ and metabolic "steady-state" is achieved with exercise within the MOD and HVY domains, in the very heavy-intensity domain (VH, where the primary component projects above critical power, but below $\dot{V}O_{2max}$), $\dot{V}O_{2p}$ continues to increase towards $\dot{V}O_{2max}$ and, if exercise is tolerated, eventually will reach $\dot{V}O_{2max}$. These non-steady-state conditions with continued rise in $\dot{V}O_{2p}$ and metabolic "instability" are associated with fatigue (i.e., an inability to generate muscle force/power (Keir et al., 2016c)) and exercise intolerance (Murgatroyd et al., 2011). Although the $\dot{V}O_{2p}$ response profile, when considered in its entirety, is different between these intensity domains, when analyzing only the primary (phase II) component of the $\dot{V}O_{2p}$ response, kinetics can be either similar or different between intensity domains. While many studies have reported $\tau\dot{V}O_{2p}$ to be similar between MOD and HVY exercise transitions (Nyberg et al., 2017, Jones et al., 2012, Koga et al., 2005, Ozyener et al., 2001, Scheuermann et al., 2001, Koga et al., 1997) or for different WR increments (ΔWR) within MOD (Keir et al., 2016b, Spencer et al., 2013, MacPhee et al., 2005), this is not unanimous (Koga et al., 2001, Koga et al., 1999, Engelen et al., 1996, Paterson & Whipp 1991).

In order to determine the underlying factors that influence $\dot{V}O_{2p}$ kinetics, studies have used a plethora of investigative techniques, such as: different gas concentrations (e.g., hypoxia, hyperoxia, hypercapnia, hyperventilation); 'priming' exercise; different ΔWRs; elevated baseline WRs (WR_{bl}); training; nutritional changes; pharmacological interventions; aging; etc., all of which challenge/alter the $\dot{V}O_{2p}$ response. However, despite all of these interventions it remains very difficult to separate out the roles of metabolic sluggishness and O_2 delivery to decisively determine the cause of the delayed activation of oxidative phosphorylation. For instance, while exercise training commonly results in a speeding of $\dot{V}O_{2p}$ kinetics (Williams et al., 2013, Murias et al., 2010a/b & 2011, McKay et al., 2009), it is well known that there are both metabolic and cardiovascular improvements that occur with training (see Hellsten & Nyberg, 2016 and Earnest et al., 2018 for review), meaning that either factor could be contributing to the faster kinetic response. Likewise, following a bout of HVY 'priming' exercise, $\dot{V}O_{2p}$ kinetics are sped on the subsequent exercise bout (Scheuermann et al., 2002, DeLorey et al., 2004 & 2007, Gurd et al., 2005 & 2006, Murias

et al., 2011b/c, Spencer et al., 2012 & 2013, Nederveen et al., 2017). However, 'priming' exercise is also known to cause an increase in both BF (O_2 delivery) to the area (Murias et al., 2011b/c, Spencer et al., 2012) as well as a heightened activation of enzymes (such as the carbohydrate rate-limiting enzyme pyruvate dehydrogenase (Gurd et al., 2006, 2008, 2009)) and oxidative substrate provision (Gurd et al., 2006, Howlett et al., 1999, Timmons et al., 1996 & 1998a/b) along the oxidative phosphorylation pathway in the active muscle. These results again preclude the ability to differentiate which mechanism is contributing to the speeding of $\dot{V}O_{2p}$ kinetics. A well designed study by Spencer et al. (2012) gave good insight into the mechanism contributing to the speeding of $\dot{V}O_{2p}$ kinetics with 'priming' exercise when they showed that in normoxia there was a significant speeding of kinetics following 'priming' exercise. However, when the subsequent bout of exercise following 'priming' was done in hypoxia, there was an elimination of the effect of 'priming'. This would implicate a role of O_2 delivery (i.e., BF) in the speeding of kinetics following 'priming', as the speeding of the metabolic pathways that would have occurred as a result of the 'priming' bout was not able to cause a speeding of $\dot{V}O_{2p}$ kinetics.

In addition to the above observation, Spencer et al. (2012) also found that kinetics were only sped when $\tau\dot{V}O_{2p}$ for the first exercise bout (unprimed) was > 20 s, a finding that supports the theory of an "O_2 delivery dependence/independence 'threshold.'" This theory posits that beyond a certain point the limitation on $\dot{V}O_{2p}$ kinetics shifts from an O_2 independent limitation (metabolic 'sluggishness') when $\tau\dot{V}O_{2p}$ is small (e.g., < ~20 s), to an O_2 dependent limitation (O_2 delivery) when $\tau\dot{V}O_{2p}$ is larger (e.g., > ~20 s) (Poole & Musch, 2010).

More recently, studies investigating the effect of an elevated WR_{bl} on $\dot{V}O_{2p}$ kinetics have consistently shown that $\tau\dot{V}O_{2p}$ is larger when transitioning from an elevated WR_{bl} (Nederveen et al., 2017, Keir et al., 2016a/b, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001), with a concomitant increase in $\dot{V}O_{2p}$ gain ($\Delta\dot{V}O_{2p}/\Delta WR$) of both the primary component (G_p) and total response (G_T; Nederveen et al., 2017, Keir et al., 2016a/b, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001). Furthermore, in recent studies from our lab, Keir et al. (2016a/b) revealed that with increasing WR_{bl} $\tau\dot{V}O_{2p}$ increased in a curvilinear fashion ($\tau\dot{V}O_{2p}$ increased to a larger extent than the increase in WR_{bl}) with G_p increasing linearly. This linear increase in G_p could be the result of the recruitment of less

efficient muscle fibres (type IIa & IIb/x), or a decrease in the Gibbs free energy released from ATP hydrolysis of those muscle fibres that were already recruited prior to the elevated WR_{bl} (Wust et al., 2014, Bowen et al., 2011), or some other presently unknown factor(s). In addition to the potential role that type IIa & IIb/x fibres play on the G_p response, the more glycolytic nature of these fibres than type I (oxidative) fibres, also may serve to augment the metabolic sluggishness of the oxidative pathways (Crow & Kushmerick 1982, Pringle et al., 2003a/b) causing a slower kinetic response. Furthermore, BF to muscles that are composed of predominantly type II fibres is lower compared to those muscles that are primarily composed of type I fibres (McDonough et al., 2004, Behnke et al., 2003). Therefore, in addition to being less efficient, type IIa & IIb/x muscle fibres also may have slower kinetics because of a metabolic sluggishness or a less than adequate O_2 delivery.

Blood Flow Kinetics

To investigate the role of O_2 delivery on $\dot{V}O_{2p}$ kinetics, studies have often utilized Doppler ultrasound to continuously monitor blood velocity and vessel diameter for the calculation of bulk BF at the level of the conduit artery (Gill et al., 1979, Hoskins et al., 1990). However, quantifying the BF response is confounded by asynchronous changes in the blood velocity profile that are brought about by changes in the driving pressure from the cardiac cycle (HR; i.e., systole and diastole) and changes in the intramuscular pressure from the muscle contraction-relaxation cycle (CR; Radegran 1997, Radegran & Saltin 1998, Osada 2004). Owing to these fluctuations, the blood velocity signal is typically averaged over either the duration of the HR cycle (MacDonald et al., 1998 & 2001, Hughson et al., 1996) or the duration of the muscle CR cycle (MacPhee et al., 2005, duManoir et al., 2010). However, both of these techniques still produce considerable variability of the BF profile. For instance, when analyzing by HR cycle, if an entire HR cycle occurs within the muscle relaxation phase (when BF is unimpeded by high intramuscular pressure) it will result in a much greater blood velocity than when a HR cycle occurs during the muscle contraction phase (when BF is occluded by the increased intramuscular pressure compressing the vasculature) (see Figure 1.2). Alternatively, when blood velocity is averaged over the muscle CR cycle, the blood velocity values will be greater if there are more systolic phases (e.g., four), than a CR cycle that has fewer systolic phases (e.g., three) (see Figure 1.2). This variation in the number of

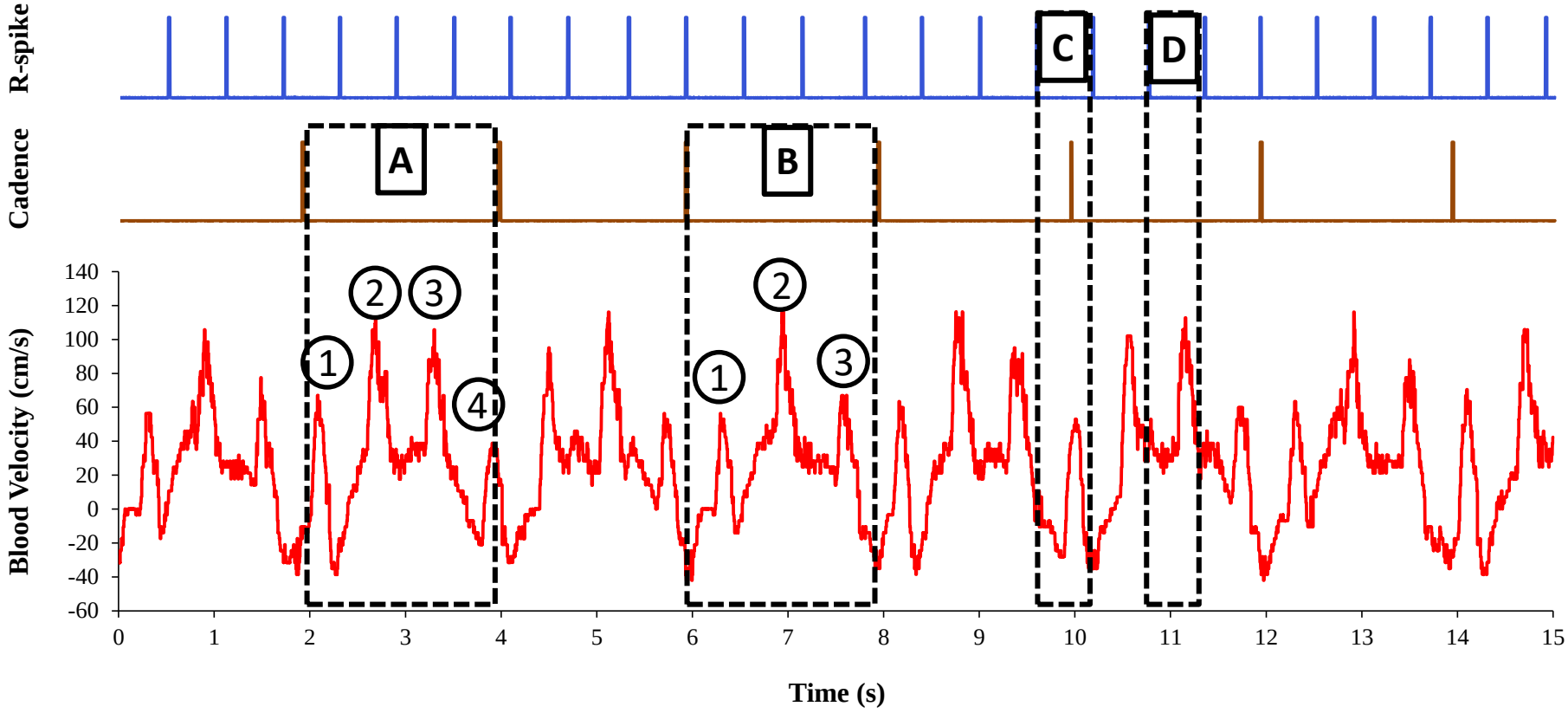

Figure 1.2. Blood velocity tracing displaying muscle contraction cadence and R-spike of heart rate (HR). Note the boxes displaying a single contraction-relaxation (CR) cycle in which there are four (box A) or three (box B) systolic phases of HR; Also, note the boxes displaying a single HR cycle during a muscle contraction phase (low velocity signal; box C) and muscle relaxation phase (high velocity signal; box D).

systolic phases during a muscle CR cycle will commonly occur due to the variability of HR, especially during increasing exercise intensities when the R-R interval durations of the HR cycle are reduced. In an effort to reduce this variability in the blood velocity values, it has become common practice to average the blood velocity signal over multiple consecutive HR or CR cycles (Holland et al., 1998, Shoemaker et al., 1996), which has been shown to decrease the coefficient of variation of the BF response (Osada 2004). However, these studies evaluated "steady-state" exercise, and therefore, it is unknown how these different analysis techniques will influence the BF profile during non-steady-state conditions when having good temporal resolution of the BF signal is crucial. Therefore, while the methods of averaging over multiple CR or HR cycles provides a steady and reliable BF signal, consideration must be given to the effect that this may have on the non-steady-state phase of BF. Due to the transitory nature of this phase, an increase in the duration of the averaging cycle will result in the inclusion of data that is on either side of the exercise transition, as well as a decrease in the number of data points in the transition phase. These issues may then affect the ability to confidently and accurately characterize BF kinetics.

To facilitate the increased demands of the muscle mitochondria, O_2 delivery (via BF) has been shown to increase in a linear manner with metabolic demand (i.e., $\dot{V}O_{2p}$) at a ratio of approximately 5-to-1 (Andersen & Saltin 1985). This increase in BF to the active musculature is accomplished 'centrally' by an increase in $\dot{Q}_T$ (HR and stroke volume) and 'peripherally' by an increase in vascular conductance (VC, principally by vasodilation). These central and peripheral factors create an increase in MAP, through the combination of an increase in $\dot{Q}_T$ and vasoconstriction in the less metabolically active tissues, which further augments BF to the active musculature. In response to a step-increase in exercise intensity, BF to the exercising muscle has been shown to increase in an exponential-like manner towards a new steady-state. This increase in BF is similar to the response profile of $\dot{V}O_{2p}$, which means that quantifying its kinetic response will give valuable insight into O_2 delivery and its role on $\dot{V}O_{2p}$ kinetics (Saltin 1985, Wesche 1986, Wesche & Walloe 1988). Upon the initiation of rhythmic exercise, there is an immediate increase in BF (phase I), which after a brief period (~15-20 s) gives rise to a secondary rise in the BF response (phase II) before achieving a steady-state (phase III) (Shoemaker & Hughson, 1999). Unlike the response of

$\dot{V}O_{2p}$ in which the initial rapid increase (phase I) in the response is attributable to increased pulmonary BF, and is temporally dissociated from $\dot{V}O_{2m}$ by the time it takes blood to transit from the muscle to the lungs, the initial increase in BF (phase I) occurs in real time. This initial increase in BF is attributed to the mechanical action of the active musculature on the vasculature (a 'muscle pump'; Tschakovsky & Hughson, 2000, Folkow et al., 1970, Sheriff et al., 1993, Stegall 1966) and to a rapid vasodilatation within the active muscle due to the effect of metabolites from the active muscle on the vasculature (Corcondilas et al., 1964, Shoemaker et al., 1998, Tschakovsky et al., 1996 & 2004). The increase in BF during phase II has been attributed to vasodilation that matches the metabolic demand of the muscle (for potential contributing vasodilators, see the *Exercise Blood Flow* section). In phase III, however, the characterization of BF kinetics is complicated when exercise is performed in the HVY domain. While there is a clear slow component in the $\dot{V}O_{2p}$ response in the HVY domain, for the BF response some studies report the presence of a slow component (Jones et al., 2012, DeLorey et al., 2007, Koga et al., 2005, Paterson et al., 2005b), while others report no discernible slow component (Nyberg et al., 2017, Fukuba et al., 2004). These inconsistent findings further demonstrate the need for good temporal resolution of BF in response to exercise.

In those studies that quantify both $\dot{V}O_{2p}$ and BF kinetics, the response of conduit artery BF has often been seen to be faster than that of $\dot{V}O_{2p}$ (MacDonald et al., 1998, Fukuba et al., 2004, MacPhee et al., 2005, Koga et al., 2005, Endo et al., 2005, Harper et al., 2006, Jones et al., 2012, Schlup et al., 2015), which would seem to shift the limiting factor on $\dot{V}O_{2p}$ kinetics away from O_2 delivery and toward a metabolic sluggishness. However, this is not a unanimous finding as several studies have observed no difference between $\tau\dot{V}O_{2p}$ and τBF (Grassi et al., 1996, Hughson et al., 1996, MacPhee et al., 2005, Paterson et al., 2005a, duManoir et al., 2010, Jones et al., 2012, McNarry et al., 2014, Nyberg et al., 2017), suggesting that O_2 delivery to the muscle could be controlling the rate of adjustment of $\dot{V}O_{2p}$.

While the response of $\dot{V}O_{2p}$ to varying ΔWRs has received a great deal of attention, relatively few studies have investigated the response of BF to increasing ΔWRs. Those studies that have investigated BF kinetics of exercise transitions to different ΔWRs have reported no difference in the values for τBF across ΔWRs (Murphy et al., 2018, Nyberg et al., 2017,

MacPhee et al., 2005, Hughson et al., 1996), even when ΔWRs were compared between MOD and HVY (McNarry et al., 2014, Jones et al., 2012, Koga et al., 2005). However, several of these studies utilized protocols in which a small muscle mass is examined (forearm or calf; Hughson et al., 1996, Saunders et al., 2005, Nyberg et al., 2017, Murphy et al., 2018), and therefore, there is little challenge to central haemodynamics. Furthermore, most of these studies use only two different transitions, which limits the ability to resolve the behaviour of BF kinetics within and across different exercise-intensity domains. Presently, the only known studies that have investigated more than two transitions (3 transitions: 60%, 80%, 100% maximal WR (WR_{max}; Nyberg et al., 2017); and 30%, 50%, 70% maximal voluntary contraction (MVC; Murphy et al., 2018)), showed no difference in BF kinetics. However, as both of these studies used a small muscle mass (calf (Murphy et al., 2018) and forearm (Nyberg et al., 2017)), it is uncertain whether these findings would apply across dynamic exercise for a large muscle mass. Additionally, apart from their findings on kinetics, Nyberg et al. (2017) also noted that BF amplitude (ΔBF) did not increase proportionately between WRs, with ΔBF increasing from 60% to 80% WR_{max}, but no difference was seen in ΔBF between 80% and 100%, implying that the relationship between WR and BF may not be linear. It also should be noted, however, that all of these aforementioned studies investigated BF kinetics at the level of the conduit artery, and therefore, do not necessarily reflect what is happening with O_2 delivery at the site of O_2 exchange in the microvasculature.

In the only study at present to directly investigate the effect of an elevated WR_{bl} on bulk BF, MacPhee et al. (2005) found that the kinetics of femoral artery BF were slowed, along with slowed $\dot{V}O_{2p}$ kinetics, when alternate-leg KE exercise was initiated from an elevated WR_{bl}. This finding could, therefore, suggest that either a delayed utilization of O_2 in the muscle results in a slowed delivery of O_2 (i.e., BF) to the muscle, or that the rate of increase in BF is insufficient, yielding a delayed attainment of $\dot{V}O_{2p}$ steady-state. MacPhee and colleagues (2005) also found that HR, VC, and deoxy[Hb + Mb] kinetics were slower from an elevated WR_{bl}, with a smaller increase in leg BF for a given increase in $\dot{V}O_{2p}$ ($\Delta BF/\Delta\dot{V}O_{2p}$) also seen when WR_{bl} was elevated. However, in a study by Saunders et al. (2005) investigating forearm VC there was no difference seen between a low or high WR_{bl} on the phase II τ of forearm VC, although phase I τ was larger when WR_{bl} was elevated.

Muscle Deoxygenation

The use of near-infrared spectroscopy (NIRS) allows for the quantification of muscle deoxygenation through the emitting of near-infrared light into the skeletal muscle. This near-infrared light is able to detect both oxygenated (Oxy[Hb + Mb]) and deoxygenated (deoxy [Hb + Mb]) haemogolbin and myoglobin within the field of interrogation of the NIRS probe (see Mancini et al., 1994, Boushel & Piantadosi 2000 for review). Any change in the deoxy[Hb + Mb] signal, therefore, is indicative of a change in the balance of the local O_2 delivery-to-O_2 utilization ratio, with an increase in the deoxy[Hb + Mb] signal indicating an increase in O_2 utilization without a proportionate increase in O_2 delivery, leading to an increase in O_2 off-loading from haemoglobin and myoglobin. This makes deoxy[Hb + Mb] a feasible, and commonly employed surrogate measure, for O_2 extraction within the muscle.

During a step-transition in WR, deoxy[Hb + Mb] increases in an exponential-like manner to a new steady-state (for those WRs in MOD or HVY). However, prior to this increase in the deoxy[Hb + Mb] signal, there is typically a brief period (termed the TD) in which the deoxy[Hb + Mb] signal remains unaltered from baseline, or exhibits a slight undershoot (duManoir et al., 2010). In this phase of the response, $\dot{V}O_{2m}$ (O_2 utilization) is increasing, but an unchanging deoxy[Hb + Mb] signal would suggest that there is equal or, when an 'undershoot' is present, greater increase in O_2 delivery (BF) to the area. Following the exponential-like increase in deoxy[Hb + Mb], an 'overshoot' in the response is frequently observed in which the deoxy[Hb + Mb] signal briefly exceeds its ultimate steady-state value. Such a response is indicative of a slow O_2 delivery to the area, necessitating that the muscle rely more heavily on O_2 extraction for its O_2 needs for a brief period of time (Bauer et al., 2007, Barbosa et al., 2011, Bowen et al., 2012, 2013). When used in combination with $\dot{V}O_{2p}$, the analysis of the deoxy[Hb + Mb] signal, and its kinetics, assists in elucidating the role of O_2 delivery on $\dot{V}O_{2p}$ kinetics. Previous studies employing NIRS have shown that when an exercise transition is initiated from a common low WR_{bl} to increasing ΔWRs there is an increase in the amplitude of deoxy[Hb + Mb] (Δdeoxy[Hb + Mb]) along with the increase in $\Delta\dot{V}O_{2p}$ (MacPhee et al., 2005, Keir et al., 2016b), implying an increased reliance on O_2 extraction to help meet the O_2 demand of the muscle with larger ΔWRs. However, the rate of adjustment of $\dot{V}O_{2p}$ and deoxy[Hb + Mb] to increasing ΔWRs is not different, with $\tau\dot{V}O_{2p}$

and mean response time (MRT; TD + τ) of deoxy[Hb + Mb] being similar across different ΔWR transitions (MacPhee et al., 2005, Keir et al., 2016b). This could suggest that a larger ΔWR does not influence the contribution of O_2 extraction to the $\dot{V}O_{2p}$ response, implying that O_2 delivery is not limiting in this situation. Alternatively, when a WR transition is initiated from an elevated WR_{bl}, while keeping a constant ΔWR, there is a higher $\Delta\dot{V}O_{2p}$ while Δdeoxy[Hb + Mb] is either similar across transitions (Keir et al., 2016b, Nederveen et al., 2017) or increases with increasing WR_{bl} (MacPhee et al., 2005). Such findings suggest that either O_2 delivery increases sufficiently such that there is not an increased reliance on O_2 extraction to meet O_2 needs between transitions, or there is an insufficient increase in BF (O_2 delivery) causing the muscle to extract more O_2 to meet O_2 demand. While $\tau\dot{V}O_{2p}$ has been consistently seen to increase with increasing WR_{bl}, deoxy[Hb + Mb] kinetics generally show a decrease in TD and increase in τdeoxy[Hb + Mb] resulting in either no difference in MRT deoxy[Hb + Mb] across WR_{bl}s (Keir et al., 2016b, Nederveen et al., 2017), or an increase in MRT deoxy[Hb + Mb] with increasing WR_{bl} (MacPhee et al., 2005). An unchanging or increasing MRT deoxy[Hb + Mb] relative to an increasing $\tau\dot{V}O_{2p}$ suggests that O_2 delivery to the muscle is more than sufficient throughout the transition such that there is not an increased reliance on O_2 extraction. This would then suggest that the slowing of $\dot{V}O_{2p}$ kinetics with increasing WR_{bl} is due to a limitation within the cell, a metabolic 'sluggishness.' However, as deoxy[Hb + Mb] is a means of quantifying the ratio of O_2 delivery-to-O_2 utilization, caution should be taken when using it to infer BF.

Microvascular Blood Flow

While conduit artery BF gives an indication of overall O_2 delivery to the whole muscle, determination of the role of O_2 delivery on $\dot{V}O_{2m}$ kinetics requires an understanding of the pressure gradient for O_2 at the site of O_2 exchange with the muscle, the microvasculature. A higher partial pressure of O_2 (PO_2) in the blood of the microvasculature will increase the diffusivity of O_2 into the muscle for use in oxidative phosphorylation. However, while bulk (conduit artery) BF can be reliably and non-invasively measured by Doppler ultrasound, due to issues of practicality, the quantification of microvascular/capillary BF ($\dot{Q}_{cap}$) is much more challenging to measure in humans, especially during exercise. To this end, the use of NIRS has been used as an indirect way of determining $\dot{Q}_{cap}$. By using the deoxy[Hb + Mb] signal

as a surrogate for O_2 extraction (a-vO_{2diff}; Grassi et al., 1996), and coupling this with $\dot{V}O_{2p}$ (a proxy for $\dot{V}O_{2m}$), $\dot{Q}_{cap}$ to the area of interrogation can be estimated through rearrangement of the Fick equation as shown (Ferreira et al., 2005d):

$$\text{Qcap} = \frac{VO2p}{[HHb]}$$

Using this method, several studies have reported the response of $\dot{Q}_{cap}$ to be slower than that of $\dot{V}O_{2p}$ (Schlup et al., 2015, Harper et al., 2006) or tightly coupled to the $\dot{V}O_{2p}$ response (Ferreira et al., 2005d, Buchheit et al., 2009). Additionally, coupled with the results that bulk BF kinetics were faster than both $\dot{V}O_{2p}$ and $\dot{Q}_{cap}$ kinetics, these findings also show that $\dot{Q}_{cap}$ and conduit artery BF are not equivalent. Therefore, efforts should be made to accurately estimate both bulk BF and $\dot{Q}_{cap}$ kinetics when investigating the role of O_2 delivery on $\dot{V}O_{2p}$ kinetics.

Overview of Studies

Numerous studies have been undertaken to investigate the contributing factors to the delayed activation of oxidative phosphorylation during exercise. These studies have come to the general consensus that $\dot{V}O_2$ kinetics are limited by a metabolic sluggishness of activation and metabolic "instability" along the oxidative pathways inside the muscle cell, to insufficient O_2 delivery to the active muscle, or to a combination of these factors. Recent studies from our lab and others have utilized the forcing functions of elevated WR_{bl}s and different ΔWRs to investigate the effect of differing muscle recruitment patterns on $\dot{V}O_{2p}$ kinetics. However, these studies have focused mainly on the $\dot{V}O_{2p}$ response, with relatively few studies having directly looked at the role of BF (O_2 delivery) on $\dot{V}O_{2p}$ kinetics during the various WR_{bl} and ΔWR transitions. In addition, those studies that have investigated BF kinetics typically have used only one or two transitions within the MOD or HVY domain. Therefore, the primary objective of this thesis is to investigate the relationship amongst BF, $\dot{V}O_{2p}$, and deoxy[Hb + Mb] by using multiple WR_{bl} and ΔWR transitions throughout the intensity domains to more fully characterize the effect of BF kinetics on $\dot{V}O_{2p}$ kinetics.

Chapter II of this thesis investigates the efficacy of different analysis techniques on predicting the BF kinetic parameter estimates. While the $\dot{V}O_{2p}$ signal is commonly calculated breath-by-breath, there has not been a consensus on how to quantify the BF signal. Most studies average the BF signal over the duration of either the HR cycle or the muscle CR cycle to compensate for the fluctuations in BF caused by both processes. However, the effect of each of these averaging techniques on BF kinetics has not been investigated. Therefore, before examining the effect of different metabolic perturbations on BF kinetics, it was necessary to discern an appropriate method for quantifying the BF response.

Chapter III examines the effect of transitions from a common baseline metabolic rate using multiple ΔWRs on BF, $\dot{V}O_{2p}$, and deoxy[Hb + Mb] kinetics and describes the relationship of these variables across the different ΔWRs that were used. Additionally, this study sought to determine whether the associations between conduit artery BF, deoxy[Hb + Mb], $\dot{Q}_{cap}$, HR, MAP, and VC change in their influences on the BF and $\dot{V}O_{2p}$ response. It was hypothesized that when exercise transitions are initiated from a common low WR_{bl}, the primary component of $\dot{V}O_{2p}$ and BF kinetics would be similar across a broad range of increasing ΔWRs. Furthermore, it was expected that the amplitude of the response for $\dot{V}O_{2p}$, bulk BF, deoxy[Hb + Mb], HR, MAP, $\dot{Q}_{cap}$, and VC will all increase proportionally with increases in ΔWR.

Chapter IV examines the effect of common exercise transitions (i.e., same ΔWR) initiated from increasing baseline metabolic rates on BF, $\dot{V}O_{2p}$, and deoxy[Hb + Mb] kinetics and describes the relationship of these variables across the many WR_{bl}s investigated. Furthermore, this study again explores the relationship of bulk BF and $\dot{V}O_{2p}$ with deoxy[Hb + Mb], $\dot{Q}_{cap}$, HR, MAP, and VC. It was hypothesized that both BF and $\dot{V}O_{2p}$ kinetics would become progressively slower with increasing WR_{bl}, with the gain of these responses also becoming progressively larger.

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CHAPTER II: Data analysis technique influences blood flow kinetics parameter estimates for moderate- and heavy-intensity exercise transitions

Introduction

With an increase in muscle oxygen utilization ($\dot{V}O_{2m}$) brought about by exercise, there is an increase in convective (bulk) muscle blood flow (BF) and O_2 diffusion to facilitate a greater delivery of O_2 to the muscle mitochondria. Muscle conduit artery BF can be assessed continuously and noninvasively using Doppler ultrasonography, which provides measures of conduit artery blood velocity and diameter (Gill 1979 & 1985, Hoskins 1990). Although conduit arteries interrogated by ultrasonography (e.g., femoral, brachial) demonstrate very little variation in diameter during dynamic exercise (MacPhee et al., 2005, Fukuba et al., 2004), the instantaneous blood velocity fluctuates with the cardiac cycle and muscle contraction-relaxation (CR) cycles, consequent to variations in arterial driving pressure, intramuscular pressure and conductance changes within the smaller resistance vessels (Radegran 1997, Radegran & Saltin 1998, Osada 2004). In this way, the instantaneous blood velocity during rhythmic exercise is greatest when systole (high arterial pressure) coincides with muscle relaxation (low intramuscular pressure and resistance) and lowest when diastole occurs during muscle contraction (Osada & Radegran 2006, Radegran & Saltin 1998). Furthermore, the time course of these fluctuations can change in response to a change in heart rate (HR) or CR cadence, with a speeding of either HR or CR producing shorter interval durations, and thereby more frequent fluctuations in blood velocity during exercise (Osada 2004). To minimize the phasic effect of the heart and skeletal muscle CR cycles on BF measurements, it is common practice to integrate the blood velocity signal over a single or multiple CR or cardiac cycles (using the beat-by-beat (R-R interval) HR). This decreases the variability of the BF-time response (less variability with averaging more cycles) during steady-state exercise conditions (Osada 2004, Holland et al., 1998, Shoemaker et al., 1996) independent of whether CR cycle or HR are used (Osada & Radegran 2006). In examining these averaging techniques, Osada (2004) found that BF averaged over many (10-30) CR cycles resulted in less variability than those BF values determined by either HR or over fewer (1-2) CR cycles, concluding that at least 10-CR cycles should be used to obtain accurate

steady-state BF values. While these established methods provide a good estimation of BF during steady-state exercise, it presently is unclear how averaging affects the dynamic profile of BF during the non-steady-state where greater temporal resolution of the signal is required.

During a step-transition in exercise intensity, $\dot{V}O_{2m}$ and pulmonary $\dot{V}O_2$ ($\dot{V}O_{2p}$) increase in an exponential-like manner to meet the increased metabolic demands of the active skeletal muscle (Krustrup et al., 2009). The time course of adjustment of $\dot{V}O_2$ reflects aerobic system dynamics and can be described by the time constant of the fundamental (phase II) $\dot{V}O_2$ response (i.e., $\tau\dot{V}O_2$) or by the mean response time of the overall $\dot{V}O_2$ response (MRT = τ + TD). Like $\dot{V}O_2$, with step-changes in work rate (WR), BF also increases in an exponential-like manner and thus quantification of its kinetics provide valuable insight into the muscle bulk O_2 delivery and the relationship between BF and $\dot{V}O_2$ (Saltin 1985, Wesche 1986, Walloe & Wesche 1988). With transitions into both moderate- (MOD, below lactate threshold (LT)) and heavy-intensity domains (HVY, above LT) the $\dot{V}O_{2p}$ increases to a new "steady-state," but in HVY, the "steady-state" is delayed compared to MOD because of an additional $\dot{V}O_{2p}$ requirement compared to MOD. This extra O_2 cost of exercise, termed the '$\dot{V}O_{2p}$ slow component,' reflects a decrease in exercise efficiency. Although these differing responses are well-recognized for $\dot{V}O_{2p}$, the observed response of BF to HVY exercise is much more ambiguous on the existence of a slow component-like response, with some studies quantifying a slow component in the BF response (Paterson et al., 2005a/b), while others are not able to discern a BF slow component (Fukuba et al., 2004). In order to accommodate this uncertainty in the existence of a BF slow component, some authors have used the same time frame as that of the $\dot{V}O_{2p}$ slow component in order to quantify a slow component in BF (Endo et al., 2005, DeLorey et al., 2007), while others have chosen to not quantify a BF slow component (Nyberg et al., 2017).

In those studies investigating BF kinetics, the blood velocity profile has been analyzed by either the HR cycle (MacDonald et al., 1998 & 2001, Hughson et al., 1996) or the muscle CR cycle (MacPhee et al., 2005, duManoir et al., 2010). However, both of these techniques are affected by the interaction of various factors including arterial and intramuscular pressure fluctuations as well as reductions in interval duration brought about by exercise-induced increases in HR or an increase in CR cadence (i.e., decreased R-R interval or CR interval,

respectively). As with steady-state exercise, to reduce the variability in the BF signal caused by the interaction of these factors, the data can be averaged over several HR or CR 'cycles'. However, during the "non-steady-state" associated with exercise transitions, as the averaging "window" is widened to include more HR or CR cycles, the fidelity of the transition profile is reduced which impairs the characterization of the BF kinetic response.

The different sources of variability in the blood velocity signal amongst these analysis techniques could distort the profile and thus, the estimation of the rate of adjustment of the BF response, and the confidence in the estimated parameters (e.g., 95% confidence interval (CI_{95}), a measure of the goodness of fit of the modeled response to the data). Therefore, the purpose of the present study was to determine whether: i) parameter estimates for BF kinetics (baseline, steady-state, amplitude, MRT, and CI_{95}) are altered consequent to HR and CR induced "distortions" in blood velocity and analysis technique used; ii) different averaging periods (1 vs 2 vs 5 HR or CR cycles; 'binned' vs. 'rolling' averages) produce different kinetics parameter estimates, and; iii) each analysis technique can similarly predict kinetic parameter estimates between moderate- (MOD) and heavy-intensity (HVY) exercise transitions.

Methods

Subjects

Eight healthy men (age, 26.8 ± 4.3 yrs (mean ± SD); mass, 83.5 ± 8.3 kg; height, 182.8 ± 5.1 cm) volunteered to participate in this study. Participants were informed of the experimental protocol and provided written informed consent for all procedures that had been approved previously by The University of Western Ontario Ethics Committee for Research on Human Subjects in accordance with the Declaration of Helsinki.

Protocol

All participants were familiarized with 'knee-extension' (KE) exercise on a custom-built alternate-leg knee-extension ergometer (see MacPhee et al., 2005) where muscle activity was confined mainly to the quadriceps muscle group, with minimal contribution from other muscle groups. A seatbelt was fastened around the participant's waist to minimize hip

movement during exercise. Participants maintained a cadence of 30 contractions per minute (cpm), with feedback displayed on a computer screen in front of them. Each participant performed an incremental KE exercise test to the limit of tolerance for determination of $\dot{V}O_{2peak}$ and estimated LT (the point at which $\dot{V}CO_2$, ventilation and end-tidal PO_2 began to increase out of proportion to $\dot{V}O_2$ without a concomitant decrease in end-tidal PCO_2). The LT was used to identify the moderate- (MOD, < LT) and heavy-intensity (HVY, > LT) exercise domains to better study intensity-specific physiological and metabolic responses. After 4 min of baseline (3 W) KE exercise, the WR increased by 3 W each minute until a cadence of 30 cpm could no longer be maintained. The WRs corresponding to MOD and HVY in this study were 24 W and 66 W, respectively. Each exercise transition protocol began with 6 min of baseline KE exercise at 3 W followed by an instantaneous increase in WR to either MOD or HVY, with each transition lasting 8 min. Each protocol was repeated 3-4 times to increase the signal-to-noise ratio (Gill 1985).

Measurements

Pulsed-wave and echo-Doppler ultrasound (GE Vingmed, System Five, 4-5MHz, 60° angle of insonation) were used to continuously monitor muscle conduit artery blood velocity and artery diameter, respectively, throughout the exercise protocol. The ultrasound probe was held over the common femoral artery, 2-3 cm proximal to the artery bifurcation to minimize turbulence, but distal to the inguinal ligament to avoid BF to the inguinal region as previously suggested (MacDonald et al., 1998, Radegran & Saltin 1998). The ultrasound gate was positioned in the center of the artery and maximized for complete insonation of the vessel cross-section. Mean blood velocity (MBV) measures were processed by a Neurovision Transcranial Doppler System (Multigon Industries Inc.) and saved on a separate computer using PowerLab (ADInstruments, LabChart 7) for offline analysis. Echo-Doppler ultrasound was continuously recorded throughout the protocol and later used to determine artery diameter. Every 60 s measures of artery diameter were taken in triplicate during a diastole in the muscle relaxation phase and averaged to yield a single vessel diameter at each time point.

Data Analysis

For each individual trial, the instantaneous MBV signal (antegrade and retrograde combined) was integrated in correspondence to a single R-R interval (HR1) and muscle CR cycle (CR1) using PowerLab software (see Figure 2.1). The BF data generated from these methods were averaged subsequently into: i) 'bins' of 2 and 5 cycles (e.g., 2 and 5 CR cycles (CR2b and CR5b)) and; ii) 'rolling' averages of 2 and 5 cycles, with the averaging 'window' progressing by a single cycle for each new point (e.g., HR2r, HR5r). This resulted in 10 different data processing techniques.

Leg femoral artery BF was calculated as:

$$BF\ (L/min) = \pi r^2 \bullet MBV\ (cm/s) \bullet 0.6$$

where r is the femoral artery radius (cm), MBV is the mean blood velocity (cm/s), and 0.6 is a factor to correct units to L/min.

Single exercise transitions were edited and data lying 3 SDs outside the local mean were removed (Keir et al., 2014, MacPhee et al., 2005, Lamarra et al., 1987). Trial repetitions for each subject were linearly interpolated on a second-by-second basis, ensemble-averaged to yield a single response for each transition (MOD and HVY), time-aligned and subsequently averaged into 5 s time bins. Data were modeled using a monoexponential nonlinear, least squares regression fitting function:

$$BF(t) = bl + amp\ (1-e^{-[(t-TD)/\tau]})$$

where BF(t) is blood flow at any time, t; bl is baseline BF (BF_{bl}; at 3 W); amp is the amplitude of the increase in BF (ΔBF) above BF_{bl}; TD is the time delay; and τ is the time constant (equivalent to the time it takes to reach 63% of ΔBF). End-exercise BF values were determined over the last 2 min of the transition. No discernible 'slow component' was seen in any of the HVY transitions, therefore both transitions (MOD and HVY) were fit with a monoexponential function from the first data point following the imposition of the higher WR to the end of exercise (8 min). The TD was locked at '0' to prevent negative values for this parameter. Because these fitting boundaries were used (i.e., fitting the entire transition, and no TD), it was decided to report the time course of BF adjustment as MRT rather than τ.

The coefficient of variation of BF_{bl} (CV_{bl}) was calculated from the ensemble averaged BF profile over the 2 min period prior to the transition.

Statistics

The kinetic parameter estimates for each condition were analyzed by a two-way repeated measures ANOVA (exercise intensity x analysis technique). A significant F ratio was further analyzed using Tukey's post hoc analysis to determine where differences were located. All statistical analyses were performed using SigmaPlot (Systat Software Inc., San Jose, CA, USA). Statistical significance was accepted at $p < 0.05$. All values are reported as mean ± SD.

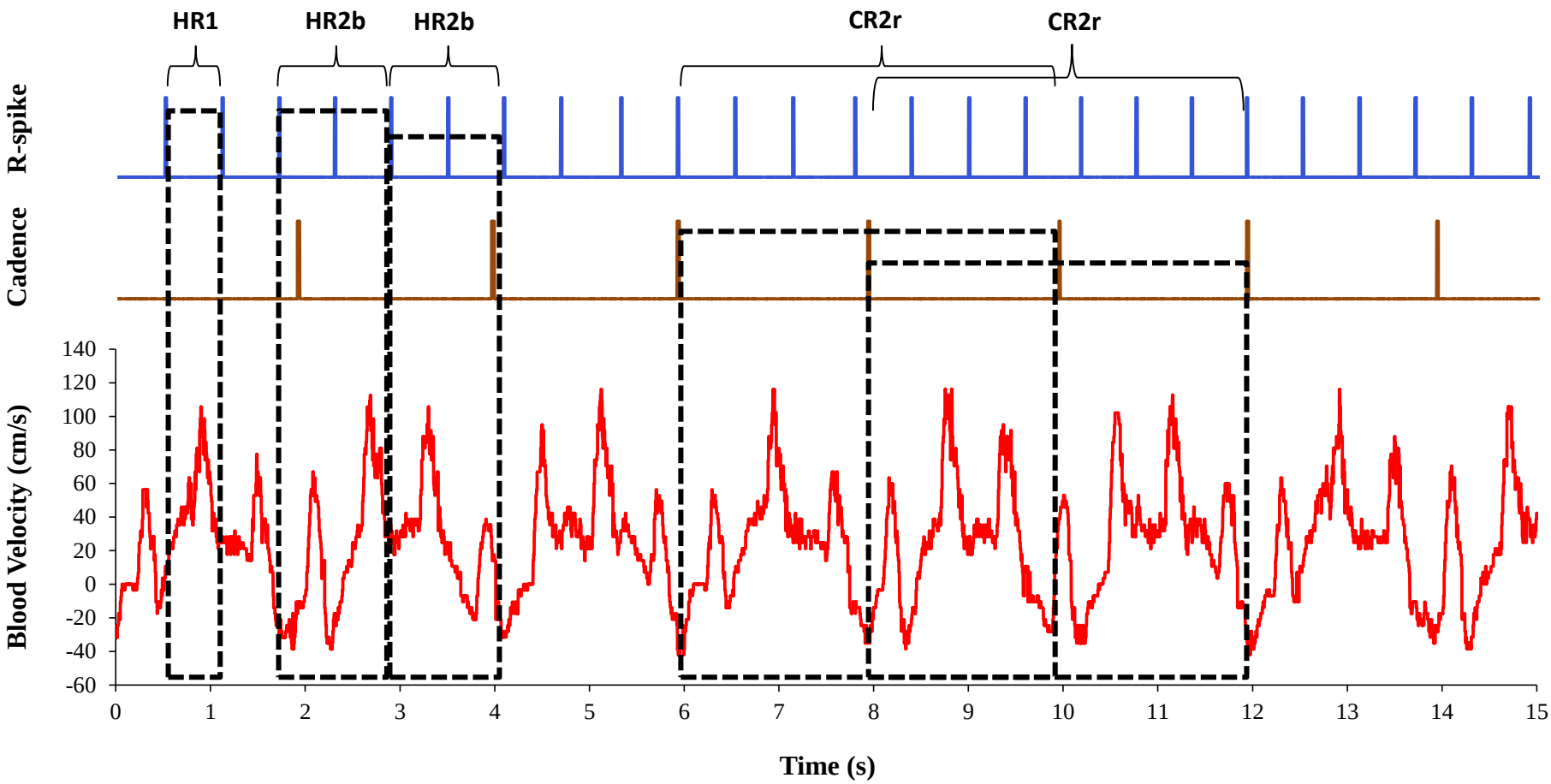

Figure 2.1. Overview of analysis techniques. HR1, integrating blood velocity within a single heart rate (HR) cycle; HR2b, integrating blood velocity within 2 consecutive HR cycles then moving the window forward to the next 2 consecutive HR cycles and integrating those 2 consecutive HR cycles; CR2r, integrating blood velocity signal within 2 consecutive muscle contraction-relaxation (CR) cycles then moving the window forward by a single CR cycle and integrating those 2 consecutive CR cycles. See text for further explanation. CR, contraction-relaxation cycles; HR, R-R intervals of heart rate; r, rolling average; b, binned average.

Results

The raw data response profiles for each of the CR and HR analysis techniques of a representative subject are shown in Figure 2.2. Subject characteristics are presented in Table 2.1 (with $\dot{V}O_{2p}$ and WR values determined from the step-incremental KE exercise protocol).

Steady-state BF and parameter estimates for BF kinetics are presented in Table 2.2. For analysis of BF_{bl} and CV_{bl}, MOD and HVY trials were combined together as both transitions were initiated from the same 3 W baseline and no between-condition differences for BF_{bl} were observed ($p > 0.05$). In general, CV_{bl} was similar amongst conditions with the exception that CV_{bl} was smaller ($p < 0.05$) for CR5b than nearly all other conditions (except CR5r, in which there was only a trend; $p = 0.07$).

Values for ΔBF from the modeled best-fit response (i.e., not calculated by end-exercise minus BF_{bl}) are displayed in Table 2.2. As expected, ΔBF was greater ($p < 0.05$) in HVY than MOD across all analysis techniques. In HVY, ΔBF tended to be higher in the CR compared to the HR analysis techniques (with the highest ΔBF found in CR1 (1.86 ± 0.41) and the lowest found in HR2r (1.68 ± 0.45)) There were no differences in ΔBF values between analysis techniques in MOD ($p > 0.05$).

The estimated MRT BF in MOD was ~30% smaller ($p < 0.05$) for CR1 compared to most other analysis techniques (Table 2.2; a trend ($p < 0.10$) was seen between CR1 vs. CR2r and HR2b). In HVY, the MRT BF was similar (~ 39 s) amongst all analysis techniques (Table 2.2).

The CI_{95}, which provides a measure of confidence in the estimated parameters, tended to be lower (or at least similar) in the CR compared to the HR analyses. From Table 2.2 it can be seen that the CI_{95} for CR1 tended to be lowest ($p < 0.05$), while HR1 was highest ($p < 0.05$) amongst analysis techniques for both MOD and HVY.

The difference between the "model-derived" end-exercise BF and the actual "data-determined" end-exercise BF responses, reflecting the accuracy of the model prediction, was ~ 60 mL/min (range: 30-100 mL/min) in MOD and ~ 130 mL/min (range: 80-210 mL/min)

in HVY (Table 2.2), with none of the analysis techniques achieving a value of '0' (which would be indicative of modeling technique precisely predicting the 'true' end-exercise BF values). In comparing the difference between the 'model-determined' steady-state BF value and the 'data-determined' end-exercise BF value (the average from the data points in the last 30 s of the transition) within each analysis technique, there were no differences amongst analysis techniques within MOD or HVY ($p > 0.05$; Table 2.2). However, in comparing how well the modeled data predicted the end-exercise data between MOD and HVY exercise within each technique, those analysis techniques that used a 'rolling' average (HR2r, HR5r, CR2r, and CR5r) were significantly closer in predicting the end-exercise BF value in MOD ("missing the mark" by only 30-40 mL/min) compared to HVY ("missing the mark" by 140-210 mL/min; $p < 0.05$; Table 2.2).

Table 2.1. Subject characteristics.

Age (yrs)	$\dot{V}O_{2peak}$ (L/min)	WR_{peak} (W)	LT (L/min)	MOD WR (% LT)	RCP (L/min)	HVY WR (% RCP)
26.8 (4.3)	1.78 (0.21)	83 (8)	1.03 (0.26)	84 (71-99)	1.42 (0.18)	95 (74-103)

LT, lactate threshold; WR, work rate; RCP, respiratory compensation point. Values are: mean (SD; except for MOD WR and HVY WR: mean (range)).

Table 2.2. Values of baseline blood flow (BF_{bl}), baseline coefficient of variation (CV_{bl}), blood flow amplitude (ΔBF), mean response time (MRT), MRT confidence interval (CI_{95}), and difference between model-derived (steady-state) and data-derived (end-exercise; SS-EE) across the different analysis techniques for moderate- (MOD) and heavy-intensity (HVY) exercise.

Variable		HR1	HR2r	HR2b	HR5r	HR5b	CR1	CR2r	CR2b	CR5r	CR5b
BF_{bl} (L/min)	Avg	0.99 (0.29)	0.98 (0.28)	0.98 (0.29)	0.98 (0.28)	0.98 (0.28)	0.97 (0.28)	0.97 (0.27)	0.97 (0.27)	0.97 (0.27)	0.97 (0.27)
CV_{bl} (%)	Avg	5.1 * (1.2)	5.3 * (1.9)	5.2 * (2.0)	5.2 * (1.8)	4.8 * (1.6)	5.3 * (1.9)	5.2 * (1.8)	5.2 * (1.6)	4.7 (1.7)	4.1 (1.5)
ΔBF (L/min)	MOD	0.78 (0.16)	0.74 (0.16)	0.75 (0.16)	0.75 (0.17)	0.76 (0.17)	0.77 (0.20)	0.76 (0.18)	0.76 (0.18)	0.76 (0.18)	0.77 (0.18)
	HVY **δ**	1.71 *† (0.36)	1.68 *†‡# (0.45)	1.73 * (0.40)	1.76 * (0.47)	1.76 * (0.41)	1.86 (0.41)	1.77 (0.45)	1.81 (0.42)	1.75 * (0.45)	1.78 (0.45)
MRT (s)	MOD	44 * (32)	42 * (25)	41 (24)	43 * (26)	42 * (24)	30 (20)	41 (24)	39 (23)	43 * (26)	42 * (25)
	HVY	37 (14)	37 (14)	33 (11)	41 (13)	39 (14)	40 (9)	40 (13)	40 (13)	40 (14)	41 (14)
CI_{95} (s)	MOD	19 (11)	15 * (9)	15 * (8)	15 * (9)	14 * (8)	11 *†‡# (4)	14 * (9)	13 * (9)	13 * (8)	12 *†‡ (8)
	HVY	21 (5)	17 * (6)	15 * (4)	14 * (6)	14 * (4)	11 *†‡ (4)	12 *† (6)	11 *†‡ (4)	12 *† (6)	10 *†‡# (5)
SS – EE (L/min)	MOD	0.10 (0.09)	0.03 (0.02)	0.08 (0.07)	0.04 (0.02)	0.09 (0.09)	0.06 (0.05)	0.03 (0.02)	0.07 (0.07)	0.04 (0.02)	0.07 (0.07)
	HVY	0.12 (0.09)	0.21 **δ** (0.15)	0.08 (0.05)	0.17 **δ** (0.13)	0.11 (0.11)	0.12 (0.10)	0.15 **δ** (0.12)	0.11 (0.10)	0.14 **δ** (0.13)	0.11 (0.15)

Significantly different ($p < 0.05$) from the: *, largest value (or smallest value for CV_{bl} Avg and MRT MOD) within that work rate (MOD or HVY) for that variable; †, 2nd largest value within that work rate (MOD or HVY) for that variable; ‡, 3rd largest value within

that work rate (MOD or HVY) for that variable; #, 4th largest value within that work rate (MOD or HVY) for that variable; δ, significantly different from MOD for that analysis technique. CR, contraction-relaxation cycle; HR, heart rate cycle; r, rolling average; b, binned average. Values are: mean (SD).

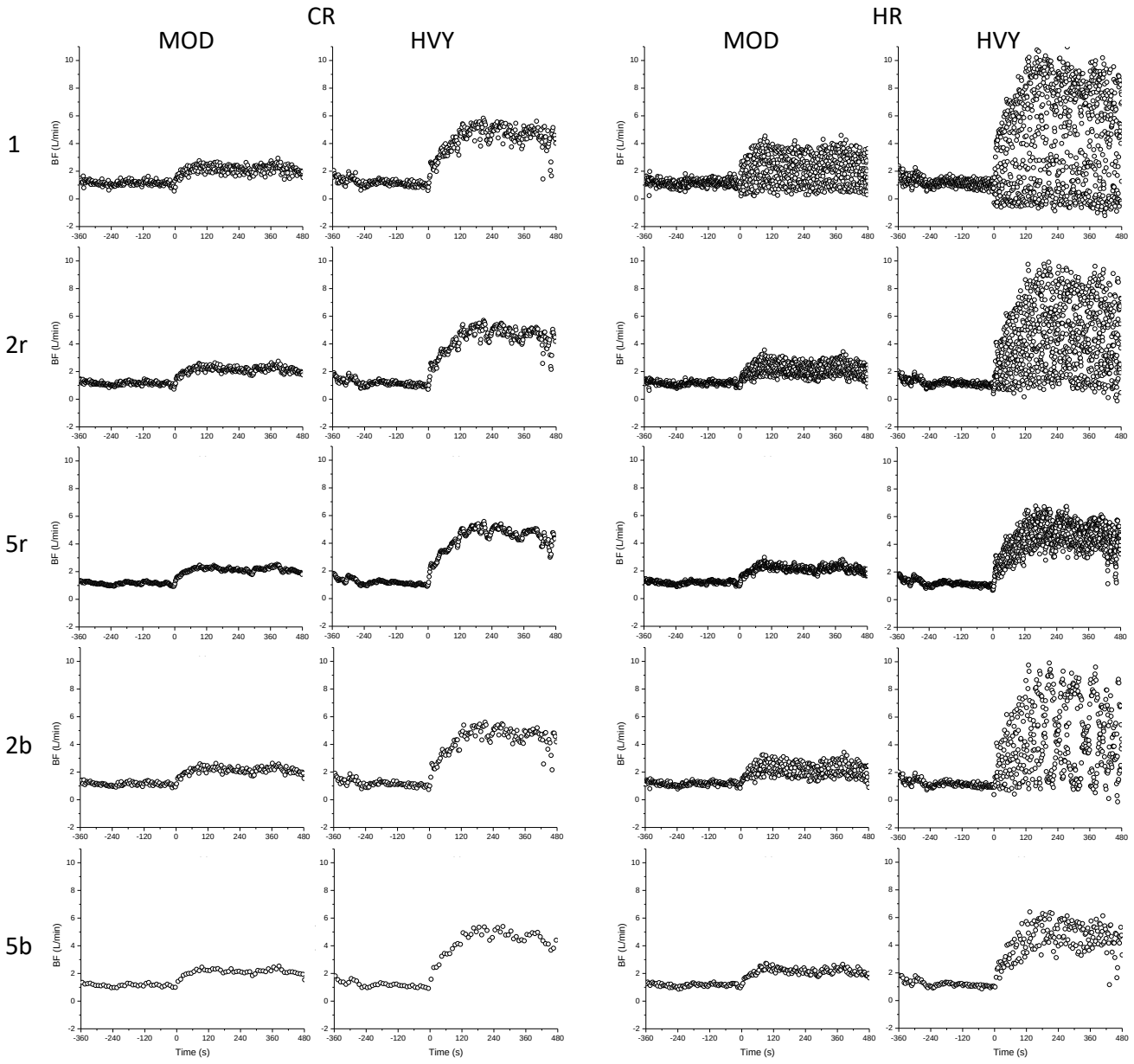

Figure 2.2. The raw data response profiles for each of the analysis techniques for a single moderate- (MOD) and heavy-intensity (HVY) exercise trial of a representative subject. CR, blood flow averaged over the contraction-relaxation cycle; HR, blood flow averaged over the cardiac cycle; 1, single cycle average; 2b, blood flow averaged over 2 cycles 'binned' together; 2r, blood flow averaged over 2 cycles 'rolled' together, etc.

Discussion

When fitting the dynamic profile of the BF response, a central goal is collecting good data to accurately quantify the true physiological response. However, despite the variability in the blood velocity signals and vascular diameter caused by underlying HR and CR cycles, there does not appear to be an accepted "gold standard" method for analyzing (or quantifying) the blood velocity or BF signal, and thus establish a "true" profile in blood velocity or BF. To help elucidate a prospective 'best method' for BF analysis, this study used the same data sets collected during transitions to a MOD and HVY WR, to investigate the effect of different data sorting techniques (based on heart rate (HR) or contraction-relaxation (CR) cycles) and data averaging "intervals" (a single cycle, or using 'binned' or 'rolling' averages over 2 or 5 HR or CR cycles) on estimates of steady-state BF and the parameters associated with the dynamic adjustment of BF during transitions to constant-load exercise (i.e., BF kinetics). The main findings of the present study were: i) MRT BF estimated using a single muscle contraction-relaxation cycle (CR1) was smaller than most other analysis techniques in MOD, but no other differences in BF kinetics were seen amongst any of the other analysis techniques for both MOD and HVY; ii) the steady-state CI_{95} was largest when using a single cardiac cycle (HR1) and lowest when using a single contraction-relaxation cycle (CR1) for BF analysis compared to all other analysis techniques in both MOD and HVY; iii) analysis by CR1 resulted in a higher (or similar) ΔBF response than the other analysis techniques in HVY, but not in MOD; and iv) analyzing data using the 'rolling' average techniques (HR2r, HR5r, CR2r, CR5r) resulted in a significant difference between MOD and HVY protocols when looking at the difference between the 'model determined' and the 'data determined' steady-state BF values.

The variability of the BF_{bl} signal revealed that the analysis technique using the longest averaging periods (i.e., CR5b, ~10 s interval durations) was associated with the smallest CV_{bl} (4.1%) compared to the other analysis techniques. This agrees with findings of previous studies showing that longer averaging periods resulted in less variability in the BF signal (Osada & Radegran 2006, Osada 2004, Shoemaker et al., 1996). However, although variability in BF values was lowest in CR5b, the steady-state BF_{bl} was similar amongst all analysis techniques (Table 2.2). This agrees with previous studies in which steady-state BF

during exercise was similar when assessed using a single HR or CR cycle (Osada & Radegran 2006, Radegran 1997). Therefore, during "steady-state" conditions, the calculated BF appears independent of the analysis technique.

In addition to estimating the steady-state BF responses to a given $\dot{V}O_2$ or WR, quantifying the dynamic profile of BF during transitions between the steady-states of $\dot{V}O_2$ or WR provides additional information on BF control, but requires more care with the analysis due to the changing HR and BF requirements associated with the exercise transient. In the present study during MOD transitions, the estimated MRT BF was smallest (~30 s) when analyzed using a single contraction-relaxation cycle (CR1) compared to the other analysis techniques (~42 s; range: 39-44 s). However, during HVY transitions, the estimated MRT BF was similar (~39 s; range: 33-41 s) independent of the analysis technique used (Table 2.2). That an analysis-related difference in estimated MRT BF was seen in MOD, but not in HVY, could potentially be attributed, in part, to the higher ΔBF in HVY compared to MOD. It previously was suggested for $\dot{V}O_2$ that a small response amplitude is associated with a lower signal-to-noise ratio, which makes it difficult to accurately or confidently model the data response profile (Casaburi et al., 1989, Fawkner & Armstrong 2007). Therefore, the smaller ΔBF associated with MOD may adversely impact the modeling and confidence in estimating the parameters for BF kinetics in this exercise domain. Alternatively, this analysis-related difference between MOD and HVY for quantifying MRT BF may be influenced by the fitting equation used in HVY, as a monoexponential may not accurately represent this response. However, in the present study, there were no trials in which there was a discernible slow component (or phase I), and thereby a monoexponential fit was deemed the most appropriate option.

The ΔBF values in MOD were similar across all analysis techniques, whereas in HVY ΔBF was generally greater when using CR than HR analysis techniques, with CR1 being the largest while HR2r was smallest amongst ΔBF values (Table 2.2). These findings imply that either BF_{bl} values or end-exercise steady-state BF values were different between analysis techniques. While there were no significant differences in any BF_{bl} values across analysis techniques, CR1 steady-state BF values were significantly greater than HR2r (data not shown). While the reason for this finding is not readily apparent, it is possible that a wider

spread of data (i.e., a larger CI_{95}) around the local mean for HR analysis techniques compared to CR analysis techniques may have skewed the steady-states to be lower or higher, respectively.

The CI_{95} characterizes the uncertainty about the true value of a population parameter (Curran-Everett & Benos 2004), and therefore, in the present study, provides some indication of the variability in the BF response. The CI_{95} for HR1 in both MOD and HVY was significantly larger than seen in any of the other analysis techniques. Overall, in both MOD and HVY, the CI_{95} for the CR analysis techniques were generally smaller than those for HR. These findings are likely due to the HR analysis techniques having shorter – and changing – time intervals within which BF is analyzed, which may lead to more frequent and larger fluctuations in the BF signal because of rhythmic changes in intramuscular pressure related to the CR cycle (see Figure 1.2 'box C' and 'box D') (Osada & Radegran 2006). If so, this response might become more pronounced during the transition period when HR is adjusting to the new, higher WR. With the CR analysis techniques, longer averaging periods (e.g., minimum ~2 s) – relative to the R-R intervals of the cardiac cycle (e.g., HVY minimum ~0.52 s (HR ~115 bpm) and MOD minimum ~0.73 s (HR ~82 bpm)) – will attenuate the size and frequency of fluctuations in MBV and BF. These findings agree with previous studies that have shown that analyses based on HR compared to CR tend to be more variable (Osada & Radegran 2006, Radegran 1997). Furthermore, Osada & Radegran (2006) found a negative linear correlation ($r = 0.995$) between the coefficient of variation (CV) for BF and the duration of a single beat-to-beat cardiac cycle. In contrast, there was no relationship when the CV of BF was analyzed with respect to the duration of a single muscle CR cycle, with the CV tending to be lower when analyzed using the CR duration compared to the HR duration (Osada & Radegran 2006). Similarly, the present study found a trend for a negative correlation between CI_{95} and interval duration for both MOD ($p = 0.10$; data not shown) and HVY ($p = 0.06$; data not shown). Although much of the variability in the BF signal is attributable to physiological sources (e.g., cardiac cycle, vascular occlusion from muscle contraction), variability also can be ascribed to the data collection process. Radegran (1997) stated that while insonation failures are readily detected and edited (or removed) when analyzing with a single CR cycle, when analyzing with a single HR cycle these errors are 'hidden' within the large natural variability of the BF signal (see Figure 2.2 – HR1), and

therefore are incorporated into the response profile. In contrast, although a single CR cycle can allow for easier detection of insonation failures, it would be plausible that over multiple cycles these same errors from insonation failures could be incorporated into the overall BF response. When averaging over longer time periods (e.g., ~10 s (CR5r and CR5b)) the negative impact caused by a brief technical measurement failure (e.g., ~1-2 s) could be 'minimized' by inclusion of error-free signal of longer duration (~8-9 s). Nonetheless, aside from eliminating these errors, a goal in fitting the dynamic profile of a response is collecting good data such that the resulting profile is representative of the underlying 'true' physiological response, allowing one to have confidence in the modeled parameter estimates. The finding that BF kinetics estimates had less variability and a smaller CI_{95} for analyses based on CR rather than HR provides support for its use in fitting BF kinetics.

The issue of variability in the BF signal during the non-steady-state phase is clearly seen by its effect on the 'model-determined' steady-state BF compared to the 'data-determined' average end-exercise BF within each analysis technique. While the differences between 'model' and 'end-exercise' steady-state BF responses were similar across analysis techniques within MOD and HVY, the difference was greater in HVY compared to MOD when using a 'rolling' average (HR2r, HR5r, CR2r, and CR5r; Table 2.2). This "variability" in the predictive ability of 'rolling' averages between MOD and HVY exercise would suggest that these techniques are less reliable for fitting BF responses when comparing transitions across more than one exercise intensity domain.

An underlying limitation in the present study is that the modeling of the BF responses in MOD and HVY are restricted by the lack of a 'gold standard' for quantifying BF kinetics and thus comparing the accuracy of different methods. Therefore, the present study chose to gauge the accuracy of these various analysis techniques based on data variability (e.g., CI_{95}) and by observing whether there were any statistical 'outliers' between analysis techniques for the different parameter estimates (e.g., BF_{bl}, ΔBF, MRT). However, it should be noted that, while desirable, having BF fitting techniques that agree with each other or produce less variability does not denote that a fitting technique is 'correct.' Nevertheless, the findings of this study show that certain BF analysis techniques can produce different kinetic parameter estimates, and therefore consideration to how BF is quantified is warranted.

In the present study the TD was constrained to '0.' This seemed reasonable as the MRT values for BF were uncharacteristically large with negative TDs – when TD was free to vary – which cannot be representative of the true physiological response. While this approach is not desirable, as it eliminates a fitting parameter from a study investigating fitting techniques and simultaneously constrains the remaining parameters (i.e., ΔBF and MRT), it has been supported previously (McNarry et al., 2014). It is suggested that future studies take this limitation into consideration when fitting the BF response, and that by not constraining the TD to '0' there may be either an inflated MRT (with a large, negative TD), or an alternative analysis technique that may be more accurate in fitting the BF data.

The effect of contraction frequency was not investigated in the present study but has been shown to impact BF variability, with higher contraction frequencies (60 cpm) showing greater variability than lower frequencies (30 cpm) at low WRs (10-20 W), but not at higher WRs (30-40 W; Osada 2004). In the present study a contraction frequency of 30 cpm was used, as this has been consistently used in our lab (Chin et al., 2013, 2010, duManoir et al., 2010, DeLorey et al., 2007, MacPhee et al., 2005, Paterson et al., 2005a/b), and so care should be taken when fitting BF responses using other contraction frequencies, especially at lower WRs. In the present study, averaging periods of up to 5 CR cycles were chosen so as to limit the maximum duration of averaging periods to no more than 10 s (2 s duty cycle x 5 cycles = 10 s), as longer averaging periods will result in i) fewer data within the transition phase, and ii) more 'mixing/crossover' of baseline and steady-state data with data from the transition phase. Shoemaker et al. (1996) drew attention to this by showing that BF variability was significantly decreased by averaging over their 3 s duty cycle compared to averaging with HR, but that no further improvements were seen by averaging over 5-60 s. These authors point out that averaging over the duty cycle "reduced the exercise-induced variability optimally while maintaining sufficient time resolution for time course studies." Although there are many options for both cadence and duty cycle beyond the parameters used in the present study (one cadence (30 cpm) and three averaging periods (1, 2, and 5 cycles)), the present study has, nonetheless, provided good criteria for consideration (e.g., HR vs. CR, 'binned' vs. 'rolling', single vs. multiple cycle averaging) when estimating BF kinetics.

In conclusion, the results of the present study extend upon the findings of previous studies, which have looked at steady-state BF values, by investigating the effect of different analysis techniques ("triggered" by HR and CR) and averaging methods ('binned' or 'rolling' over 2 or 5 cycles) on the non-steady-state, transition, phase of the BF response profile. While there is no 'gold standard' for BF analysis that could be used to verify the best estimate of BF and whether any of the present analysis techniques is 'correct' or 'better' than the others, the findings of the present study strongly suggest that analysis technique can influence BF kinetics parameter estimates. Based on the findings of this study, it is suggested that CR1 may be a good analysis technique as CR analysis techniques were associated with lower variability (small CI_{95}) than HR analysis techniques. Furthermore, those techniques using 'rolling' averages resulted in discrepancies between model- and data-determined end-exercise BF values between MOD and HVY exercise, meaning that within the CR analysis techniques, the methods of CR2r and CR5r may be poor analysis techniques. Lastly, a shorter averaging period (1 vs. 2 or 5) will also serve to minimize the 'overlap' of data in steady-state and non-steady-state phases of the transition.

Acknowledgements

We gratefully thank each of our participants for their time and effort in this study. We thank Brad Hansen for his technical support of the lab equipment. We also thank Maureen MacDonald and her lab for their guidance on measuring blood flow.

Grants: This study was supported by the National Science and Engineering Research Council of Canada (NSERC) research and equipment grant

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CHAPTER III: The effect of increasing work rate amplitudes from a common metabolic baseline on the kinetic response of $\dot{V}O_{2p}$, blood flow, and muscle deoxygenation

Introduction

During exercise there is an increase in ATP utilization by the active skeletal muscle, with a larger increase in exercise intensity necessitating a larger energy requirement. The greater energy needs ultimately are provided by oxidative phosphorylation, which relies on the delivery of O_2 to the muscle via blood flow (BF). During steady-state exercise there is a linear relationship between muscle O_2 utilization ($\dot{V}O_{2m}$) and BF (Andersen & Saltin 1985). However, upon a step-increase in external work rate (WR), pulmonary O_2 utilization ($\dot{V}O_{2p}$; reflective of $\dot{V}O_{2m}$) does not increase instantaneously, but instead increases in an exponential manner towards a new steady-state (Whipp & Wasserman 1972, Grassi et al., 1996 Rossiter 2011, Poole & Jones 2012), with the rate of this response quantified by the time constant (τ). The delay in attaining steady-state $\dot{V}O_{2p}$ is believed to be due to either a delay in the activation of oxidative enzymes (metabolic 'sluggishness') or an inadequate delivery of O_2 to the muscle (i.e., BF) or some combination of both (for review see Poole & Jones 2012, Rossiter 2011, Tschakovsky & Hughson 1999).

The use of Doppler ultrasound allows for the continual monitoring of both blood velocity and vessel diameter for the determination of conduit artery (bulk) BF (Gill 1979 & 1985, Hoskins 1990). BF also is described as increasing exponentially to meet the increased metabolic demands of the active skeletal muscle, in a fashion similar to the $\dot{V}O_{2p}$ response (Saltin 1985, Wesche 1986, Walloe & Wesche 1988). This increase in BF is accomplished 'centrally' by an increase in cardiac output ($\dot{Q}_T$; via an increase in heart rate (HR) and stroke volume) and mean arterial pressure (MAP), and 'peripherally' by an increase in vascular conductance (VC; via an increase in vasodilation) (for review see Saltin et al., 1998, Joyner & Casey 2015). In general, many studies (MacDonald et al., 1998, Fukuba et al., 2004, MacPhee et al., 2005, Koga et al., 2005, Endo et al., 2005, Harper et al., 2006, Jones et al., 2012, Schlup et al., 2015), but not all (Grassi et al., 1996, Hughson et al., 1996, MacPhee et al., 2005, Paterson et al., 2005a, duManoir et al., 2010, Jones et al., 2012, McNarry et al.,

2014, Nyberg et al., 2017), report muscle conduit artery BF kinetics as being faster than $\dot{V}O_{2p}$ kinetics. The assessment of BF and BF kinetics in many of these studies commonly is made at the level of the conduit artery, and thus the dynamic changes may not accurately reflect BF and O_2 delivery responses occurring in the muscle microcirculation at the red blood cell–myocyte interface. Measures of local muscle oxy-/deoxygenation changes during transitions to exercise can be studied noninvasively using near-infrared spectroscopy (NIRS). NIRS-derived muscle deoxygenation (deoxy [Hb + Mb]; reflecting deoxygenated haemoglobin and myoglobin) provides information on muscle fractional O_2 extraction, or local microvascular O_2 delivery-to-local $\dot{V}O_{2m}$ ratio, within the volume of muscle interrogated by NIRS (DeLorey et al., 2003, Grassi et al., 2003, Grassi & Quaresima 2016). Ferreira et al. (2005b) demonstrated that deoxy[Hb + Mb] in combination with $\dot{V}O_{2p}$ (as a proxy for $\dot{V}O_{2m}$) could provide an estimate of microvascular/capillary BF ($\dot{Q}_{cap}$) kinetics. Using this method, Barstow and colleagues (Harper et al., 2006; Schlup et al., 2015) reported that at the onset of knee-extension exercise, the kinetics of femoral artery blood flow (measured using Doppler ultrasonography) were faster than the adjustment of $\dot{V}O_{2p}$, however the kinetics of $\dot{Q}_{cap}$ were either slower (Harper et al., 2006) or similar to $\dot{V}O_{2p}$ kinetics (Ferreira et al., 2005b; Schlup et al., 2015). These findings of similar or slower $\dot{Q}_{cap}$ kinetics suggest that in order for $\dot{V}O_{2p}$ to increase faster than the rate at which O_2 is being delivered to the muscle there would have to be a significant deoxygenation within the muscle microvasculature which presumably slows O_2 diffusion into muscle.

Although the kinetics of $\dot{V}O_{2p}$ during transitions to increasing ΔWRs has received a great deal of attention, the dynamics of BF adjustments during these transitions has received less attention. Those studies that have examined BF responses to different intensities, generally, report no difference in BF kinetics between transitions of increasing ΔWRs (Nyberg et al., 2017, McNarry et al., 2014, Jones et al., 2012, Koga et al., 2005, MacPhee et al., 2005, Radegran & Saltin 1998, Hughson et al., 1996), with no difference in $\dot{V}O_{2p}$ kinetics across these different ΔWRs either (Nyberg et al., 2017, Jones et al., 2012, Koga et al., 2005, MacPhee et al., 2005). However, several of these studies investigated exercise using a small muscle mass (e.g., forearm, calf) in which there is little challenge to central haemodynamics (Hughson et al., 1996, Saunders et al., 2005, Nyberg et al., 2017, Murphy et al., 2018). Additionally, nearly all of these studies have investigated transitions of only two different

ΔWRs (with only a few investigating the differences between MOD and HVY (McNarry et al., 2014, Jones et al., 2012, Koga et al., 2005) and, therefore, it is not known whether these findings hold true for transitions extending beyond two different ΔWRs, or if there is a hitherto unseen relationship (e.g., linear, curvilinear, sigmoidal, parabolic, etc.) between increasing ΔWRs and kinetics. In one of the few studies that has investigated more than two transitions (3 transitions; 60, 80, 100% max WR) it was found that although the kinetic response of BF amongst WRs did not differ, and the BF amplitude (ΔBF) was greater for exercise at 80% compared to 60% max WR, there was no difference in ΔBF for exercise at 80 and 100% WR max (Nyberg et al., 2017). However, in this study small muscle mass exercise (i.e., forearm) was performed, and whether findings of similar kinetics and an attenuated increase in ΔBF with increasing ΔWR persists in a larger muscle mass is not known. In a study using single leg knee-extension exercise, Radegran & Saltin (1998) reported no difference in the BF time constant (τBF) during exercise transitions to four different ΔWRs (10, 30, 50, 70 W), although they did report a tendency (not significant) for the time to reach 50% of the response amplitude to be longer at higher ΔWRs.

Studies from our lab investigating exercise transitions from a common light-intensity baseline metabolic rate, but increasing ΔWRs for leg-cycling exercise, reported that although the phase II $\dot{V}O_{2p}$ amplitude increased progressively with increasing ΔWR, the $\dot{V}O_{2p}$ time constant did not change (being ~22 s; Keir et al., 2016b; Spencer et al., 2013). However, BF was not measured in these studies. Therefore, the purpose of the present study was to investigate, using alternate-leg knee-extension exercise, the kinetics of $\dot{V}O_{2p}$ and conduit artery BF initiated from a common light-intensity baseline metabolic rate, but with increasing ΔWRs encompassing a range of moderate- to very heavy-intensity domains of exercise. We hypothesized that when exercise transitions were initiated from a common light-intensity baseline, the time constant (τ) for the primary component of both BF and $\dot{V}O_{2p}$ would be unchanging across increasing ΔWRs, but τBF would be consistently smaller than $\tau\dot{V}O_{2p}$ within the different transitions. Similarly, it was hypothesized that within each of the variables of deoxy[Hb + Mb], HR, MAP, $\dot{Q}_{cap}$, and VC, the rate of adjustment would be similar across increasing ΔWRs.

Methods

Subjects

Ten healthy male volunteers (25.6 ± 4.5 yrs (mean ± SD); 85.5 ± 8.6 kg; 184.1 ± 5.3 cm) participated in this study. Subjects were informed of the experimental protocol and were free to withdraw at any time without penalty. Subjects gave written informed consent with all procedures approved by The University of Western Ontario Ethics Committee for Research on Human Subjects in accordance with the Declaration of Helsinki.

Protocol

All participants were familiarized with 'knee-extension' on a custom-built alternate-leg knee-extension (KE) ergometer (see Bell et al., 2001) in which muscle activity was confined to the quadriceps muscle group while the hamstrings were relaxed throughout exercise. A seatbelt was fastened around the participant's waist to minimize hip movement during exercise. Participants were instructed to maintain a cadence of 30 contractions per minute (cpm) which was visualized on a computer screen in front of them. Each participant performed an incremental KE exercise test to the limit of tolerance which consisted of 4 min baseline (3 W) KE exercise, after which the WR increased by 3 W each minute until a cadence of 30 cpm could no longer be maintained. On a separate day, each subject also performed a ramp incremental exercise test to the limit of tolerance on a cycle ergometer (see Keir et al., 2016b). Each incremental test was used to determine $\dot{V}O_{2peak}$, estimated LT (the point at which $\dot{V}CO_2$, ventilation and end-tidal PO_2 ($P_{ET}O_2$) began to increase out of proportion to $\dot{V}O_2$ without a concomitant decrease in end-tidal PCO_2 ($P_{ET}CO_2$)), and respiratory compensation point (RCP; the point at which ventilation and $P_{ET}O_2$ displayed a sharper increase, and $P_{ET}CO_2$ displayed a decrease, relative to $\dot{V}O_2$) for each mode of exercise. These boundaries were used to identify the moderate- (MOD; < LT), heavy- (HVY; > LT to < RCP), and very heavy-intensity (VH; primary component > RCP, but < $\dot{V}O_{2peak}$) exercise domains. Five different exercise transitions were performed on the KE ergometer in which each transition began with 6 min KE exercise from a common baseline WR of 3 W followed by an instantaneous increase to a ΔWR of either 21 (Δ_{21}), 30 (Δ_{30}), 42 (Δ_{42}), 51 (Δ_{51}), or 63 (Δ_{63}) W (see Figure 3.1), with each transition lasting 8 min. The order of

transitions was randomized and at least 24 hrs was allowed between trials. Each transition was repeated 3-5 times to increase the signal-to-noise ratio (Gill 1985).

Measurements

$\dot{V}O_{2p}$: Gas exchange was measured breath-by-breath using volume turbinometry and mass spectrometry as described previously (Keir et al., 2016a/b). Briefly, inspired and expired airflow and volumes were measured by a bidirectional turbine and pneumotach (Hans Rudolph, model 4813; total dead space was 150 mL) which were calibrated before each test with a syringe of known volume (3.0 L) and the pneumotach was adjusted for zero flow. Gas concentrations were sampled continuously and measured by a mass spectrometer (AMIS 2000, Innovision, Lindvedvej, Denmark) which was calibrated before each test using precision-analyzed gas mixtures. The time delay between an instantaneous square-wave change in gas concentration and its detection by the mass spectrometer was determined electronically by computer. Data were collected at a frequency of 100 Hz and transferred to a computer which aligned gas concentrations and expired volumes to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using the algorithms of Swanson (1980).

BF: Pulsed-wave and echo-Doppler ultrasound (GE Vingmed, System Five, 4-5MHz, 60° angle of insonation) were used to continuously monitor blood velocity and artery diameter, respectively, throughout the exercise protocol. The ultrasound probe was held over the common femoral artery, 2-3 cm proximal to the artery bifurcation to minimize turbulence, but distal to the inguinal ligament to avoid BF to the inguinal region as previously reported (MacDonald et al., 1998, Radegran & Saltin 1998). The ultrasound gate was positioned in the center of the artery and maximized for complete insonation of the vessel cross-section. Mean blood velocity measures were processed by a Neurovision Transcranial Doppler System (Multigon Industries Inc.) and saved on a separate computer using PowerLab (ADInstruments, LabChart 7) for later analysis. Echo-Doppler ultrasound was continuously recorded throughout the protocol, saved and used to determine artery diameter. Every 60 s measures of artery diameter were taken in triplicate during diastole in the muscle relaxation

phase and averaged to yield a single vessel diameter at each time point. BF was calculated by the equation:

$$BF\ (L/min) = \text{blood velocity (cm/s)} \bullet \pi \bullet (\text{diameter (cm)}/2)^2 \bullet 0.12$$

where 0.12 is the conversion factor of 's' into 'min' (i.e., x 60) for, mL/min into L/min (i.e., ÷ 1000), and BF for both legs (i.e., x 2). Due to the inherent variability in blood velocity from the cardiac cycle and muscle contraction (Radegran 1997, Osada & Radegran 2006), the present study calculated BF by taking an average of blood velocity over a single muscle contraction-relaxation cycle which has been proposed as an appropriate technique to determine BF kinetics (see Chapter II).

NIRS and microvascular blood flow (Q_{cap}): Local deoxy [Hb + Mb] (in addition to oxyhaemoglobin (and myoglobin; oxy[Hb + Mb]), total haemoglobin (and myoglobin; $_{TOT}$[Hb + Mb]), and tissue oxygenation index (TOI)) was measured in the vastus lateralis muscle using NIRS (Oxiplex TS, Model 92505, ISS, Champaign, USA) as described previously (Keir et al., 2016b). Briefly, NIR optodes were housed in a rigid optically dense plastic holder (connected to laser diodes and photomultiplier tube by optical fibers) with two parallel rows of light emitter fibers (one row emitting at a wavelength of 690 nm and the other row at 828 nm) and one detector fiber bundle; the source-detector separations for this probe were 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths. The NIRS probe was secured to the skin overlying of the proximal portion of the vastus lateralis muscle (approximately 1/3rd the distance from the greater trochanter to the lateral epicondyle of the femur), with great care taken to ensure the same placement for all trials. An optically-dense black vinyl cover and elastic wrap were used to minimize the intrusion of extraneous light and prevent movement of the probes, while allowing free leg movement. The raw signals for deoxy[Hb + Mb], oxy[Hb + Mb], $_{TOT}$[Hb + Mb], and TOI were corrected for skin and adipose tissue thickness (which were measured by Doppler ultrasound) by linear regression of the Hb_{tot} signal against adipose tissue thickness, in accordance with the method of Bowen et al. (2013). Microvasclar BF ($\dot{Q}_{cap}$) was calculated by rearranging the Fick equation, using $\dot{V}O_{2p}$ (after correcting for the cardiodynamic component (phase I)) to quantify $\dot{V}O_{2m}$, and

deoxy[Hb + Mb] as a surrogate for a-vO_{2diff} ($\dot{Q}_{cap}$ = $\dot{V}O_{2p}$/deoxy[Hb + Mb]), as described previously (Harper et al., 2006, Ferreira et al., 2005b).

HR: A Polar HR monitor connected to Powerlab (ADInstruments Inc., Colorado Springs, CO) was used to continuously record HR.

MAP: Systolic and diastolic blood pressure (SBP and DBP) were measured manually on the left arm (at the level of the heart) by a trained technician using a sphygmomanometer and stethoscope, and were used to calculate MAP, where MAP = 1/3 SBP + 2/3 DBP. Measures of BP were taken at rest, twice during baseline KE (at 2 min and 5 min), and at the 30 s time point of each minute during the transition. BP values were corrected to the level of the common femoral artery by an adjustment of 2 mmHg per inch vertical difference between the level of the heart and the common femoral artery (Sia 2003). Femoral artery VC was calculated as BF/MAP. Due to the infrequent measures of MAP and issues of precisely time-aligning both BF and MAP, the iterated fits of BF and MAP were used to calculate VC.

Lactate: Blood was sampled from the earlobe during one of the transitions for each of the individual WRs – at a timepoint 2 min prior to the completion of the exercise transition – for determination of the blood lactate concentration using a lactate analyzer (Lactate Scout, Sports Resource Group, Hawthorne, NY).

Data Analysis

Individual trials for $\dot{V}O_{2p}$ and BF were edited for aberrant data (lying 3 SDs outside the local mean were removed; Lamarra et al., 1987). Data for all variables were linearly interpolated on a second-by-second basis, with data from like transitions for each subject then being ensemble-averaged to yield a single response for each transition and subsequently averaged to 5 s time bins. Data were modeled by a monoexponential nonlinear, least squares regression fitting procedure:

$$Y(t) = bl + amp\ (1\text{-}e^{-[(t\text{-}TD)/\tau]})$$

where *Y* represents $\dot{V}O_{2p}$, BF, deoxy[Hb + Mb], MAP, VC, or HR at any time (*t*); bl is the steady-state baseline of *Y*; amp is the "steady-state" increase in *Y* above bl; TD is the time delay; and τ is the time constant (equivalent to the time taken to reach 63% of amplitude).

For $\dot{V}O_{2p}$, only data within the primary (phase II) component were included in the fitting window (i.e., excluding the 'cardiodynamic' component (phase I) and the 'slow component' when exercise was above LT). Phase I was determined by extending the fit window backward from ~35 s until τ, χ^2, and confidence interval began to increase, as described elsewhere (Rossiter et al., 2001). Similarly, for those transitions into HVY and VH (determined from analyzing the LT in the ramp incremental test, and confirmed by lactate values), the slow component was determined by extending the fit window forward from ~90 s (a time point that was clearly a part of the primary component) until τ, χ^2, and confidence interval began to increase. The $\dot{V}O_{2p}$ slow component was quantified as the difference between the phase II steady-state $\dot{V}O_{2p}$ and end-exercise $\dot{V}O_{2p}$ (averaged over the last 30 s of the exercise transition).

Data for BF were fit from the first time point after the transition to the time point that corresponded with the end of the phase II $\dot{V}O_{2p}$ response (τBF), with TD fixed at 0 s. Additionally, because previous studies have suggested uncertainty on the manifestation of a slow component in the BF profile (Endo et al., 2005, Fukuba et al., 2004), BF data were also fit to the end of the exercise transition for quantification of the mean response time (MRT), with both measures (τBF and MRT) being commonly reported in the literature (Jones et al., 2012, Koga et al., 2005, Endo et al., 2005, Fukuba et al., 2004).

The deoxy[Hb + Mb] response was fit from the end of the calculated TD (CTD; the first value after the transition in which deoxy[Hb + Mb] increased 1 SD above the pre-transition baseline value) to one of three options depending on the deoxy[Hb + Mb] response profile: 1) if an 'overshoot' was present, the response was fit to the peak before the decline in the profile; 2) for those profiles displaying a constant gradual increase after an initial plateau in the response, the response was fit until just prior to the gradual increase; 3) for those profiles not displaying an 'overshoot' or a discernible gradual increase, the response was fit to ~90

s, a method that has been employed previously (Buchheit et al., 2009, Ferreira et al., 2005a). The kinetics of deoxy[Hb + Mb] was quantified by the MRT (CTD + τ).

Data for all transitions of HR were fit from the first time point after the transition to 92 s, as this time point produced the most consistent fit for all trials of all subjects. To avoid negative time delays the TD was locked at '0' for every fit.

The MAP profile was fit from the first data point after the transition (30 s) until the last data point in the transition (450 s).

The VC profile was fit from the beginning of the transition to either the end of exercise or to the peak of the overshoot (if an overshoot was present). When an overshoot was present its amplitude was calculated as the difference between the peak of the response and end-exercise.

A ratio of the increase in BF relative to the increase in $\dot{V}O_{2p}$ ($\Delta BF/\Delta\dot{V}O_{2p}$) was calculated by dividing the amplitude of the full response of BF by the amplitude of the full response of $\dot{V}O_{2p}$.

Statistics

The kinetic parameters for each variable were analysed by a one-way repeated measures ANOVA. A significant F ratio was further analysed using Tukey's post hoc analysis to determine where differences were. All statistical analyses were performed using SigmaPlot (Systat Software Inc., San Jose, CA, USA). Statistical significance was accepted at $p < 0.05$. All values are reported as mean ± SD.

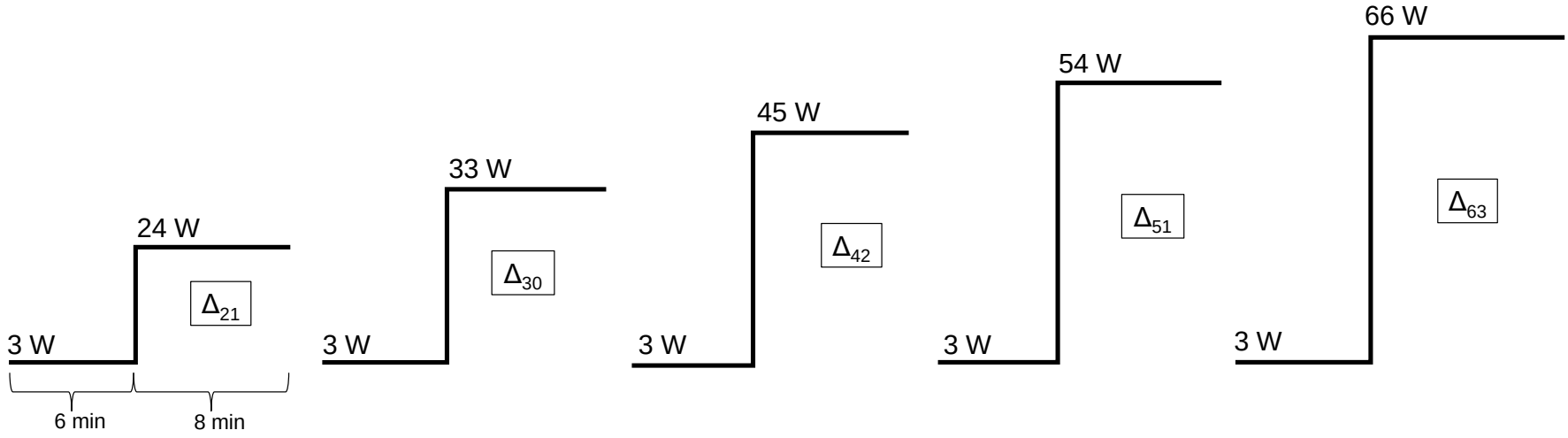

Figure 3.1. Protocol schematic.

Results

Values for $\dot{V}O_{2p}$ and WR from the incremental KE exercise test are displayed in Table 3.1, along with blood [lactate$^-$] from the constant-intensity exercise trials. An incremental exercise test on a cycle ergometer resulted in a peak $\dot{V}O_{2p}$ of 3.99 ± 0.86 L/min at a peak WR of 378 ± 72 W, with a LT and RCP of 2.30 ± 0.53 L/min and 3.11 ± 0.62 L/min, respectively.

$\dot{V}O_{2p}$: The group mean $\dot{V}O_{2p}$ profiles for each exercise transition are displayed in Figure 3.2A. Baseline $\dot{V}O_{2p}$ (~0.55 ± 0.003 L/min) was similar ($p > 0.05$) for all transitions. As expected, the phase II $\dot{V}O_{2p}$ amplitude ($\Delta\dot{V}O_{2p}$) increased linearly with increasing ΔWR ($p < 0.05$; Figure 3.3A). The primary $\dot{V}O_{2p}$ gain (G_p) was similar amongst exercise transitions ($p > 0.05$; Figure 3.3B), however, due to an increase in the slow component amplitude at the larger ΔWRs ($p < 0.05$; Figure 3.3C) the overall $\dot{V}O_{2p}$ gain (G_T) was greater at the larger ΔWRs ($p < 0.05$; Figure 3.3D). The phase II $\tau\dot{V}O_{2p}$ was similar at lower exercise transitions, but increased ($p < 0.05$) at the higher exercise transitions ($p < 0.05$; Figure 3.3E).

BF: The group mean BF response profiles for each transition are displayed in Figure 3.2B. Baseline BF was similar ($p > 0.05$) across all transitions (~1.96 ± 0.04 L/min). There was a linear increase in ΔBF with ΔWR ($p < 0.05$; for both phase II (Figure 3.4A) and over the entire transition (data not shown)). However, when comparing the steady-state or end-exercise BF vs. $\dot{V}O_{2p}$ relationship, a curvilinear increase in BF was observed (Figure 3.5A). This relationship was similarly seen when the increase in ΔBF was adjusted relative to the increase in metabolic demand (i.e., $\Delta BF/\Delta\dot{V}O_{2p}$), in which transitions Δ_{51} and Δ_{63} both had smaller increases in BF relative to metabolic demand than transitions Δ_{21}, Δ_{30}, and Δ_{42} ($p < 0.05$; Figure 3.5B). The MRTs for the overall BF response were greater for the two largest (Δ_{51} and Δ_{63}) than the two smallest transitions (Δ_{21} and Δ_{30}; $p < 0.05$; Figure 3.4B), however, when only the 'primary component' for BF was considered the τBF was similar across all transitions (Figure 3.4C). For most transitions τBF was smaller than $\tau\dot{V}O_{2p}$ ($p < 0.05$), the exception being the Δ_{21} transition where only a trend was observed ($p = 0.08$; Figure 3.5C).

deoxy[Hb + Mb]: The group mean response profiles of deoxy[Hb + Mb] for each transition are shown in Figure 3.2C. The baseline values for deoxy[Hb + Mb] were not different between exercise transitions (~41.7 ± 0.6 μM; $p > 0.05$). There was a gradual increase in the 'primary' deoxy[Hb + Mb] amplitude (Δdeoxy[Hb + Mb]) with increasing ΔWR, with the 3 highest ΔWRs being larger than the lowest ΔWR ($p < 0.05$; Figure 3.6A). The overall deoxy[Hb + Mb] G_T (end-exercise minus baseline) was similar across all exercise transitions ($p > 0.05$; Figure 3.6B). The MRT deoxy[Hb + Mb] was smaller ($p < 0.05$) for higher (Δ_{51}, Δ_{63}) than lower (Δ_{21}) transitions ($p < 0.05$; Figure 3.6C), but the τdeoxy[Hb + Mb] was similar amongst all exercise transitions (data not shown; $p > 0.05$).

[Hb_{tot}]: The group mean response profiles for [Hb_{tot}] are shown in Figure 3.2D. The baseline values for [Hb_{tot}] were similar between exercise transitions (~81 μM; $p > 0.05$). While the amplitude of [Hb_{tot}] (Δ[Hb_{tot}]) was greater ($p < 0.05$) in Δ_{63} than most other transitions (Figure 3.7A), there were no differences in end-exercise [Hb_{tot}] values across transitions ($p > 0.05$; data not shown). As a result, the G_T for [Hb_{tot}] was smaller at the larger ΔWRs than the lower ΔWRs ($p < 0.05$; Figure 3.7B).

HR: The group mean HR response profiles for each transition are show in Figure 3.8A. Baseline HR was similar across all exercise transitions (~72 ± 1 bpm; $p > 0.05$). The amplitude of HR (ΔHR) for the 'primary' component increased linearly with increasing ΔWR ($p < 0.05$; Figure 3.9A). The τHR was similar across all exercise transitions ($p > 0.05$; Figure 3.9B).

MAP: The group mean response profiles for MAP are shown in Figure 3.8B. Baseline MAP was similar across all transitions (93 ± 1 mmHg; $p > 0.05$). The amplitude of MAP (ΔMAP) was larger ($p < 0.05$) at higher than lower ΔWRs ($p < 0.05$; Figure 3.10A), while the MAP G_T was larger at Δ_{51} and Δ_{63} than Δ_{30} ($p < 0.05$; Figure 3.10B). Although an ANOVA showed no differences ($p > 0.05$) in MRT MAP across ΔWRs, a significant linear correlation was observed between MRT MAP and ΔWR (Pearson correlation coefficient, $r^2 = 0.31$; $p < 0.05$; Figure 3.10C).

VC: The group mean response profiles for VC are shown in Figure 3.8C. Baseline VC was similar across all exercise transitions (~15.7 ± 0.3 mL/min/mmHg; $p > 0.05$). The VC

'primary' amplitude (ΔVC) increased linearly with increasing ΔWR ($p < 0.05$; Figure 3.11A). The overall VC G_T was greater in the two smallest (Δ_{21}, Δ_{30}) compared to the two largest transitions (Δ_{51}, Δ_{63}; $p < 0.05$; Figure 3.11B). A small 'overshoot' in the VC response was observed which was more apparent at the highest (Δ_{63}) compared to lower transitions (Δ_{21}, Δ_{30}, Δ_{42}; $p < 0.05$; Figure 3.11C). In general, the MRT VC was variable and similar for all transitions (Figure 3.11D).

$\dot{Q}_{cap}$: The group mean response profiles for $\dot{Q}_{cap}$ are shown in Figure 3.8D. Baseline $\dot{Q}_{cap}$ was not different amongst transitions (~0.028 ± 0.001 arbitrary units; $p > 0.05$). The $\dot{Q}_{cap}$ amplitude ($\Delta\dot{Q}_{cap}$; determined as the difference between baseline and end-exercise) was larger at the higher ΔWRs ($p < 0.05$; Figure 3.12A). No differences were seen in the $\dot{Q}_{cap}$ G_T values across exercise transitions ($p > 0.05$; Figure 3.12B).

Table 3.1. Values from incremental exercise test and lactate values at various work rates for knee-extension (KE) exercise.

WR_{peak} (W)	$\dot{V}O_{2peak}$ (L/min)	LT (L/min)	RCP (L/min)	Lactate (mM)					
				3 W	24 W	33 W	45 W	54 W	66 W
87 (15)	1.84 (0.28)	1.03 (0.26)	1.42 (0.18)	1.6 (0.2)	1.8 (0.4)	2.0 (0.5)	2.6 (0.5)	3.0 (0.6)	4.5 (1.1)

Values are mean (SD). WR, work rate; LT, lactate threshold; RCP, respiratory compensation point.

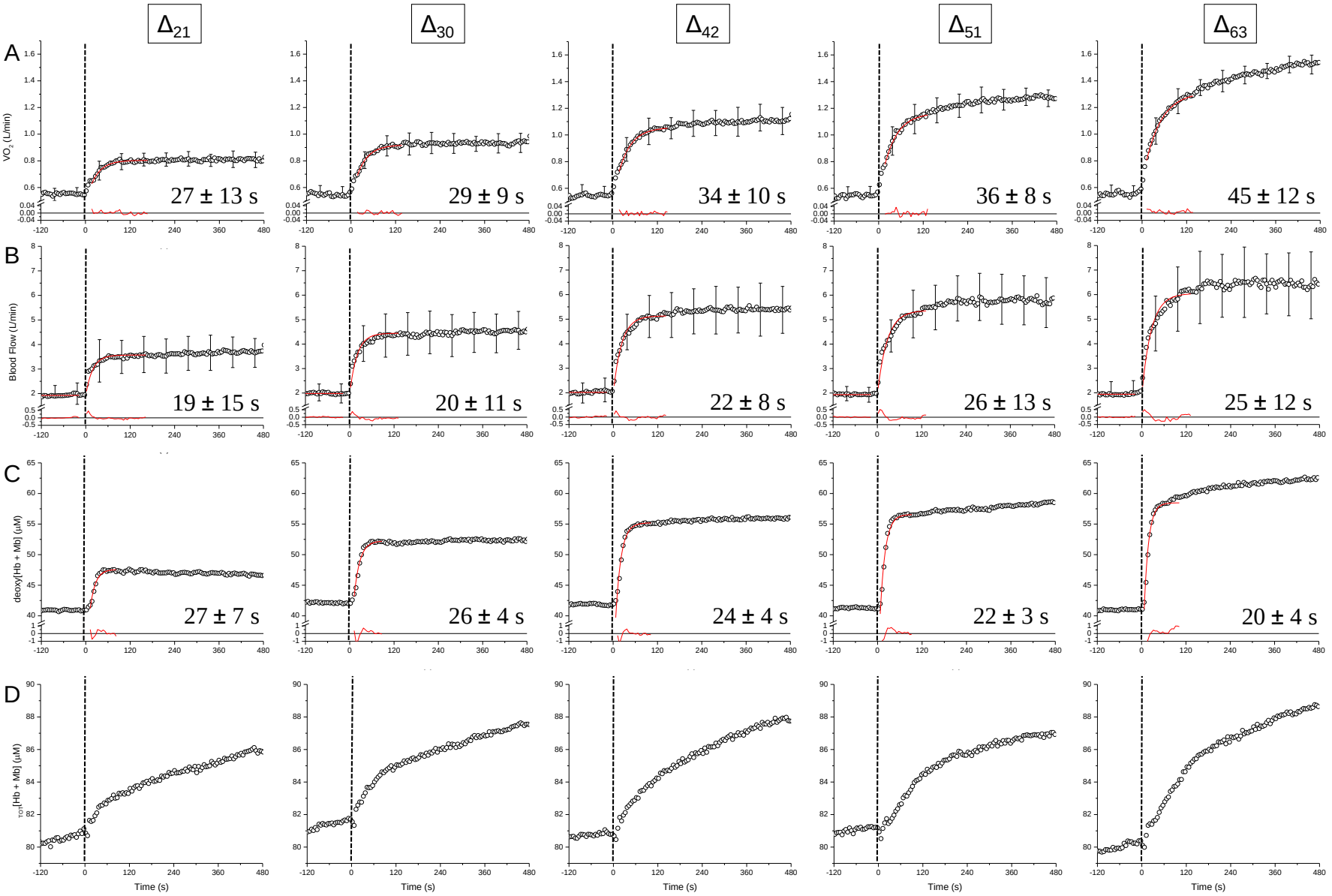
Δ21
Δ30
Δ42
Δ51
Δ63
A
B
C
D
VO2 (L/min)
Blood Flow (L/min)
deoxy[Hb + Mb] (μM)
TOI[Hb + Mb] (μM)
27 ± 13 s
29 ± 9 s
34 ± 10 s
36 ± 8 s
45 ± 12 s
19 ± 15 s
20 ± 11 s
22 ± 8 s
26 ± 13 s
25 ± 12 s
27 ± 7 s
26 ± 4 s
24 ± 4 s
22 ± 3 s
20 ± 4 s
Time (s)

Figure 3.2. Group mean responses (with SD bars; note, SD bars were removed on deoxy[Hb + Mb] and $_{TOT}$[Hb + Mb] for clarity) of: A, pulmonary O_2 uptake ($\dot{V}O_{2p}$); B, blood flow; C, muscle deoxygenation (deoxy[Hb + Mb]); and D, total haemoglobin + myoglobin ($_{TOT}$[Hb + Mb]) for trials Δ_{21}-Δ_{63}. Vertical dashed lines indicate the onset of the exercise transition (time = 0 s). The group mean phase II kinetic responses for each trial are superimposed on the data (red lines, fit by a monoexponential using group mean parameter estimates). Residuals for the monoexponential fit are displayed (in red) about the Y = 0 line. Values for the time constant (τ) of $\dot{V}O_{2p}$, and BF, and mean response time (MRT) deoxy[Hb + Mb] (mean ± SD) are displayed inset in each graph. Δ, work rate amplitude.

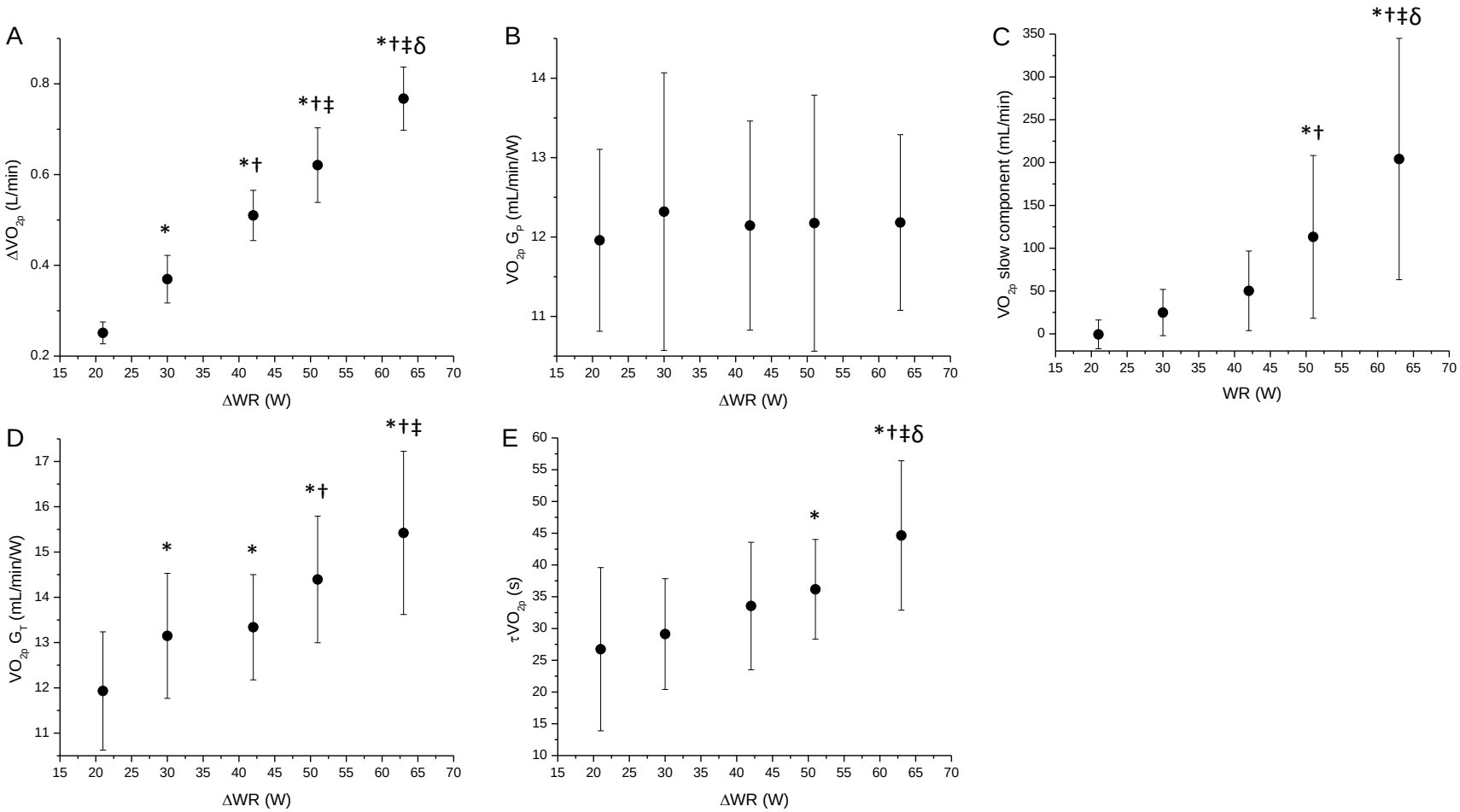

Figure 3.3. Pulmonary O_2 uptake ($\dot{V}O_{2p}$) parameter estimates from a 3 W baseline to the various work rate amplitudes (ΔWR) of each trial (Δ_{21}-Δ_{63}) for, A: $\dot{V}O_{2p}$ amplitude of the primary component; B: $\dot{V}O_{2p}$ gain of the primary component; C: $\dot{V}O_{2p}$ slow component amplitude; D: $\dot{V}O_{2p}$ gain of the full response; and E: $\dot{V}O_{2p}$ time constant. Significant difference ($p < 0.05$) from: *, Δ_{21}; †, Δ_{30}; ‡, Δ_{42}; δ, Δ_{51}. G, gain of the primary ($_p$) and total ($_T$) response; τ, time constant; W, watts.

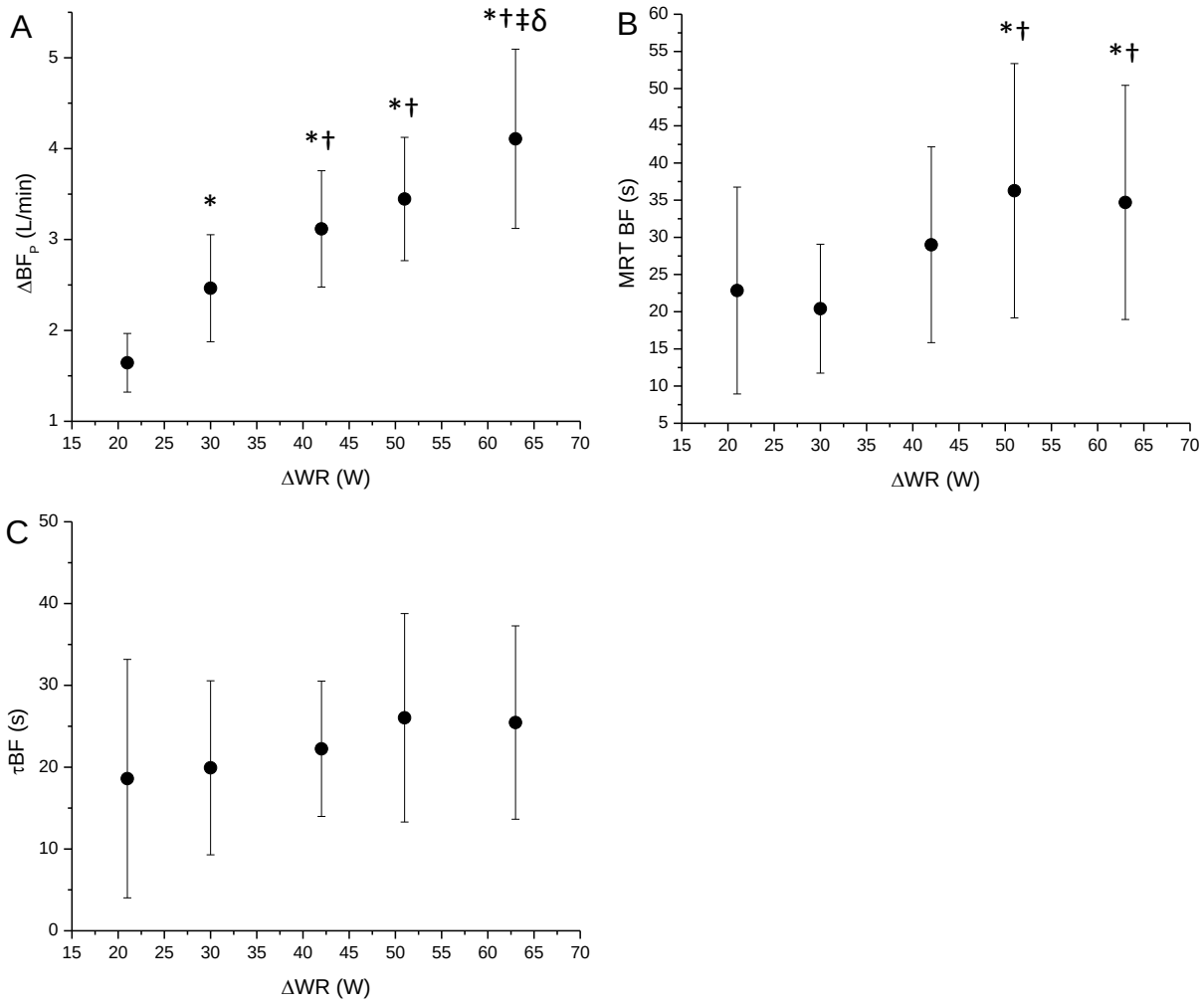

Figure 3.4. Blood flow (BF) parameter estimates from a 3 W baseline to the various work rate amplitudes (ΔWR) of each trial (Δ_{21}-Δ_{63}) for, A: BF amplitude of the primary component (determined from $\dot{V}O_{2p}$ primary component); B: time constant for BF fit to the entire transition; C: time constant for BF fit to the same time point as $\dot{V}O_{2p}$. Significant difference ($p < 0.05$) from: *, Δ_{21}; †, Δ_{30}; ‡, Δ_{42}; δ, Δ_{51}. ΔBF_p, amplitude of the primary component of BF; MRT, mean response time of the entire BF response; τ BF, time constant for the BF response fit in the same time frame as the $\dot{V}O_{2p}$ primary component.

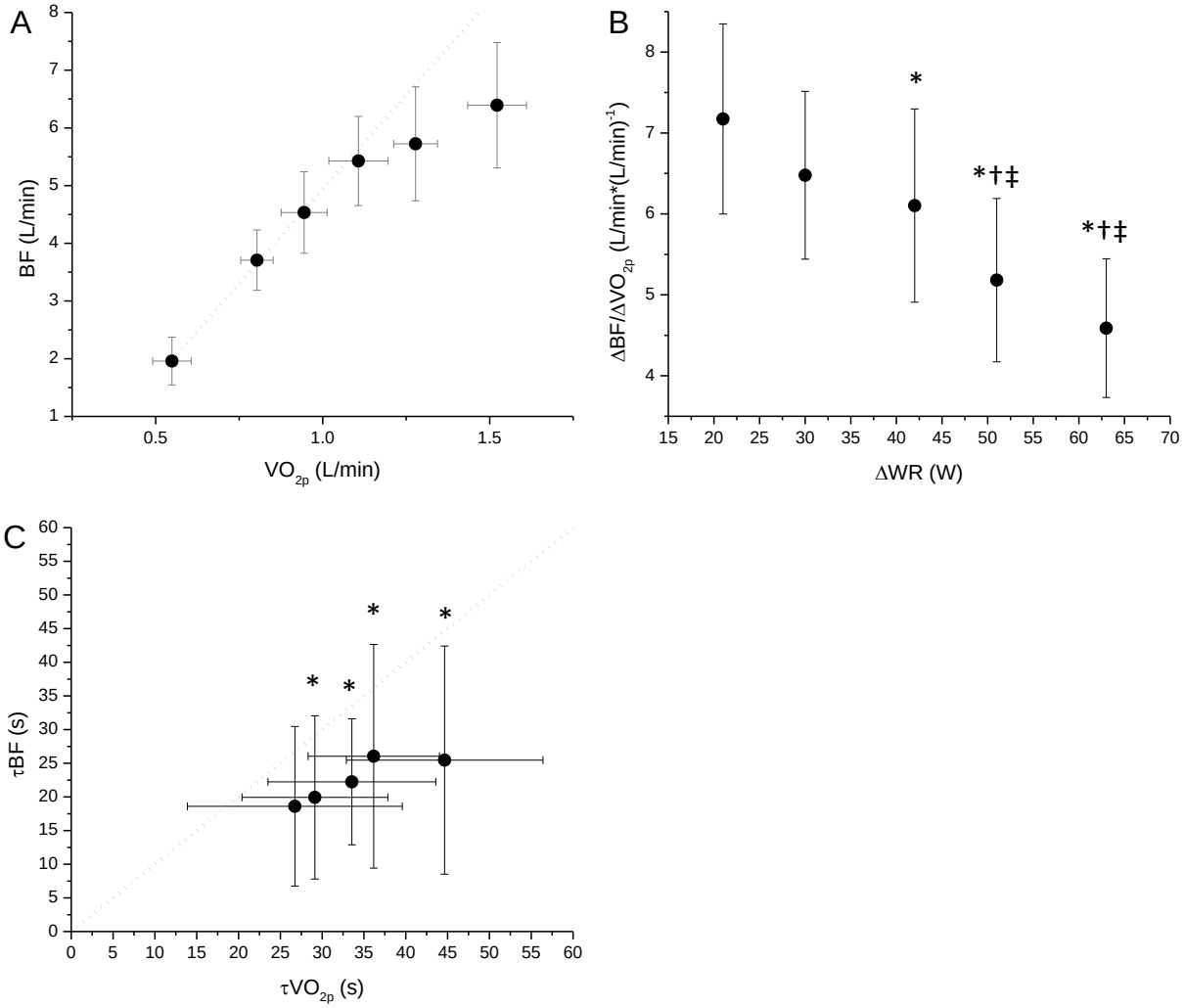

Figure 3.5. Blood flow (BF) vs. pulmonary O_2 uptake ($\dot{V}O_{2p}$) for, A: absolute end-exercise BF vs. end-exercise $\dot{V}O_{2p}$ for the different WRs (i.e., 3, 24, 33, 45, 54, 66 W); B: BF amplitude relative to the pulmonary O_2 uptake ($\dot{V}O_{2p}$) amplitude for the different ΔWRs; C: time constant of the primary (phase II) component of the various transitions (i.e., Δ_{21}-Δ_{63}). Symbols in panel B denote a significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}; ‡, bl_{24}; δ, bl_{33}. For panel C, * denotes a significant difference ($p < 0.05$) between τBF and $\tau\dot{V}O_{2p}$ for that transition. Dotted line in panel A denotes linear regression of the lower 3 transitions ($y = 6.5499x - 1.6133$); dotted line for panel C denotes line of identity ($y = x$). τ, time constant for the primary component.

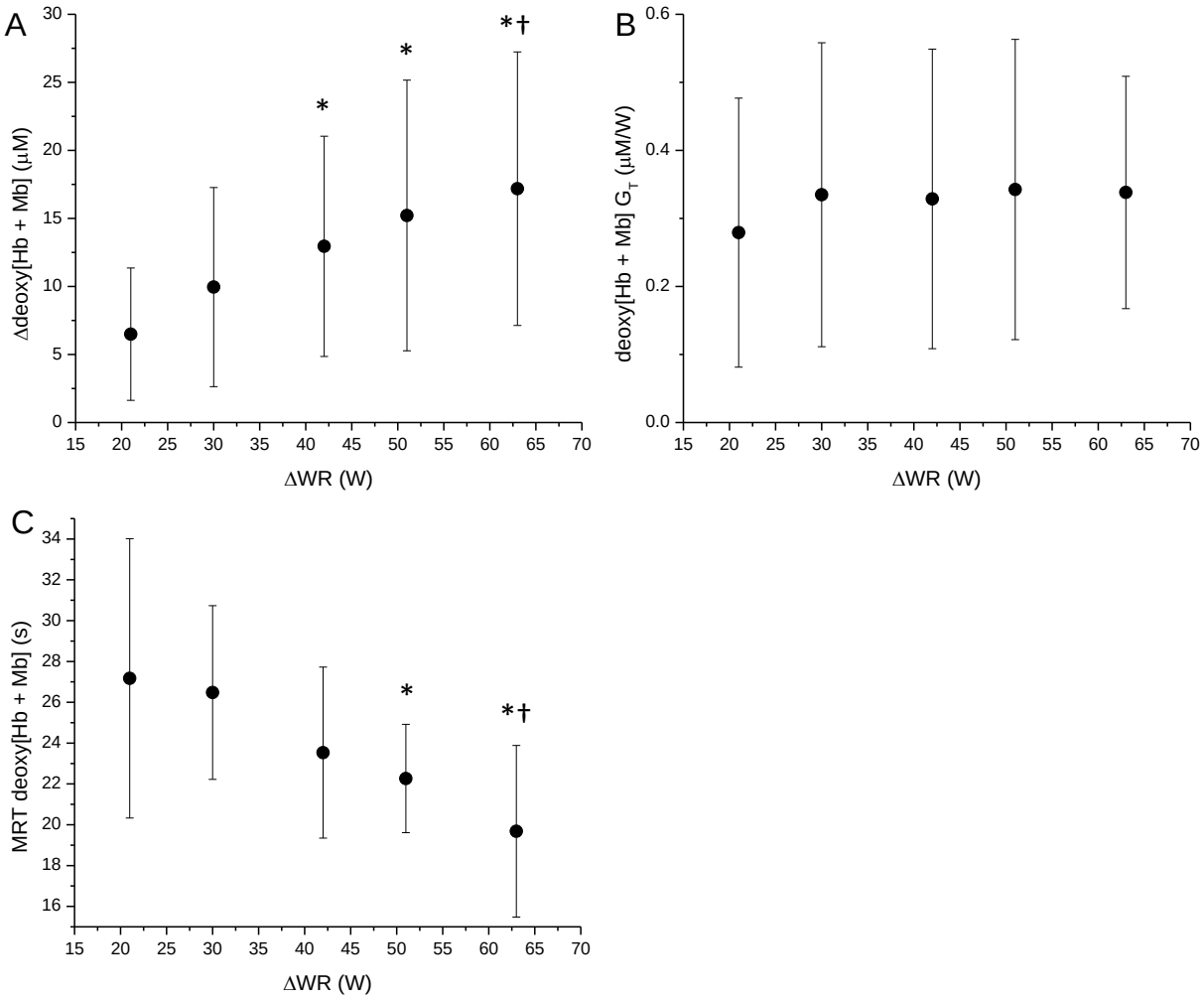

Figure 3.6. Muscle deoxygenation (deoxy[Hb + Mb]) parameter estimates from a 3 W baseline to the various work rate amplitudes (ΔWR) of each trial (Δ_{21}-Δ_{63}) for, A: deoxy[Hb + Mb] amplitude of the 'primary' component; B: deoxy[Hb + Mb] gain of the entire transition; C: mean response time (time constant + time delay) for deoxy[Hb + Mb]. Significant difference ($p < 0.05$) from*, Δ_{21}; †, Δ_{30}. G_T gain of the total response; MRT, mean response time; μM, micromolar; W, watts.

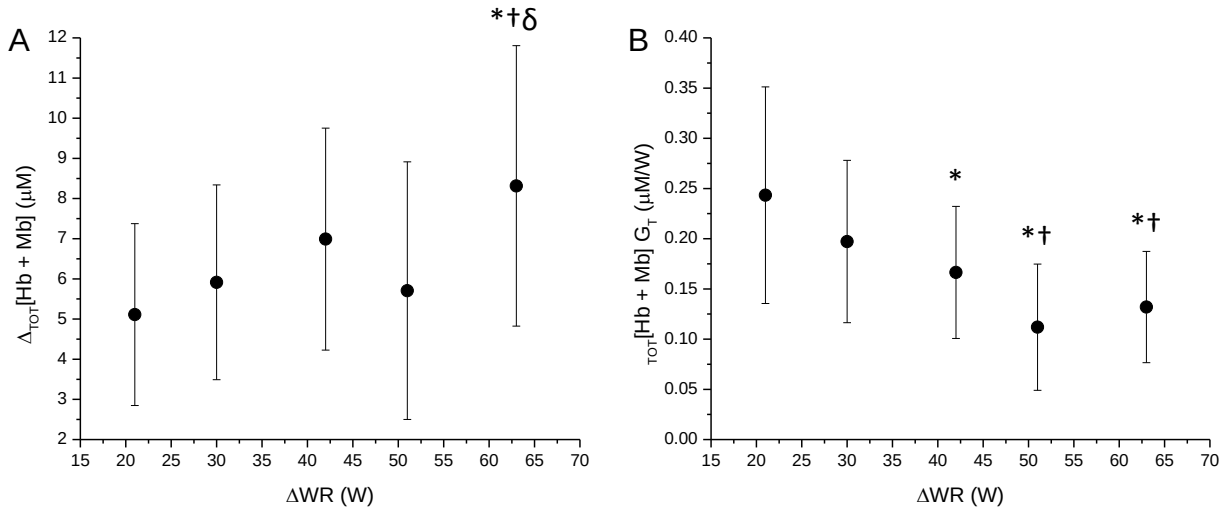

Figure 3.7. Total haemoglobin (and myoglobin; $_{TOT}$[Hb + Mb]) parameter estimates from a 3 W baseline to the various work rate amplitudes (ΔWR) of each trial (Δ_{21}-Δ_{63}) for, A: [Hb_{tot}] amplitude; B: gain in [Hb_{tot}] for the entire transition. Significant difference ($p < 0.05$) from: *, Δ_{21}; †, Δ_{30}; δ, Δ_{51}. G_T, gain of the total response; μM, micromolar.

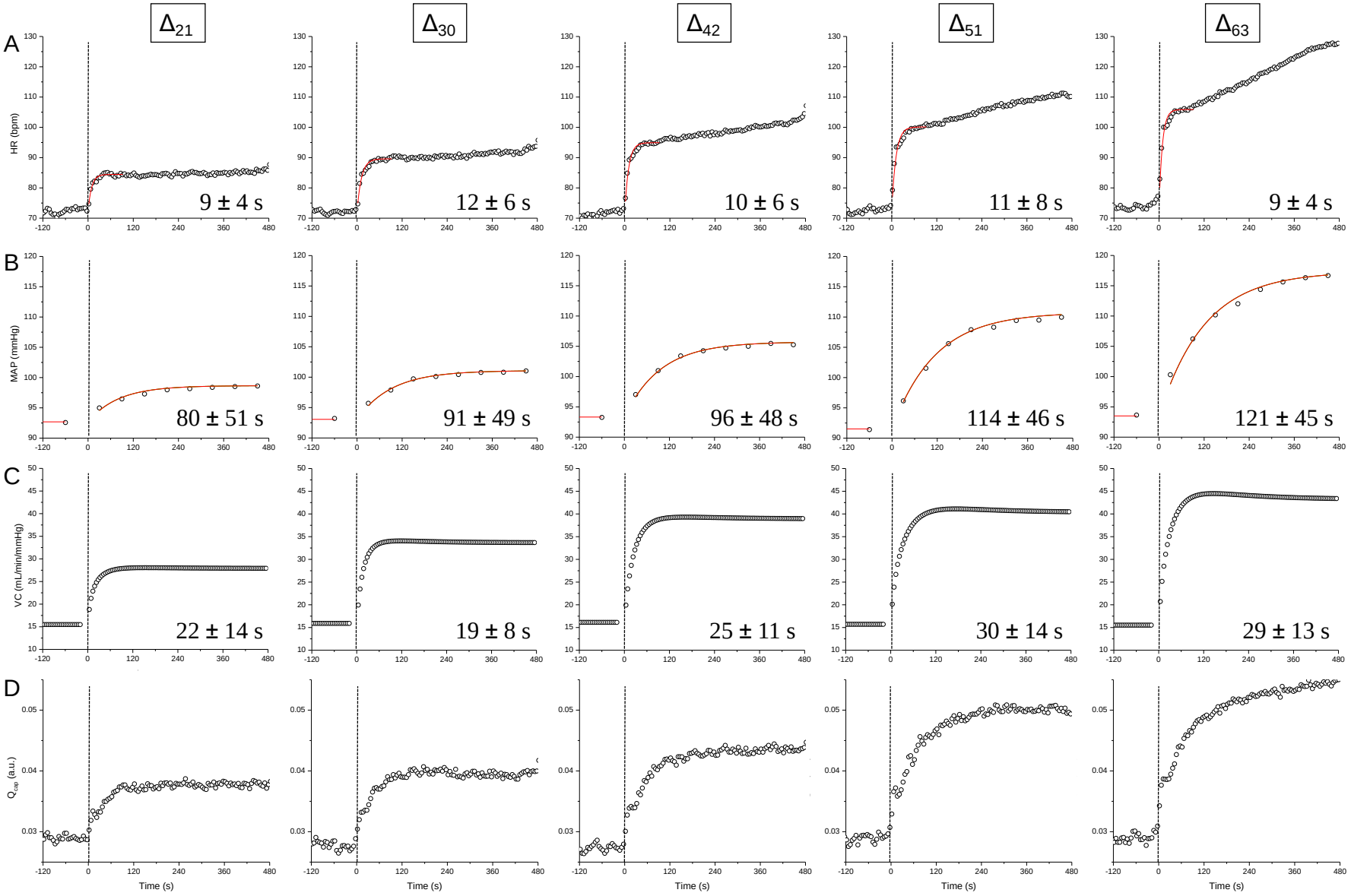
Δ21
Δ30
Δ42
Δ51
Δ63
A
B
C
D
HR (bpm)
MAP (mmHg)
VC (mL/min/mmHg)
Qcap (a.u.)
9 ± 4 s
12 ± 6 s
10 ± 6 s
11 ± 8 s
9 ± 4 s
80 ± 51 s
91 ± 49 s
96 ± 48 s
114 ± 46 s
121 ± 45 s
22 ± 14 s
19 ± 8 s
25 ± 11 s
30 ± 14 s
29 ± 13 s
Time (s)

Figure 3.8. Group mean response profiles of: A, heart rate (HR); B, mean arterial pressure (MAP); C, vascular conductance (VC); and D, microvascular blood flow ($\dot{Q}_{cap}$) for trials Δ_{21}-Δ_{63}. Vertical dashed lines indicate the onset of the exercise transition (time = 0 s). The group mean kinetic responses for HR and MAP are superimposed on the data (red lines, fit by a monoexponential using group mean parameter estimates). Values for τHR, mean response time (MRT) MAP, and MRT VC (mean ± SD) are displayed inset in each graph. bpm, beats per minute; a.u., arbitrary units; Δ, work rate amplitude.

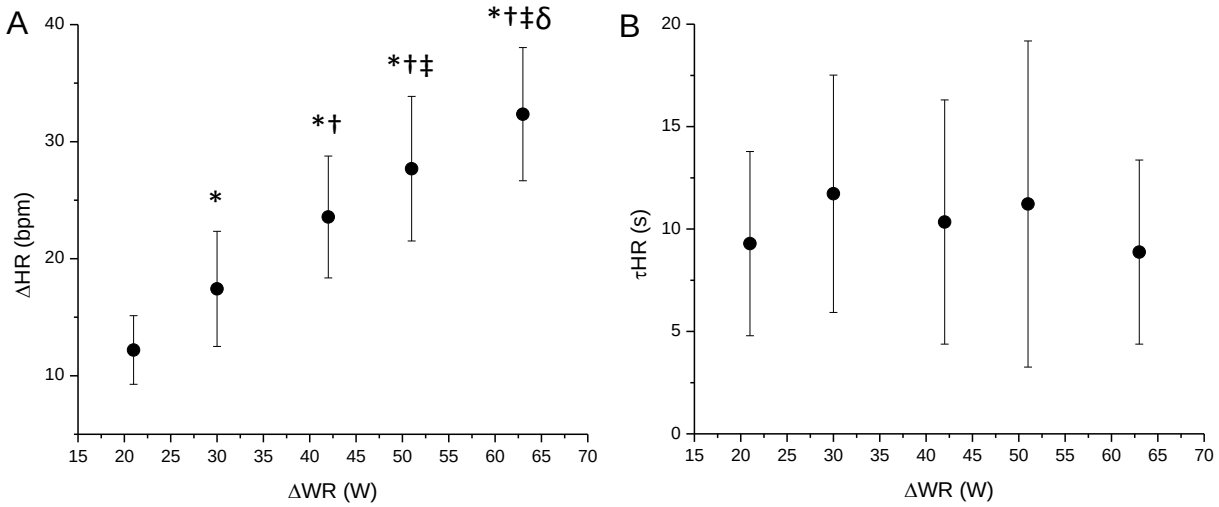

Figure 3.9. Heart rate (HR) parameter estimates from a 3 W baseline to the various work rate amplitudes (ΔWR) of each trial (Δ_{21}-Δ_{63}) for, A: HR amplitude of the 'primary' component; B: time constant for the HR 'primary' component. Significant difference ($p < 0.05$) from: *, Δ_{21}; †, Δ_{30}; ‡, Δ_{42}; δ, Δ_{51}. τ, time constant.

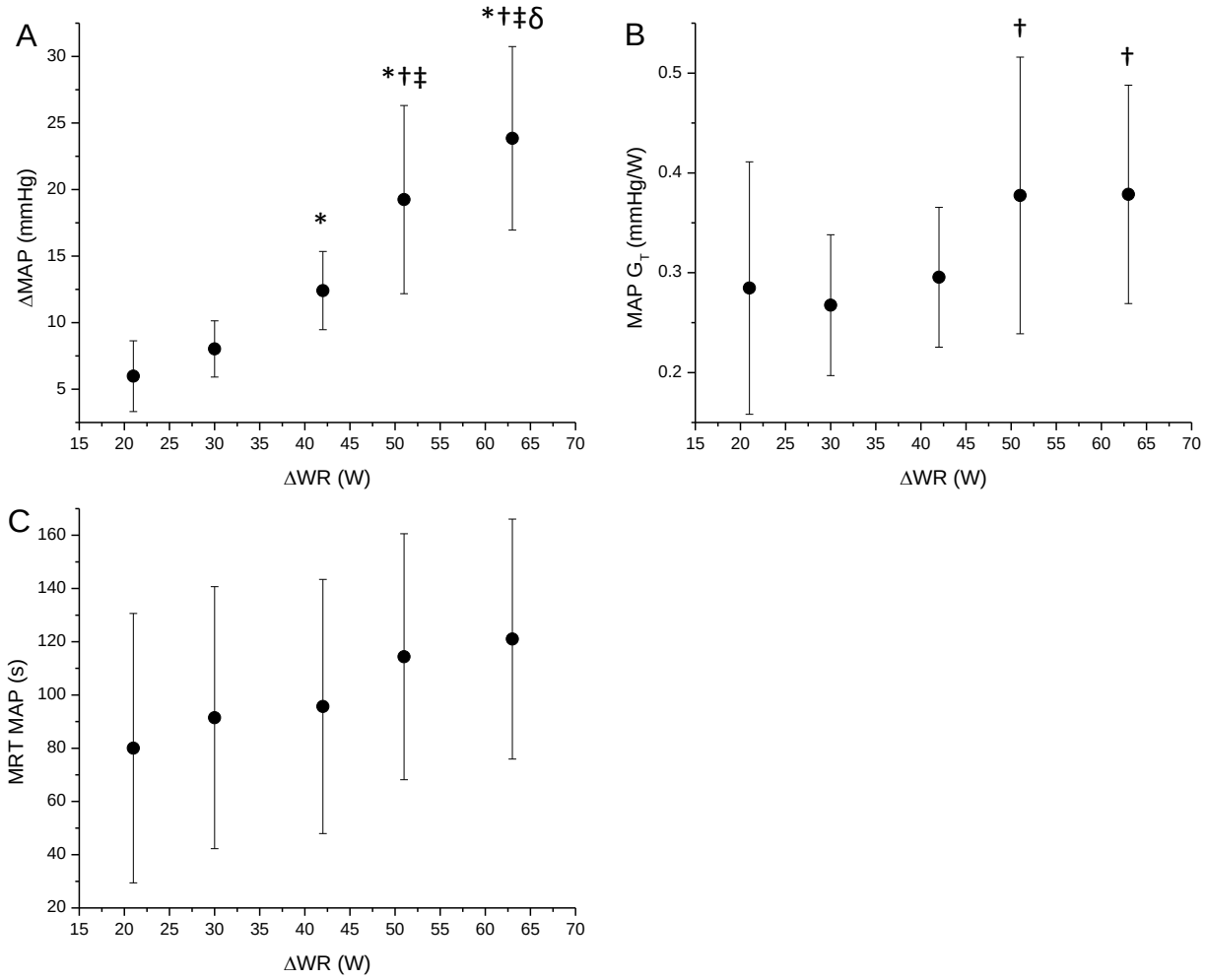

Figure 3.10. Mean arterial pressure (MAP) parameter estimates from a 3 W baseline to the various work rate amplitudes (ΔWR) of each trial (Δ_{21}-Δ_{63}) for, A: MAP amplitude of the entire transition; B: gain in MAP of the entire transition; C: time constant of the entire transition for MAP. Significant difference ($p < 0.05$) from: *, Δ_{21}; †, Δ_{30}; ‡, Δ_{42}; δ, Δ_{51}. G_T, gain of the total response; MRT, mean response time; W, watts.

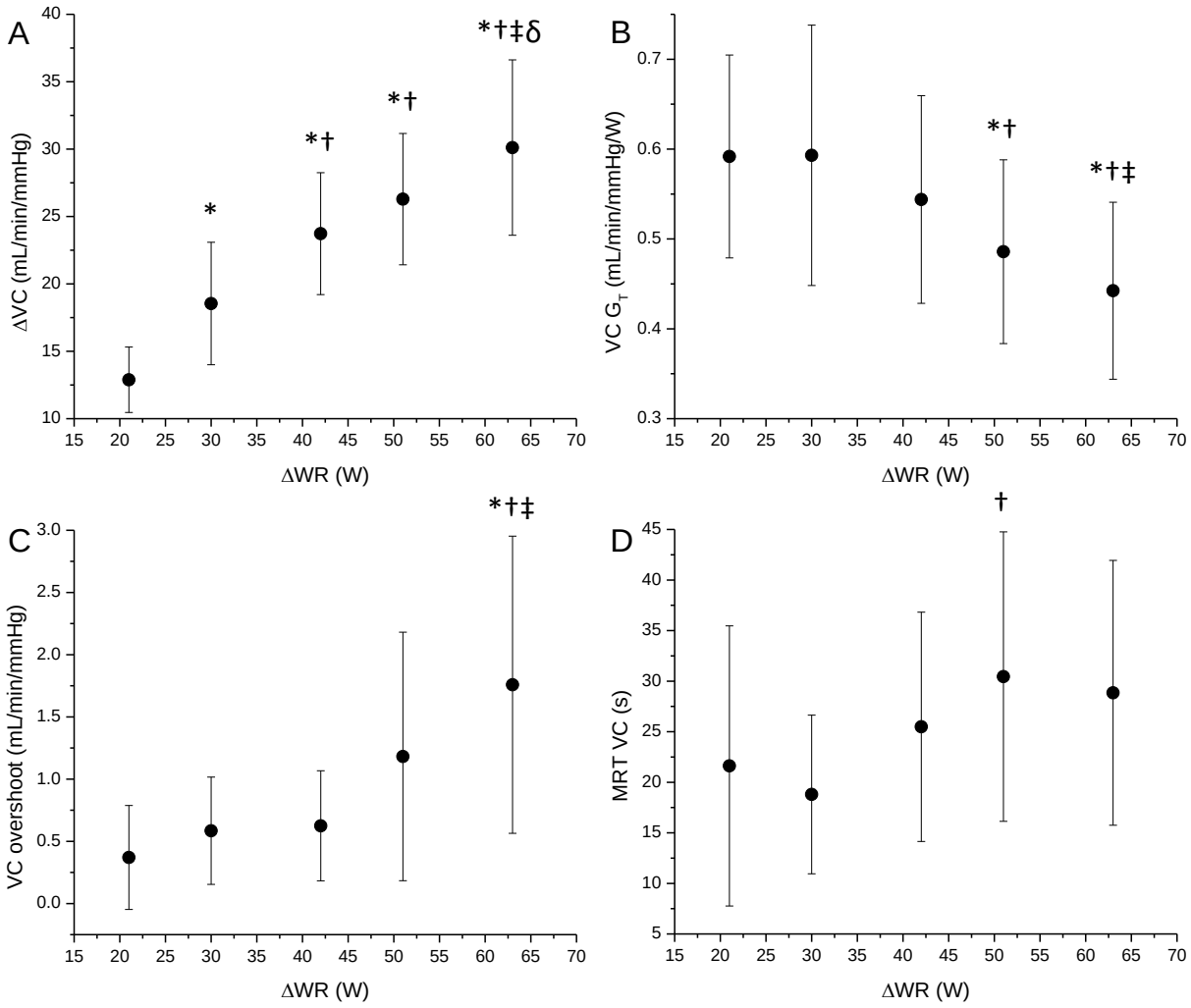

Figure 3.11. Vascular conductance (VC) parameter estimates from a 3 W baseline to the various work rate amplitudes (ΔWR) of each trial (Δ_{21}-Δ_{63}) for, A: VC amplitude of the 'primary' component; B: gain of VC for the entire transition; C: amplitude of the overshoot of VC; D: time constant of the VC response. Significant difference ($p < 0.05$) from: *, Δ_{21}; †, Δ_{30}; ‡, Δ_{42}; δ, Δ_{51}. G_p, gain of the primary component of the response; MRT, mean response time.

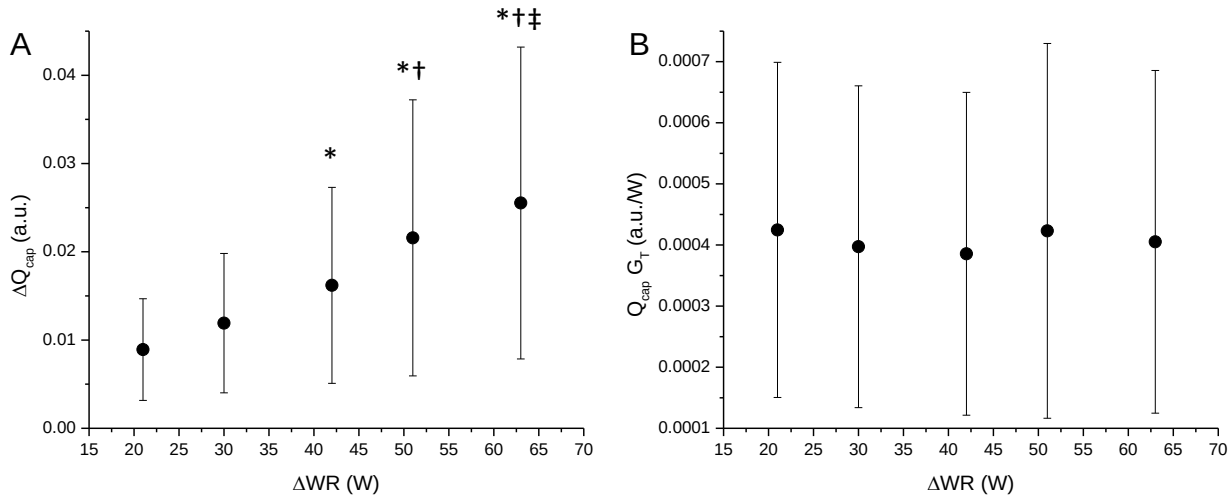

Figure 3.12. Calculated microvascular blood flow ($\dot{Q}_{cap}$) parameter estimates from a 3 W baseline to the various work rate amplitudes (ΔWR) of each trial (Δ_{21}-Δ_{63}) for, A: $\dot{Q}_{cap}$ amplitude; B: gain in $\dot{Q}_{cap}$ for the entire transition. Significant difference ($p < 0.05$) from: *, Δ_{21}; †, Δ_{30}; ‡, Δ_{42}. G_T, gain of the total response; a.u., arbitrary units.

Discussion

In the present study, alternate-leg, knee-extension (KE) exercise was used to study step-transitions of progressively higher WRs all initiated from the same, common low baseline metabolic rate. With this approach we were able to study, in an integrative manner, the dynamic adjustments of $\dot{V}O_{2p}$, femoral (conduit) artery BF, and deoxy[Hb + Mb]. Also, by including measures of MAP, we were able to calculate VC and $\dot{Q}_{cap}$. Combined, these measures provided information on the relationship amongst muscle O_2 utilization and muscle O_2 delivery during exercise transitions spanning across multiple exercise domains (i.e., from MOD to HVY to VH). The main findings of the present study were that during transitions in KE exercise from a light-intensity baseline, but increasing ΔWRs to within the MOD-to-VH-intensity domains: i) the kinetics of $\dot{V}O_{2p}$ became progressively slower, without corresponding changes in femoral artery BF kinetics (τBF), (although there was a trend for BF kinetics to become slower when measured over the entire transition (MRT BF)); ii) while the amplitude of both $\dot{V}O_{2p}$ ($\Delta\dot{V}O_{2p}$) and femoral artery BF (ΔBF) increased with increasing ΔWR, the $\Delta BF/\Delta\dot{V}O_{2p}$ became smaller, which coincided with a greater increase in muscle deoxygenation amplitude (increased Δdeoxy[Hb + Mb]) and a more rapid rate of muscle deoxygenation (lower MRT deoxy[Hb + Mb]) iii) τHR was similar across ΔWRs; and iv) MRT VC tended to become larger with increasing ΔWR.

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

The finding in the present study that phase II $\tau\dot{V}O_{2p}$ becomes larger (i.e., kinetics become slower) during transitions to increasing ΔWRs is in agreement with some (Koga et al., 2001, Koga et al., 1999, Engelen et al., 1996, Paterson & Whipp 1991), but not all studies (Nyberg et al., 2017, Keir et al., 2016b, Spencer et al., 2013, Jones et al., 2012, Koga et al., 2005, MacPhee et al., 2005, Ozyener et al., 2001, Scheuermann et al., 2001, Koga et al., 1997). In many previous studies where no differences in $\dot{V}O_{2p}$ kinetics were reported between MOD and HVY, leg cycling exercise was used (Keir et al., 2016b, Spencer et al., 2013, Ozyener et al., 2001, Scheuermann et al., 2001, Koga et al., 1997) which involved a relatively larger muscle mass and several other muscle groups including the quadriceps, hamstrings, and gluteal muscles, and the muscles of the anterior and posterior lower leg (Hug & Dorel 2009).

In the present study, the KE exercise involved a much smaller muscle mass which was restricted mainly to the quadriceps (Koga et al., 2019, Richardson et al., 1998, Andersen et al., 1985). Different exercise modalities may affect the fibre recruitment such that, for the same absolute or relative WR, a greater proportion of type I and (possibly) type II muscle fibres would be required to satisfy force/power requirements during KE compared to leg cycling exercise. Koga et al. (2005) suggested that an increased recruitment of both efficient and less efficient muscle fibres could explain their findings of a larger primary gain in the $\dot{V}O_{2p}$ response of KE vs. cycling exercise. In addition to some fibres being less efficient (particularly type II fibres), studies on isolated fibre types in rodents (Crow & Kushmerick 1982) and different fibre compositions in humans (Pringle et al., 2003a/b, Barstow et al., 2000, Barstow et al., 1996) have shown that type II fibres have slower $\dot{V}O_{2p}$ kinetics. These slower kinetics in type II fibres could be due to mechanisms intrinsic to the muscle, or an O_2 delivery limitation as type II fibres have been shown to have a lower BF supply than type I fibres (McDonough et al., 2004, Behnke et al., 2003). This could then suggest that $\dot{V}O_{2p}$ kinetics would be slower if a greater proportion of these type II fibres were to be recruited during an exercise. Additionally, during muscle contraction there likely will be an intramuscular pressure-induced compression of the vasculature causing BF occlusion (especially with isometric contraction (Bigland-Ritchie et al., 1995) and with increasing intensities of dynamic contractions (Radegran & Saltin 1998, Kagaya & Homma 1997, Walloe & Wesche 1988)). Although KE exercise is "dynamic", for similar WRs, BF may be more restricted in KE than in cycling exercise because of a more pronounced isometric component and greater intramuscular pressures within the involved muscles during the contraction phase resulting in a lower local $\dot{Q}O_2$-to-$\dot{V}O_{2m}$ (Koga et al., 2005). At higher WRs where a more forceful contraction is needed there may be greater occluding of BF, attenuating the local $\dot{Q}O_2$-to-$\dot{V}O_2$, and thereby contributing to slowing of $\dot{V}O_{2p}$ kinetics due to the impedance of O_2 delivery. Indeed, the findings in the present study of a smaller increase in $[Hb_{tot}]$ G_T with increasing ΔWR would seem to corroborate an attenuated increase in O_2 delivery at higher WRs. In addition, Hoelting et al. (2001) reported that BF was attenuated at higher intensities, with a plateau in BF reached despite continued increases in WR. Contrary to these potential differences in perfusion between exercise modes, Koga et al. (2019) reported greater muscle perfusion during HVY exercise in KE – as evidenced by

a smaller Δdeoxy[Hb + Mb], slower deoxy[Hb + Mb] kinetics, and a larger $_{TOT}$[Hb + Mb] amplitude in the vastus lateralis muscle – compared with leg cycling. Despite these findings of greater perfusion in KE than cycling exercise, Koga et al. (2019) found $\dot{V}O_{2p}$ kinetics to be similar between the two modes of exercise. Additionally, Koga et al. (2005) and Rossiter et al. (2001), both discuss finding that $\tau\dot{V}O_{2p}$ was similar between cycling and KE exercise, whereas in an earlier study by Shoemaker et al. (1994), $\dot{V}O_{2p}$ kinetics were found to be slower in KE than cycling exercise when exercising at the same absolute WR (~ 40-45 W). Koga et al. (2005) suggested that the slower $\dot{V}O_{2p}$ kinetics in KE reported by Shoemaker et al. (1994) were attributable to intensity domain differences since the KE WR was performed in the HVY domain while the cycling WR was in MOD. However, Koga et al. (2005) examined KE and leg cycling exercise in both the MOD and HVY intensity domains and reported that while the $\dot{V}O_{2p}$ MRT (for the entire response profile) was longer in KE than cycling in both the MOD and HVY domains, the primary $\tau\dot{V}O_{2p}$ was not different amongst modalities and intensities. Taken together, these findings of similar $\dot{V}O_{2p}$ kinetics between KE and cycling exercise seem to suggest that perfusion differences between the two modes did not influence $\dot{V}O_2$ kinetics. However, in their more recent study, Koga et al. (2019) also noted faster deoxy[Hb + Mb] kinetics in the rectus femoris muscle, and despite baseline values for $\dot{V}O_{2p}$, absolute WR, and $_{TOT}$[Hb + Mb] being similar between exercise modes, the baseline deoxy[Hb + Mb] was higher in both the vastus lateralis and rectus femoris in KE compared with cycling exercise. These findings indicate a smaller $\dot{Q}O_2$-to-$\dot{V}O_2$ ratio in KE vs. cycling exercise, suggesting that further study on muscle perfusion between the two exercise modes is needed.

The present study has the advantage of examining $\dot{V}O_{2p}$ and BF kinetics across a range of intensities spanning the MOD, HVY, and VH exercise intensity domains. Overall, the phase II $\tau\dot{V}O_{2p}$ (i.e., primary component) was not different with transitions to lower (MOD) ΔWRs, but became larger with transitions to the highest (HVY or VH) ΔWRs (see Figure 3.3E). For exercise transitions into the HVY and VH domains, there is a delayed onset 'slow component' that develops with time, with this slow component representing a larger proportion of overall $\dot{V}O_{2p}$ with progressively higher ΔWRs (Figure 3.3C). Given that the $\dot{V}O_{2p}$ slow component contributes to a slowing of the overall $\dot{V}O_{2p}$ response, care was taken

to exclude this component and focus only on the phase II $\dot{V}O_{2p}$ component. That this approach was successful is supported by the constancy of the $\dot{V}O_{2p}$ G_p ($\Delta\dot{V}O_{2p}/\Delta WR$) across WRs, which would not have been the case if the $\dot{V}O_{2p}$ slow component was included in the response.

BF

One of the main goals of the present study was to investigate the kinetic response of bulk (conduit artery) BF to increasing ΔWRs. We observed a slower response of BF kinetics to the larger ΔWRs, with an overall increase in MRT BF (and a trend for an increase in τBF) with increasing ΔWR. This is somewhat contrary to the findings of previous studies which report no differences in BF kinetics in response to transitions of different ΔWRs (Nyberg et al., 2017, McNarry et al. 2014, Jones et al., 2012, Koga et al., 2005, MacPhee et al., 2005). However, in studies that found no differences in BF kinetics, the group average values tended to be slower (by 2-12 s) for larger ΔWR transitions (McNarry et al., 2014; Jones et al., 2012, Koga et al., 2005, Hughson et al., 1996). The slower BF responses at higher ΔWRs in the present study could be related to higher intramuscular pressures, and thus greater resistance to BF, with higher muscle forces required of the higher intensities. Indeed, previous studies have shown a rapid, large increase in BF immediately after the cessation of exercise, consistent with a significant reduction in impedance to BF (van Beekvelt et al., 2001, Shoemaker et al., 1994). Alternatively, rather than a mechanical limitation to flow, the slower BF response may be a consequence of the slower $\dot{V}O_{2m}$ kinetics associated with the higher ΔWRs. A slower response of $\dot{V}O_{2m}$ by recruited muscle fibres would be expected to result in a delayed increase in microvascular BF to those areas in the muscle. This may then slow the BF response upstream at the conduit artery where BF was measured in the present study. This slowing of bulk BF kinetics coincides well with the slowing of $\dot{V}O_{2p}$ kinetics observed in the present study, suggesting that the two variables could be influencing each other during the transitory phase of exercise. However, in the present study the kinetics of conduit artery BF were faster than, or similar to, those of $\dot{V}O_{2p}$ (Figure 3.5C), which agrees with previous findings reported in the literature in which BF kinetics are faster (Schlup et al., 2015, Jones et al., 2012, Harper et al., 2006, Endo et al., 2005, Koga et al., 2005, MacPhee et al., 2005, MacDonald et al., 1998) or similar to $\dot{V}O_{2p}$ kinetics (Nyberg et al., 2017,

McNarry et al., 2014, Jones et al., 2012, duManoir et al., 2010, Paterson et al., 2005a/b, MacPhee et al., 2005). The finding that conduit artery BF kinetics are faster than or similar to $\dot{V}O_{2p}$ kinetics (and thus $\dot{V}O_{2m}$ kinetics) suggests that at the level of the whole muscle there is adequate O_2 delivery for metabolic demand. However, from the conduit artery at the level of the whole muscle, this O_2 delivery must be subsequently distributed within the microvasculature to the active muscle fibres where O_2 exchange with the myocyte occurs. At the level of the microvasculature, Barstow and colleagues have demonstrated that estimated $\dot{Q}_{cap}$ kinetics are slower than both conduit artery BF kinetics (Schlup et al., 2015, Harper et al., 2006) and $\dot{V}O_{2p}$ kinetics (Schlup et al., 2015, Harper et al., 2006, Ferreira et al., 2005), suggesting that the dynamics of local muscle BF and O_2 delivery potentially might be limiting to $\dot{V}O_{2m}$.

$\dot{V}O_{2p}$ and BF

The present study also found that the increase in BF was not proportional to the increase in metabolic demand across exercise intensity domains, with the $\Delta BF/\Delta\dot{V}O_{2p}$ ratio decreasing linearly from ~7-to-1 to ~4.5-to-1 as ΔWR increased from 21 W to 63 W (Fig 3.5B). While the increase in the BF signal reflects conduit artery (bulk) BF to primarily the active knee extensor muscles in both thighs, the increase of the $\dot{V}O_{2p}$ reflects increases in O_2 utilization of the working quadriceps muscles and, to a lesser extent, the increasing O_2 utilization of the cardiac and respiratory muscles. Therefore, it is possible that the decrease in $\Delta BF/\Delta\dot{V}O_{2p}$ is due to an increased O_2 utilization of the cardiac and respiratory muscles brought about by the higher WRs. However, even at the highest WR in the present study, HR and ventilation values were still well below the maximal values attained by these subjects on a cycle ergometer (~70% maximal HR and 35% maximal ventilation; data not shown), indicating that their contribution to $\dot{V}O_{2p}$ should still be quite small. Accepting this decrease in the BF-to-$\dot{V}O_{2p}$ ratio as emanating primarily from the exercising muscle, the attenuation of an increase in bulk BF with increasing ΔWR may be related to an increase in sympathetic activity that accompanies higher WRs. An increase in sympathetic activity with increasing ΔWR would cause increased vasoconstriction, particularly in the non-exercising muscle/tissues (see Delp & O'Leary 2004 for review), which would cause a decrease in BF to those areas. In the present exercise model, in which BF to the entire lower limb is being

measured, but only the quadriceps are exercising, it would be expected that the vasculature in the quadriceps would undergo vasodilation (as a result of metabolic factors 'overcoming' the sympathetic drive for vasoconstriction; i.e., "sympatholysis" (see Joyner & Casey 2015 for review)), while the relatively large amount of non-exercising muscle in the lower limbs (e.g., hamstrings, calves, etc.) would be expected to undergo vasoconstriction as a result of increased sympathetic output. This could create a situation in which a considerably greater proportion of the vasculature in the lower limbs that is undergoing vasoconstriction than vasodilation, leading to an overall attenuation of the increase in BF at higher ΔWRs. Alternatively, this reduction of the BF-to-$\dot{V}O_{2p}$ ratio with increasing ΔWR may be related to an attenuated increase in BF associated with greater muscle tensions and mechanical compression of the vasculature, as suggested by Hoelting et al. (2001). In line with this, Shoemaker et al. (1994) observed an increase in BF following exercise cessation, suggesting that BF was inadequate during exercise because of an impedance created by the contracting muscle. Van Beekvelt et al. (2001) found a similar increase in BF at the cessation of exercise, with this hyperemia being larger following a higher WR, indicating that higher muscle tension causes greater vascular occlusion. Therefore, as a result of these factors, or other unknown factors affecting BF, there may not be a proportional increase in bulk BF with metabolic rate.

MAP and VC

In the present study, there was an increase in MAP with exercise, with the rate of this adjustment being similar amongst the various ΔWRs. While kinetics were unchanged, there was a linear increase in ΔMAP with ΔWR, with MAP G_T (= ΔMAP/ΔWR) also showing a larger increase at higher ΔWRs (see Figure 3.10B). This increase in MAP G_T is likely attributable to increased sympathetic output at higher ΔWRs causing an increase in both $\dot{Q}_T$ (due to an increase in HR and stroke volume), as well as an increase in resistance (due to vasoconstriction; see Delp & O'Leary 2004 for review). Reciprocal to this increase in MAP G_T, was a decrease in VC G_p with increasing ΔWR. This finding is not unexpected as vascular resistance would be increasing in other parts of the body in an effort to redistribute the body's BF to the area(s) where it is needed most (i.e., quadriceps). Consequently, the much larger area of the body that is undergoing vasoconstriction compared to the relatively

smaller proportion of the body that is vasodilating, would lead to an overall decrease in VC G_p with increasing ΔWR. The present study also saw an 'overshoot' in the VC response, reflecting an early, transient excess in BF, which was greater at higher ΔWRs (see Figure 3.11C). This VC overshoot is due to the conflation of the two factors used in the estimation of VC, BF and MAP. While BF is relatively quick in its response to reach a new steady-state, as seen by faster BF than $\dot{V}O_{2p}$ kinetics in the present study (~20-35 s; Figure 3.5C) and others (Schlup et al., 2015, Harper et al., 2006, Koga et al., 2005, MacDonald et al., 1998), the response of MAP is much slower to reach its new steady-state (~80-120 s; Figure 3.10C). The slower response of MAP may be partly attributable to the sympathetic nervous system (SNS) and its relatively slow increase in activity, which thereby, results in a slower increase in vascular resistance (i.e., vasoconstriction). Indeed, previous studies have shown sympathetic nerve activity to be unchanged during the 1st min of exercise before showing an increase in the 2nd minute of exercise (Hansen et al., 1994), with Hohimer & Smith (1979) showing that it took 1.5 min to observe a decrease in renal BF with exercise in baboons. Furthermore, although the present study did not find a significant difference amongst MRT MAP values, there was a positive linear correlation seen between MRT and ΔWR, which could suggest that the relatively slow adjustment of the SNS, which will be contributing to a larger degree at higher WRs, may also be affecting MAP kinetics. However, future studies with more detailed kinetic analysis of MAP are necessary to substantiate these observations.

deoxy[Hb + Mb]

In the present study muscle deoxygenation, deoxy[Hb + Mb], was used as a surrogate for a-vO_{2diff}. The deoxy[Hb + Mb] amplitude (Δdeoxy[Hb + Mb]; O_2 extraction) increased with increasing ΔWR reflecting a progressively widening mismatch amongst local microvascular O_2 delivery and local muscle O_2 utilization. This increase in Δdeoxy[Hb + Mb] could be associated with the muscle fibres already recruited, as they would have a higher metabolic rate, and therefore, a higher O_2 demand. Additionally, a higher ΔWR would be expected to cause a greater recruitment of less efficient muscle fibres, with these fibres known to have a smaller BF supply and rely more on O_2 extraction than more efficient muscle fibres (Ferreira et al., 2006, McDonough et al., 2005 & 2004). In line with this, Behnke and colleagues (2003) reported a faster decline in PO_2 in type II fibres than type I fibres during exercise,

which indicates a slower adjustment of O_2 delivery relative to O_2 utilization. This suggests that not only is BF supply lower in those fibres that are less efficient compared to more efficient fibres, but BF kinetics may also be slower in the less efficient fibres.

According to the Fick equation, for a given $\dot{V}O_2$, a decrease in BF (and O_2 delivery) would necessitate a greater O_2 extraction, and thus increase in Δdeoxy[Hb + Mb]. While the observed increase in Δdeoxy[Hb + Mb] with increasing ΔWR and the decrease in the conduit artery BF-to-whole body $\dot{V}O_{2p}$ ratio with increasing ΔWR (Figure 3.5B) agrees well with the Fick equation, it must be underscored that deoxy[Hb + Mb] represents the microvascular O_2 delivery-to-local muscle O_2 utilization, while BF-to-$\dot{V}O_{2p}$ represents muscle conduit artery (bulk) BF-to-whole body $\dot{V}O_2$. Therefore, the two measures, while related, must be interpreted with caution as these two sites are not equivalent, with conduit artery BF indicative of the gross delivery of O_2 to the whole muscle, while microvascular BF indicates what is occurring at the site of O_2 exchange with the myocyte.

The finding in the present study that $[Hb_{tot}]$ G_T decreased with increasing ΔWR (Figure 3.7B) would seem to further support the notion of an overall attenuation of O_2 delivery within the microvasculature at higher ΔWRs, and necessitate that the muscle rely more on O_2 extraction. As an increase in $_{TOT}$[Hb + Mb] is indicative of an increase in the volume of red blood cells (haemoglobin) within the area of interrogation of the NIRS probe, a relative decrease in this signal would suggest a lessening of the functional capillary surface area. This would then impact the ability of O_2 to diffuse into the myocyte, which could create a situation in which O_2 delivery is limiting to the $\dot{V}O_{2p}$ response. However, in the present study the kinetics of $_{TOT}$[Hb + Mb] were not analyzed as the profile did not reliably resemble a monoexponential response. Therefore, the role that O_2 diffusivity plays in the $\dot{V}O_{2p}$ kinetic response across different ΔWRs cannot be ascertained from the present study.

In addition to the greater increase in Δdeoxy[Hb + Mb] as ΔWR increased, the MRT deoxy[Hb + Mb] (reflecting O_2 extraction kinetics) became progressively smaller (i.e., faster; Figure 3.6C) despite the dynamics of $\dot{V}O_{2p}$ (reflecting muscle O_2 utilization kinetics) becoming slower at higher ΔWRs (Figure 3.2A). This suggests that at larger ΔWRs there is an inadequate microvascular BF and O_2 delivery response to the muscle, necessitating an

increased reliance on O_2 extraction to meet the metabolic demands of the muscle. This finding agrees well with Jones et al. (2012) who found that the MRT for a-vO_{2diff} (a measure of O_2 extraction) was faster at high-intensity vs. low-intensity single-leg KE exercise transitions, while kinetics of the $\dot{V}O_{2m}$ primary component were similar between the two intensities. Furthermore, several studies also observed smaller MRT deoxy[Hb + Mb] (by ~5 s) when ΔWR was greater (i.e., faster kinetics with a larger ΔWR), although their values did not reach statistical significance (Keir et al., 2016b, MacPhee et al., 2005, Ferreira et al., 2005b). These findings seem to support the assertion that at higher WRs, where there is an increased recruitment of less efficient fibres which may have an inadequate O_2 delivery-to-O_2 utilization response, there will be an increased reliance on O_2 extraction during the transitory phase.

$\dot{Q}_{cap}$

In the present study $\dot{Q}_{cap}$ was calculated by rearranging the Fick equation, using $\dot{V}O_{2p}$ and deoxy[Hb + Mb] as surrogates for $\dot{V}O_{2m}$ and a-vO_{2diff}, respectively, such that $\dot{Q}_{cap}$ = $\dot{V}O_{2p}$/deoxy[Hb + Mb]. Analysis of the $\dot{Q}_{cap}$ profiles revealed that for many of the transitions the profiles did not resemble a monoexponential response, in agreement with the $\dot{Q}_{cap}$ response profiles reported by Barstow and colleagues (Schlup et al., 2015, Harper et al., 2006), with Ferreira et al. (2005b) questioning whether the $\dot{Q}_{cap}$ profile is best quantified by a simple exponential equation. This may be a consequence of data handling and the natural conflation of the deoxy[Hb + Mb] (which reflects local deoxygenation within the largely superficial active muscle fibres, and often presents with a short time delay followed by a rapid increase in deoxy[Hb + Mb] (duManoir et al., 2010, DeLorey et al., 2007, MacPhee et al., 2005)) and $\dot{V}O_{2p}$ (after being corrected for phase I) profiles. Therefore, due to the characteristics of the $\dot{Q}_{cap}$ profiles, and the uncertainty in proper fitting technique, $\dot{Q}_{cap}$ kinetics were not analyzed in the present study. Nonetheless, it was found that the end-exercise $\Delta\dot{Q}_{cap}$ increased with increasing ΔWR, while the $\dot{Q}_{cap}$ G_T was similar across all ΔWRs. This suggests that while the bulk BF G_T decreases, at the level of the microvasculature, BF is redistributed such that there is a maintenance of the O_2 delivery-to-O_2 utilization ratio within the exercising muscle across different ΔWRs. This finding could support the idea that an increase in bulk BF is modulated by the muscle pump and rapid

vasodilation (Tschakovsky & Sheriff 2004), while microvascular BF is regulated more so by the metabolic demand of the muscle (Ferreira et al., 2005b).

Limitations

The present study sought to investigate the response of $\dot{V}O_{2p}$ and several cardiovascular parameters to predetermined ΔWRs and did not adjust for the intensity domains of the individual subjects. The ΔWRs chosen provided a large amplitude change, and thus a large signal-to-noise ratio, were tolerated such that all subjects were able to complete the entire 8 min transition at the highest ΔWR, and allowed comparisons across a range of WRs (i.e., 5 in total). However, a disadvantage of this protocol was that the same absolute ΔWR could have represented a transition into a different exercise-intensity domain for different subjects (MOD vs. HVY vs. VH; see APPENDIX III for individual subject intensity domains at various ΔWRs), with different metabolic and cardiovascular response profiles. To best compensate for this issue, care was taken to isolate the primary $\dot{V}O_{2p}$ and BF components when analyzing the kinetics of the response profiles, as it has been suggested that the parameter estimates for the primary component of $\dot{V}O_{2p}$ is comparable amongst intensity domains (see Poole and Jones 2012 for review). For future studies comparisons across multiple common end-transition intensity domains might be considered.

Although commonly used in the literature, NIRS does have several assumptions and limitations in its use, which have been well described (Barstow 2019, Grassi & Quaresima 2016, Ferrari & Quaresima 2012). Firstly, the depth of penetration of the NIRS signal into muscle tissue is dependent on and attenuated by increasing thickness of skin and adipose tissue. This and the low output power of many NIRS units restricts the volume of NIRS interrogation to the more superficial muscle fibres which have been shown to have a greater proportion of type II fibres (Lexell et al., 1983, Johnson et al., 1973), and therefore the deoxy[Hb + Mb] signal will represent a greater contribution from type II fibres. Nevertheless, the probe was carefully placed over the same area of muscle for each trial, and therefore the response, although perhaps restricted more so to type II fibres, will still reflect what is happening to deoxy[Hb + Mb] as ΔWR increases. Secondly, the field of interrogation for NIRS is small in comparison to the whole muscle and in the present study only the vastus

lateralis muscle of the quadriceps was examined, with Koga and colleagues showing that the muscles which comprise the quadriceps have differing perfusion responses (Koga et al., 2019, Okushima et al., 2015), with differences also seen between superficial and deep portions of the same muscle (Okushima et al., 2015). Because of these issues, the NIRS signal cannot be inferred as representative of the whole muscle. Thirdly, although representative of the microvasculature, the relative contribution of arterioles, venules, and capillaries to the NIRS signal cannot be ascertained. However, their contribution to tissue saturation appears to remain the same across various O_2 saturation levels (Chance et al., 2003). Additionally, the temporal profile of deoxy[Hb + Mb] has been shown to closely resemble that of a-vO_{2diff}, indicating that deoxy[Hb + Mb] provides a good estimation of O_2 extraction (Grassi et al., 1996). Lastly, the dissociation curves for Hb and Mb differ, with oxy-haemoglobin having a sigmoidal relationship in which O_2 off-loading being greater when microvascular PO_2s are between 60-10 mmHg, whereas oxy-myoglobin has a hyperbolic relationship in which O_2 off-loading does not significantly occur until very low intracellular PO_2s ($P_{50} \approx 2.5 – 3$ mmHg; Schenkman et al., 1997, Richardson et al., 1995). Due to these differences, Hb and Mb will each be desaturated to different extends depending on the microvascular and intracellular PO_2 levels. Therefore, it is difficult to determine the contribution of Mb to the deoxy[Hb + Mb] signal as we do not have reliable values for intracellular PO_2 levels during exercise. However, this should not affect the integrity of using NIRS to look at the O_2 delivery-to-O_2 utilization ratio, as the O_2 diffusion pathway as a whole should be unaffected.

However, at present, we do not have reliable values of intracellular PO_2 levels during exercise, making it difficult to determine the contribution of deoxy-Mb to the NIRS signal. While it has been shown that myoglobin contributes ~50-70% of the NIRS signal at rest, and ~70% of the change in the NIRS signal from rest to maximal exercise is from myoglobin (Davis & Barstow 2013), the diffusion pathway as a whole would be unaffected, and therefore, this should not compromise the use of NIRS in looking at the O_2 delivery-to-O_2 utilization ratio.

BP measures were taken manually using a sphygmomanometer which limited the frequency of measures to 1 min intervals. Although MAP was modeled well as a monoexponential

response, the low frequency of data collection early in the exercise transition reduced the confidence in the kinetics analysis. In addition to MAP analysis, this low frequency of data collection will also impact the confidence of fitting the VC response as MAP was required for this calculation (i.e., VC = BF/MAP).

The present study required the use of high WRs in order to achieve sufficient amplitudes (increasing the signal-to-noise ratio) as well as provide a sufficient number of transitions to compare. These high WRs meant that participants had to have a relatively high level of fitness. This is particularly pertinent when considering that those with a higher fitness (trained) have faster kinetics than less fit (untrained) individuals (Murias et al., 2011, Phillips et al., 1995, Bell et al., 2001). Further to this, some authors have proposed a "boundary" for $\dot{V}O_{2p}$ kinetics in which those subjects with a larger $\tau\dot{V}O_{2p}$ (e.g., >20 s) may be in a range in which BF is limiting to $\dot{V}O_2$, while those with a smaller $\tau\dot{V}O_{2p}$ (e.g., <20 s) have kinetics that are likely limited by a metabolic 'sluggishness' in the myocyte (see Poole & Jones 2012 for review). Therefore, the results of this study cannot be easily transferred to a less fit population, as there may be differing degrees to which BF is influencing $\dot{V}O_2$ kinetics in these instances. Additionally, due to the high WRs and the many repetitions needed in this study, consideration must also be given to the potential for a training-induced speeding of kinetics. We attempted to minimize the possibility of inducing a training response by alternating trials of higher and lower WRs throughout the study such that high WR trials were not performed on consecutive days, but the large volume (~30-35 trials) would make it hard to avoid a training effect. However, a comparison of the individual trials within each transition revealed no observable differences in $\dot{V}O_{2p}$, BF, or HR. Additionally, by randomizing the trials such that each of the 5 protocols was performed once before performing a second trial for each of the protocols, if any training effect were present it should be dissipated evenly across the different protocols, making between trials comparisons valid.

Conclusion

The present study investigated the effect that exercise transitions initiated from a common low WR baseline (3 W) to ΔWRs spanning across the MOD, HVY, and VH domains would

have on the kinetic response and interaction between $\dot{V}O_{2p}$, BF, deoxy[Hb + Mb], HR, MAP, $\dot{Q}_{cap}$, and VC during KE exercise. With this approach, it was found that $\dot{V}O_{2p}$ kinetics became progressively slower as ΔWR increased, with BF kinetics tending to slow with increasing ΔWRs. Although it is not possible to determine what might be contributing to the slowing of O_2 delivery with increasing ΔWR, the observed speeding of deoxy[Hb + Mb] kinetics would seem to suggest that the metabolic pathways within the myocyte are able to utilize O_2 more rapidly than it is delivered, with this increased reliance on O_2 extraction during the transition possibly providing some compensation to the slowing of bulk O_2 delivery. There also was a progressive decrease in the bulk BF-to-$\dot{V}O_{2p}$ ratio as ΔWR became larger. In accordance with the Fick equation, this decrease in bulk BF-to-$\dot{V}O_{2p}$ ratio appears to be 'offset' by an increase in O_2 extraction (Δdeoxy[Hb + Mb]). Further research is warranted to elucidate the contributions of bulk and microvascular BF on O_2 delivery to the muscle in response to exercise transitions of different ΔWRs.

Acknowledgements

We gratefully thank each of our participants for their time and effort in this study. We thank Brad Hansen for his technical support of the lab equipment. We also thank Maureen MacDonald and her lab for their guidance on measuring blood flow. We also extend our gratitude to Professor P. A. Robbins, University of Oxford, for providing the 'End-tidal Forcing' software for breath-by-breath pulmonary oxygen uptake measurement.

Grants: This study was supported by the National Science and Engineering Research Council of Canada (NSERC) research and equipment grant.

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CHAPTER IV: The effect of increasing baseline metabolic rate on the kinetic response of $\dot{V}O_{2p}$, blood flow, and muscle deoxygenation

Introduction

Upon a step-transition in work rate (WR), pulmonary O_2 uptake ($\dot{V}O_{2p}$; reflective of muscle O_2 utilization ($\dot{V}O_{2m}$)) increases in an exponential manner towards a new steady-state (Whipp & Wasserman 1972, Grassi et al., 1996). This response defines $\dot{V}O_{2p}$ kinetics, with the rate of this response being quantified by the time constant (τ). The cause of this delay in the $\dot{V}O_{2p}$ response is considered to be due to a delayed activation of oxidative enzymes (a metabolic 'sluggishness') or an inadequate blood flow (BF) and delivery of O_2 to the muscle, or a combination of both (see Poole & Jones 2012, Rossiter 2011, Tschakovsky & Hughson 1999 for review).

When the exercise transition is initiated from a common, light-intensity baseline WR, the rate of adjustment of the primary, phase II, component of the $\dot{V}O_{2p}$ response remains relatively constant across a range of WR amplitudes (ΔWR) (Nyberg et al., 2017, Keir et al., 2016a/b, Spencer et al., 2013, Jones et al., 2012, MacPhee et al., 2005, Koga et al., 2005, Ozyener et al., 2001), although this is not a consistent finding (Koga et al., 2001, Koga et al., 1999, Paterson & Whipp 1991). However, when exercise transitions of similar ΔWRs are initiated from elevated baseline metabolic rates, $\dot{V}O_{2p}$ kinetics become progressively slower (i.e., phase II $\tau\dot{V}O_{2p}$ becomes larger) as the baseline metabolic rate increases (Nederveen et al., 2017, Keir et al., 2016a/b, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001). Concomitant with the slowing of $\dot{V}O_{2p}$ kinetics, there also is an increase in the steady-state $\dot{V}O_{2p}$ gain ($\Delta\dot{V}O_{2p}/\Delta$WR) of both the primary component (G_p) and total response (G_T) even for exercise transitions constrained within the moderate-intensity (MOD) domain (Nederveen et al., 2017, Keir et al., 2016a/b, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001). The increase in G_T at intensities above the lactate threshold (LT) (i.e., in the heavy-(HVY), and very heavy-(VH) intensity domains) is further augmented by an additional, delayed, increase in $\dot{V}O_{2p}$ (and $\dot{V}O_{2m}$), the '$\dot{V}O_2$ slow component', which is not present at intensities below LT (i.e., in MOD). Recent studies by Keir et al. (2016a/b)

revealed that the slowing of $\dot{V}O_{2p}$ kinetics with increasing baseline metabolic rates occurred in a curvilinear fashion within intensities spanning the MOD, HVY, and VH domains, while the increases in G_p and G_T were linear (at least within MOD and HVY).

During transitions to exercise, conduit artery (bulk) BF, like $\dot{V}O_{2p}$, increases in an exponential-like manner towards a new, higher, "steady-state" level to meet the elevated metabolic demands of the active muscle (Saltin 1985, Wesche 1986, Walloe & Wesche 1988). For transitions from a low, baseline metabolic rate, conduit artery BF kinetics compared to $\dot{V}O_{2p}$ kinetics are reported to be either faster (MacDonald et al., 1998, Fukuba et al., 2004, MacPhee et al., 2005, Koga et al., 2005, Endo et al., 2005, Harper et al., 2006, Jones et al., 2012, Schlup et al., 2015), suggesting that bulk BF and O_2 delivery do not limit the adjustment of $\dot{V}O_{2p}$, or are not different (Grassi et al., 1996, Hughson et al., 1996, MacPhee et al., 2005, Paterson et al., 2005, duManoir et al., 2010, Jones et al., 2012, McNarry et al., 2014, Nyberg et al., 2017), suggesting a close matching between BF dynamics and O_2 utilization. However, in many of these studies, conduit artery, bulk, BF supplying active muscle was investigated, whereas BF in the microvasculature, where actual exchange of O_2 between the capillary and myocyte occurs, was not determined. Measures of $\dot{V}O_{2p}$ (a proxy for $\dot{V}O_{2m}$) combined with near-infrared spectroscopy (NIRS)-derived measures of muscle microvascular deoxygenation (HHb), reflecting the balance between local muscle O_2 delivery and O_2 utilization and thus fractional O_2 extraction, can be used to provide an estimate of microvascular BF ($\dot{Q}_{cap}$) by rearranging the Fick equation (Ferreira et al., 2005b; Harper et al., 2006). Using this technique, Harper et al. (2006) and Schlup et al. (2015) both reported that estimated $\dot{Q}_{cap}$ kinetics were slower than both $\dot{V}O_{2p}$ and conduit artery BF kinetics suggesting the possibility that local muscle O_2 delivery at sites distal to the main conduit artery could limit the adjustment of $\dot{V}O_{2m}$.

In a previous study, Keir et al. (2016b) reported that τdeoxy[Hb + Mb] became larger when exercise transitions were initiated from an increasing baseline metabolic rate, but the time delay (TD) prior to the deoxy[Hb + Mb] increase became smaller, resulting in no change to the overall kinetics (mean response time (MRT)). This unchanging MRT deoxy[Hb + Mb] between the different transitions (indicating that the microvascular O_2 delivery-to-O_2 utilization ratio was similar amongst transitions), coupled with a slowing of $\dot{V}O_{2p}$ kinetics

with increasing baseline WR, lead the authors to conclude that O_2 delivery is not limiting to $\dot{V}O_{2p}$ kinetics in this situation. However, these authors lacked a direct measure of BF to more directly assess the role of O_2 delivery in this situation.

MacPhee and coworkers (2005) examined the effect of an elevated baseline metabolic rate on $\dot{V}O_{2p}$ and BF responses during alternate-leg knee-extension exercise and reported that the adjustment of both $\dot{V}O_{2p}$ and bulk BF were slowed when the transition was initiated from an elevated compared to a lower baseline metabolic rate. Additionally, these authors also found a smaller increase in BF for a given increase in $\dot{V}O_{2p}$ ($\Delta BF/\Delta\dot{V}O_{2p}$) when the transition was initiated from an elevated baseline. However, in that study, only two transitions were used and both were constrained within the MOD domain where BF should not be restricted as sometimes is seen with HVY or VH exercise where O_2 delivery may be attenuated (i.e., the gain in BF is less than in MOD) based on calculations from data in previous studies (Koga et al., 2005, Van Beekvelt et al., 2001, MacDonald et al., 1998). Therefore, the purpose of the present study was to investigate the effect of increasing baseline metabolic rates spanning the MOD to HVY to VH domains with transitions of a common ΔWR on the adjustments and interrelationships between BF and $\dot{V}O_{2p}$ at exercise onset. Furthermore, we sought to characterize the dynamics of both the deoxy[Hb + Mb] and $\dot{Q}_{cap}$ responses across increasing baseline WR (WR_{bl}). Based on the studies of Keir et al. (2016a/b), we hypothesized that both $\dot{V}O_{2p}$ and BF kinetics would become progressively slower with an increasing WR_{bl} and that this would be accompanied by a progressively rising gain for both $\dot{V}O_{2p}$ and BF. Also, in line with Keir et al. (2016b), we hypothesized that MRT deoxy[Hb + Mb] would be similar across transitions, which would be consequent to a slowing of $\dot{Q}_{cap}$ kinetics with increasing WR_{bl}. Furthermore, it was hypothesized that the $\dot{V}O_{2p}$ G_p would become greater with increasing WR_{bl}.

Methods

Subjects

Ten healthy male volunteers (mean ± SD; 25.6 ± 4.5 yrs; 85.5 ± 8.6 kg; 184.1 ± 5.3 cm) participated in this study. Subjects were free to withdraw at any time without penalty. Subjects provided written informed consent with all procedures approved by The University

of Western Ontario Ethics Committee for Research on Human Subjects in accordance with the Declaration of Helsinki.

Protocol

All trials were performed on a custom-built alternate-leg knee-extension (KE) ergometer (see MacPhee et al., 2005) in which the subjects were familiarized with the 'kicking' motion. A restraining belt was fastened around the participant's waist to minimize hip movement during each trial such that the quadriceps muscle group performed the work solely while the hamstrings were relaxed throughout exercise. Participants maintained a cadence of 30 contractions per minute (cpm) which was displayed continuously on a screen in front of them. A step-incremental KE exercise test was performed for estimation of LT (the point at which there was an inflection in $\dot{V}CO_2$, ventilation, and end-tidal PO_2 ($P_{ET}O_2$) relative to $\dot{V}O_2$, without a decrease in end-tidal PCO_2 ($P_{ET}CO_2$)) and respiratory compensation point (RCP; the point at which there was a steeper increase in ventilation and $P_{ET}O_2$ relative to $\dot{V}O_2$ with a decrease in $P_{ET}CO_2$). These boundaries were used to differentiate between the MOD (< LT), HVY (> LT, but < RCP), and VH (> RCP, but < $\dot{V}O_{2peak}$) intensity domains. This test began with baseline (3 W) 'kicking' for 4 min, after which the WR was increased by 3 W each minute until a cadence of 30 cpm could no longer be maintained (i.e., limit of tolerance). Five different exercise transition protocols were performed, with each protocol beginning with 6 min of baseline 'kicking' at either 3 (bl_3), 12 (bl_{12}), 24 (bl_{24}), 33 (bl_{33}), or 45 (bl_{45}) W followed by an instantaneous increase in ΔWR of 21 W (see Figure 4.1) for each transition; each transition lasted 8 min. To increase the signal-to-noise ratio, each transition was repeated 3-5 times (Gill 1985), with the order of transitions being randomized and at least 24 hrs between trials.

Measurements

$\dot{V}O_{2p}$: A volume turbine and mass spectrometer were used to measure breath-by-breath gas exchange as described previously (Keir et al., 2016a/b). Briefly, a bidirectional turbine and pneumotach (Hans Rudolph, model 4813; total dead space was 150 mL) to measure inspired and expired airflow and volumes were calibrated before each test with a syringe of known volume (3.0 L) and the pneumotach adjusted for zero flow. A mass spectrometer (AMIS

2000, Innovision, Lindvedvej, Denmark), which was calibrated before each test using precision-analyzed gas mixtures, was used to continuously sample gas concentrations. The time delay was determined electronically by computer by measuring the time between an instantaneous square-wave change in gas concentration and its detection by the mass spectrometer. Data were collected at a frequency of 100 Hz and transferred to a computer which aligned gas concentrations and expired volumes to build a profile of each breath. Alveolar gas exchange was calculated breath-by-breath using the algorithms of Swanson (1980).

BF: Blood velocity and arterial diameter were monitored continuously throughout the exercise protocol using pulsed-wave and echo-Doppler ultrasound, respectively (GE Vingmed, System Five, 4-5MHz, 60° angle of insonation). The ultrasound probe was held over the common femoral artery, 2-3 cm proximal to the artery bifurcation to minimize turbulence, but distal to the inguinal ligament to avoid BF to this area as previously reported (McDonald et al., 1998, Radegran & Saltin 1998). The ultrasound gate was positioned in the center of the artery and maximized for complete insonation of the vessel cross-section. Mean blood velocity measures were processed by a Neurovision Transcranial Doppler System (Multigon Industries Inc.) and analyzed on a separate computer using PowerLab (ADInstruments, LabChart 7). Arterial diameter was measured in triplicate during a diastole in the muscle relaxation phase and averaged to yield a single vessel diameter every 60 s. BF was calculated by the equation:

$$\text{BF (L/min)} = \text{blood velocity (cm/s)} \bullet \pi \bullet (\text{diameter (cm)}/2)^2 \bullet 0.12$$

where 0.12 is the conversion factor of ‘s’ into ‘min’ for BF in both legs (i.e., 60 x 2) and mL/min into L/min (i.e., ÷ 1000). The blood velocity signal was averaged over a single muscle contraction-relaxation cycle, as this method has been proposed as an acceptable technique to analyze BF kinetics (see Chapter II) by taking into account the variability in the blood velocity signal created by muscle contraction and the cardiac cycle (Radegran 1997, Osada & Radegran, 2006).

NIRS and microvascular blood flow ($\dot{Q}_{cap}$): Local deoxy[Hb + Mb] (in addition to oxy[Hb + Mb], $_{TOT}$[Hb + Mb], and tissue oxygenation index (TOI)) was measured in the vastus lateralis

muscle using NIRS (Oxiplex TS, Model 92505, ISS, Champaign, USA). Briefly, NIRS optodes were housed in a rigid optically-dense, plastic holder (connected to laser diodes and photomultiplier tube by optical fibers) with two parallel rows of light emitter fibers (one row emitting at a wavelength of 690 nm and the other row at 828 nm) and one detector fiber bundle; the source-detector separations for this probe were 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths. An elastic strap was used to secure the NIRS probe over the vastus lateralis muscle approximately 1/3rd the distance between the greater trochanter and the lateral femoral condyle. The probe was then covered with an optically-dense black vinyl sheet, and wrapped with a tensor bandage to minimize the intrusion of extraneous light and prevent movement of the probes. The raw signals from NIRS (deoxy[Hb + Mb], oxy[Hb + Mb], $_{TOT}$[Hb + Mb], and TOI) were corrected for skin and adipose tissue thickness (which were measured by echo-Doppler ultrasound) by linear regression of the Hb_{tot} signal against adipose tissue thickness, in accordance with the method of Bowen et al. (2013). By using $\dot{V}O_{2p}$ (after correcting for the cardiodynamic component (phase I)) as a representation of $\dot{V}O_{2m}$, and deoxy[Hb + Mb] as a surrogate for arterial-venous O_2 difference (a-vO_{2diff}), $\dot{Q}_{cap}$ was calculated by rearranging the Fick equation ($\dot{Q}_{cap}$ = $\dot{V}O_{2p}$/deoxy[Hb + Mb]), as previously described (Harper et al., 2006, Ferreira et al., 2005b).

Heart Rate (HR): A Polar HR monitor was used to continuously record HR to Powerlab (ADInstruments Inc., Colorado Springs, CO).

Mean Arterial Pressure (MAP) and Vascular Conductance (VC): A sphygmomanometer and stethoscope were used to measure systolic and diastolic blood pressure (SBP and DBP) on the left arm (at the level of the heart) by a trained technician, with MAP being calculated as: 1/3 SBP + 2/3 DBP. Blood pressure was measured at rest, at 2 min and 5 min of baseline exercise, 30 s into the transition and at one minute intervals thereafter. To correct for hydrostatic pressure, all BP values were adjusted by 2 mmHg per inch vertical difference between the level of the heart and the common femoral artery (Sia 2003). Due to the infrequent measures of MAP, and issues of precisely time-aligning both BF and MAP, vascular conductance in the femoral artery (VC = BF/MAP) was calculated using the iterated fits of BF and MAP.

Lactate: Blood was sampled from the earlobe 2 min prior to the end of each transition for determination of the blood lactate concentration using a lactate analyzer (Lactate Scout, Sports Resource Group, Hawthorne, NY).

Data Analysis

The $\dot{V}O_{2p}$ and BF data for individual trials were cleaned by removing aberrant data points (lying 3 standard deviations outside the local mean) (Lamarra et al., 1987), linearly interpolated to second-by-second values, and like transitions were ensemble averaged to yield a single response for each transition in each subject. Data for each trial were subsequently averaged to 5 s time bins and modeled using a monoexponential nonlinear, least squares regression equation:

$$Y(t) = bl + amp\ (1\text{-}e^{-(t\text{-}TD)/\tau})$$

where Y represents $\dot{V}O_{2p}$, BF, deoxy[Hb + Mb], $\dot{Q}_{cap}$, MAP, VC, or HR at any time (t); bl is the steady-state baseline of Y; amp is the "steady-state" increase in Y above bl; TD is the time delay; and τ is the time constant (equivalent to the time taken to reach 63% of amplitude).

Baseline for each variable (except MAP) was quantified by averaging data 2 min prior to the increase in WR. For MAP, the 2 values recorded prior to each step-transition were averaged together and reported the baseline value data (as MAP was only measured once every minute throughout the exercise trial).

For $\dot{V}O_{2p}$ data, the 'cardiodynamic' component (phase I) and the 'slow component' (phase III) were excluded from the model fit leaving only the primary component (phase II). Each of these phases was determined by extending the fit window backward from ~35 s (to determine phase I) or extending the fit window forward from ~90 s (to determine phase III) until τ, χ^2, and confidence interval began to increase, as described by Rossiter et al. (2001). Transitions into HVY, in which a slow component would appear, were determined from analyzing the LT in the ramp incremental test, and confirmed by blood lactate values.

For BF, the TD was fixed at 0 s (to prevent negative values), and transitions were fit from the first time point in the transition to the time point that corresponded with the end of phase II for $\dot{V}O_{2p}$ (with this fitting strategy referred to as τBF). However, due to uncertainty on whether the BF profile contains a slow component (Endo et al., 2005, Fukuba et al., 2004), the mean response time (MRT) of the BF profile was also determined by extending the fit to the end of the exercise transition, with both measures (τBF and MRT) being commonly reported in the literature (Jones et al., 2012, Koga et al., 2005, Endo et al., 2005, Fukuba et al., 2004).

The TD for deoxy[Hb + Mb] was excluded from the response profile by fitting from the first data point in which deoxy[Hb + Mb] increased 1 SD above the pre-transition baseline value. The end-point of the deoxy[Hb + Mb] model fit was either: 1) the peak of the 'overshoot', if an 'overshoot' was present; or 2) the end of an initial plateau in the profile, for those profiles that displayed a continual gradual increase in the deoxy[Hb + Mb] response; or 3) ~90 s for those profiles not displaying an 'overshoot' or a discernible gradual increase, as has been done previously (Buchheit et al., 2009, Ferreira et al., 2005a). The kinetics of deoxy[Hb + Mb] were quantified by the MRT (CTD + τ). For those profiles that had an 'overshoot,' the 'overshoot' was quantified by summing together those data points that were in the 'overshoot' region. For those profiles that displayed a gradual increase in deoxy[Hb + Mb] after the fitting window (i.e., a slow component-like response), the amplitude of this 'slow component' was calculated as the difference between the 'phase II' model-fit and the end of the transition.

The profiles for HR and MAP were fit from the first data point in the transition until ~90 s for HR, or end-exercise for MAP. For both HR and MAP the TD was locked at '0' for every fit to avoid negative TD values.

The VC profile was fit from the first point after the transition to either the end of exercise or, when an overshoot was present, to the peak of the overshoot. When an overshoot was present its size was calculated as the difference between the peak of the overshoot and the end-exercise VC.

The BF-to-$\dot{V}O_{2p}$ ratio was calculated by dividing the amplitude of the full response of BF by the amplitude of the full response of $\dot{V}O_{2p}$.

Statistics

A one-way repeated measures ANOVA was used to analyze the kinetic parameters for each variable. When a significant F ratio was found, it was further analysed using Tukey's post hoc analysis to determine where differences were. All statistical analyses were performed using SigmaPlot (Systat Software Inc., San Jose, CA, USA). Statistical significance was accepted at $p < 0.05$. All values are reported as mean ± SD.

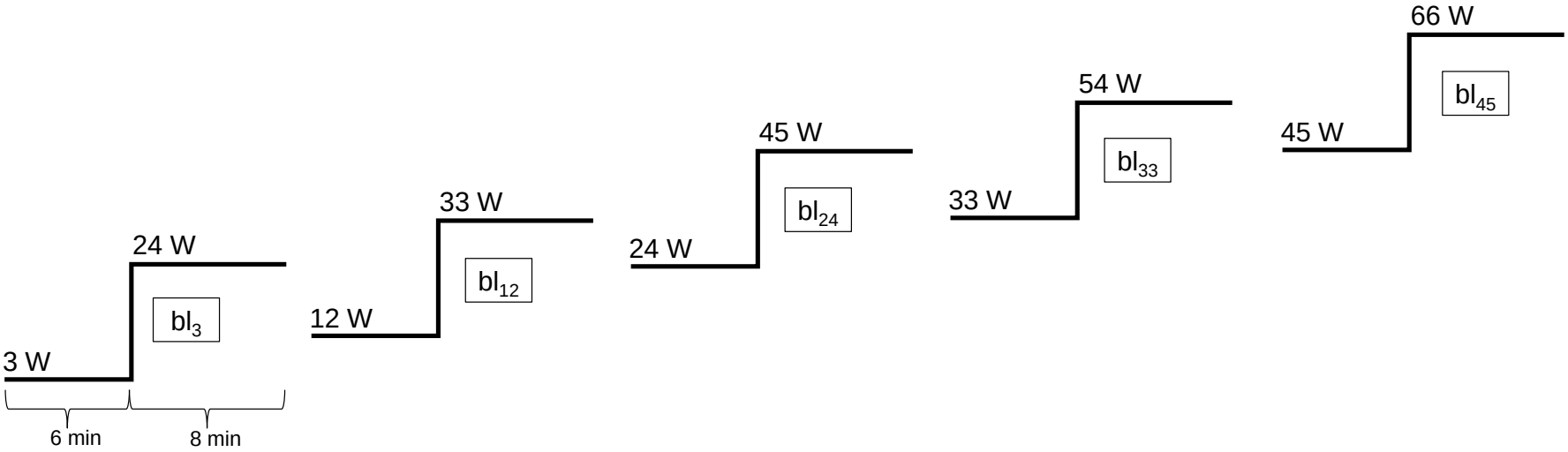

Figure 4.1. Protocol schematic.

Results

$\dot{V}O_{2p}$ and WR values from the incremental KE exercise test are displayed in Table 4.1, along with lactate values that were taken at each of the baseline (3 and 12 W) and end-exercise WRs (24, 33, 45, 54, and 66 W). For comparison, with cycle ergometry, the RI peak $\dot{V}O_{2p}$ was 3.99 ± 0.86 L/min (48.2 ± 9.0 mL/kg/min), and peak WR (WR_{peak}) was 378 ± 72 W.

$\dot{V}O_{2p}$: The group mean $\dot{V}O_{2p}$ profiles for each exercise transition are displayed in Figure 4.2A. Baseline $\dot{V}O_{2p}$ increased linearly with increasing WR_{bl} ($p < 0.05$; Figure 4.3A). Despite ΔWR being the same for all transitions, the amplitude of the primary $\dot{V}O_{2p}$ component was larger for transitions initiated from WRs bl_{33} and bl_{45} compared to WRs bl_3 and bl_{12} ($p < 0.05$; data not shown) such that the gain ($\Delta\dot{V}O_{2p}/\Delta WR$) of the primary $\dot{V}O_{2p}$ component (G_p) ($p < 0.05$; Figure 4.3B) and total $\dot{V}O_{2p}$ response (G_T) ($p < 0.05$; data not shown), generally, were larger for higher compared to lower intensity transitions. The time course of the primary component, $\tau\dot{V}O_{2p}$, were larger for transitions from higher compared to lower WR_{bl}'s ($p < 0.05$; Figure 4.3C).

BF: The group mean BF response profiles for each transition are displayed in Figure 4.2B. Baseline BF increased linearly with increasing WR_{bl} ($p < 0.05$; Figure 4.4A). End-exercise BF values increased in a curvilinear fashion with end-exercise $\dot{V}O_{2p}$ values at each WR, with the increase in BF relative to $\dot{V}O_{2p}$ decreasing at the higher transitions (Figure 4.5A). When looking at the BF amplitude corresponding to the time period of the $\dot{V}O_{2p}$ primary component, the ΔBF was smaller in the highest compared to the lower transitions ($p < 0.05$; Figure 4.4B). When the increase in ΔBF was adjusted relative to the increase in metabolic demand (i.e., $\Delta BF/\Delta\dot{V}O_{2p}$) the ratio was smaller at the higher compared to the lower transitions ($p < 0.05$; Figure 4.5B). When the estimation of BF kinetics was constrained to the time frame associated with the $\dot{V}O_{2p}$ primary component (τBF) or was fit across the entire BF response profile (MRT BF), the BF kinetics tended to be slower for the mid-intensity transitions (i.e., for transitions initiated from WR_{bl}s bl_{24} and bl_{33}) compared to lower or highest transitions ($p < 0.05$; Figures 4.4C & D). The τBF and $\tau\dot{V}O_{2p}$ were similar for lower exercise transitions, but at higher transitions τBF was lower than $\tau\dot{V}O_{2p}$ ($p < 0.05$; Figure 4.5C).

deoxy[Hb + Mb]: The group mean deoxy[Hb + Mb] profiles for each transition are shown in Figure 4.2C. Baseline deoxy[Hb + Mb] (deoxy[Hb + Mb]$_{bl}$) increased progressively with increasing WR_{bl}, and was significantly higher at the final two WR_{bl}s ($p < 0.05$; Figure 4.6A). Although the 'primary' deoxy[Hb + Mb] amplitude (Δdeoxy[Hb + Mb]) was similar for all exercise transitions ($p > 0.05$; data not shown), there was a greater slow component-like deoxy[Hb + Mb] response for the transitions from a higher WR_{bl} ($p < 0.05$; Figure 4.6B). The gain of the full deoxy[Hb + Mb] response (deoxy[Hb + Mb] G_T) was generally similar between transitions, with the exception of bl_{24} being larger than bl_3 ($p < 0.05$; Figure 4.6C). There was a trend for the lowest WR_{bl} to have an 'overshoot' that was larger than the two highest WR_{bl}s ($p = 0.06$; Figure 4.6D). The MRT deoxy[Hb + Mb] was larger at the highest (bl_{45}) compared to the lower (bl_3, bl_{12}) transitions ($p < 0.05$; Figure 4.6E).

[Hb_{tot}]: The group mean response profiles for [Hb_{tot}] are shown in Figure 4.2D. Baseline [Hb_{tot}] ([Hb_{tot}]$_{bl}$) was higher ($p < 0.05$) for the higher WR_{bl}s (Figure 4.7A). Despite ΔWR being the same amongst transitions, there was an attenuated increase in [Hb_{tot}] amplitude (Δ[Hb_{tot}]) with increasing WR_{bl} ($p < 0.05$; Figure 4.7B).

HR: Group mean HR profiles are shown in Figure 4.8A. Baseline HR increased with increasing WR_{bl}s ($p < 0.05$; Figure 4.9A). The 'primary' ΔHR was similar amongst the different transitions ($p > 0.05$; data not shown). The τHR was smaller for the transition from bl_{45} than bl_{12} ($p < 0.05$; Figure 4.9B), but no other differences were seen.

MAP: The group mean MAP responses are shown in Figure 4.8B. Baseline MAP increased with increasing WR_{bl} ($p < 0.05$; Figure 4.10A). Both MAP amplitude (ΔMAP; data not shown) and MAP gain (Figure 4.10B) were larger ($p < 0.05$) at the higher WR_{bl}'s ($p < 0.05$). The MRT MAP was similar amongst all transitions ($p > 0.05$; Figure 4.10C).

VC: The group mean VC profiles are presented in Figure 4.8C. Baseline VC increased linearly with increasing WR_{bl} ($p < 0.05$; Figure 4.11A). The VC gain (G_T; ΔVC/ΔWR) decreased progressively with increasing WR_{bl} and was significantly smaller at the highest compared to all other transitions ($p < 0.05$; Figure 4.11B). There was often an 'overshoot' in the initial VC response that was followed by a small, gradual decrease throughout the exercise transition. This 'overshoot' was significantly greater in bl_{45} than bl_{24} ($p < 0.05$;

Figure 4.11C). The MRT VC was smaller in bl_3 and bl_{45} than both bl_{24} and bl_{33} ($p < 0.05$; Figure 4.11D).

$\dot{Q}_{cap}$: The $\dot{Q}_{cap}$ response profiles for the group mean are shown in Figure 4.8D. Baseline $\dot{Q}_{cap}$, generally, was higher at the higher compared to the lower WR_{bl}'s ($p < 0.05$; Figure 4.12A). The $\dot{Q}_{cap}$ amplitude ($\Delta\dot{Q}_{cap}$, Figure 4.12B) and $\dot{Q}_{cap}$ gain (data not shown) were similar amongst transitions ($p > 0.05$).

Table 4.1. Values from incremental exercise test and lactate values at various work rates for knee-extension exercise.

WR_{peak} (W)	$\dot{V}O_{2peak}$ (L/min)	LT (L/min)	RCP (L/min)	Lactate (mM)						
				3 W	12 W	24 W	33 W	45 W	54 W	66 W
87 (15)	1.84 (0.28)	1.03 (0.26)	1.42 (0.18)	1.6 (0.2)	1.7 (0.3)	1.8 (0.4)	2.0 (0.5)	2.6 (0.5)	3.0 (0.6)	4.5 (1.1)

Values are mean (SD). WR, work rate; LT, lactate threshold; RCP, respiratory compensation point.

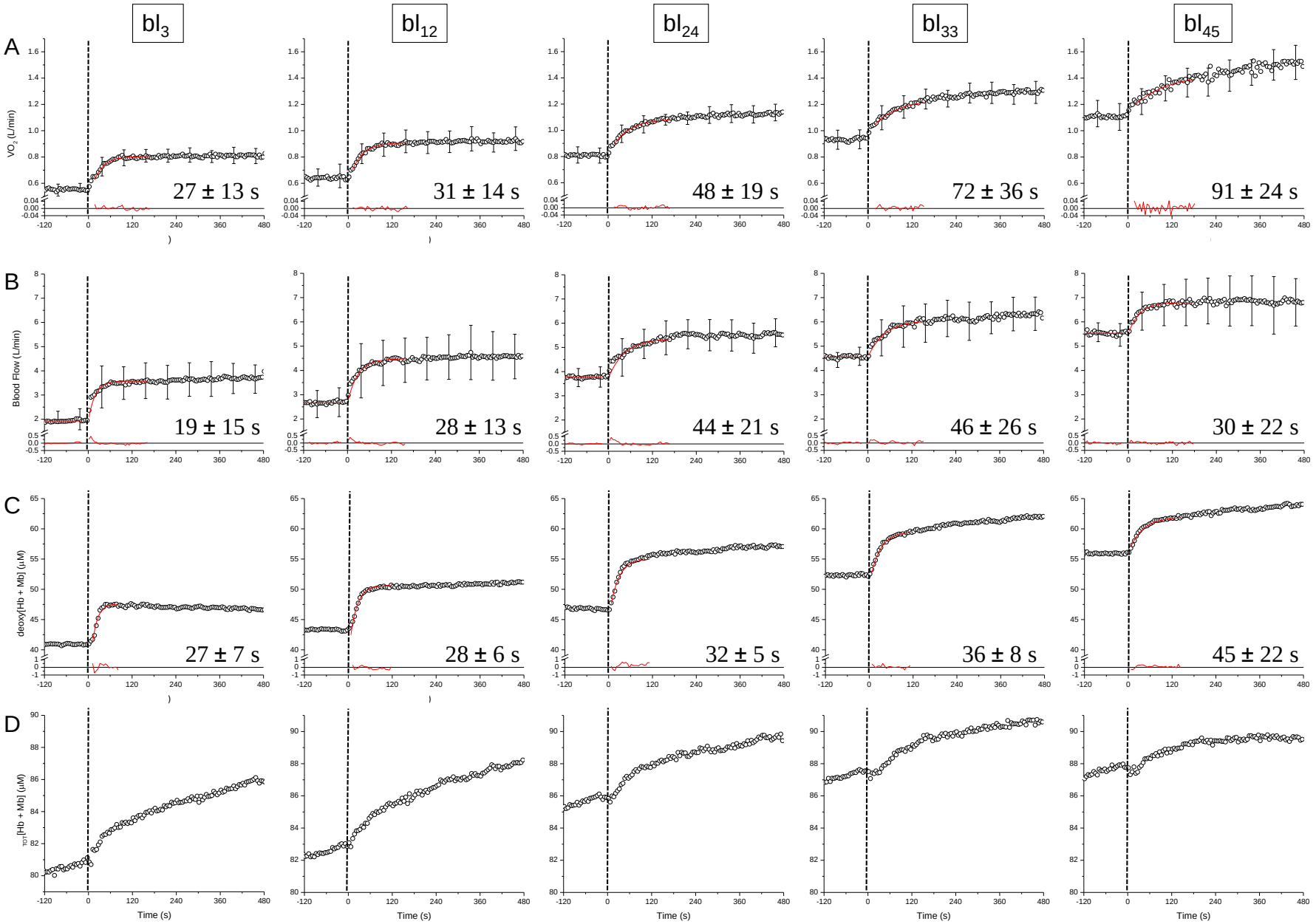
bl3
bl12
bl24
bl33
bl45
A
B
C
D
VO2 (L/min)
Blood Flow (L/min)
deoxy(Hb + Mb) (µM)
TOI[Hb + Mb] (µM)
27 ± 13 s
31 ± 14 s
48 ± 19 s
72 ± 36 s
91 ± 24 s
19 ± 15 s
28 ± 13 s
44 ± 21 s
46 ± 26 s
30 ± 22 s
27 ± 7 s
28 ± 6 s
32 ± 5 s
36 ± 8 s
45 ± 22 s
Time (s)

Figure 4.2. Group mean responses (±SD; note that for clarity, deoxy[Hb + Mb] and $_{TOT}$[Hb + Mb] SDs were not included) of: A, pulmonary O_2 uptake ($\dot{V}O_{2p}$); B, blood flow (BF); C, muscle deoxygenation (deoxy[Hb + Mb]); and D, total haemoglobin + myoglobin ($_{TOT}$[Hb + Mb]) for trials bl_3-bl_{45}. Vertical dashed lines indicate the onset of the exercise transition (time = 0 s). The group mean primary component responses for each trial are superimposed on the data (red lines, fit by a monoexponential using group mean parameter estimates). Residuals for the monoexponential fit are displayed (in red) about the Y = 0 line. Time constant (τ) values for $\dot{V}O_{2p}$, and BF, and mean response time (MRT) values for deoxy[Hb + Mb] (mean ± SD) are displayed inset in each graph.

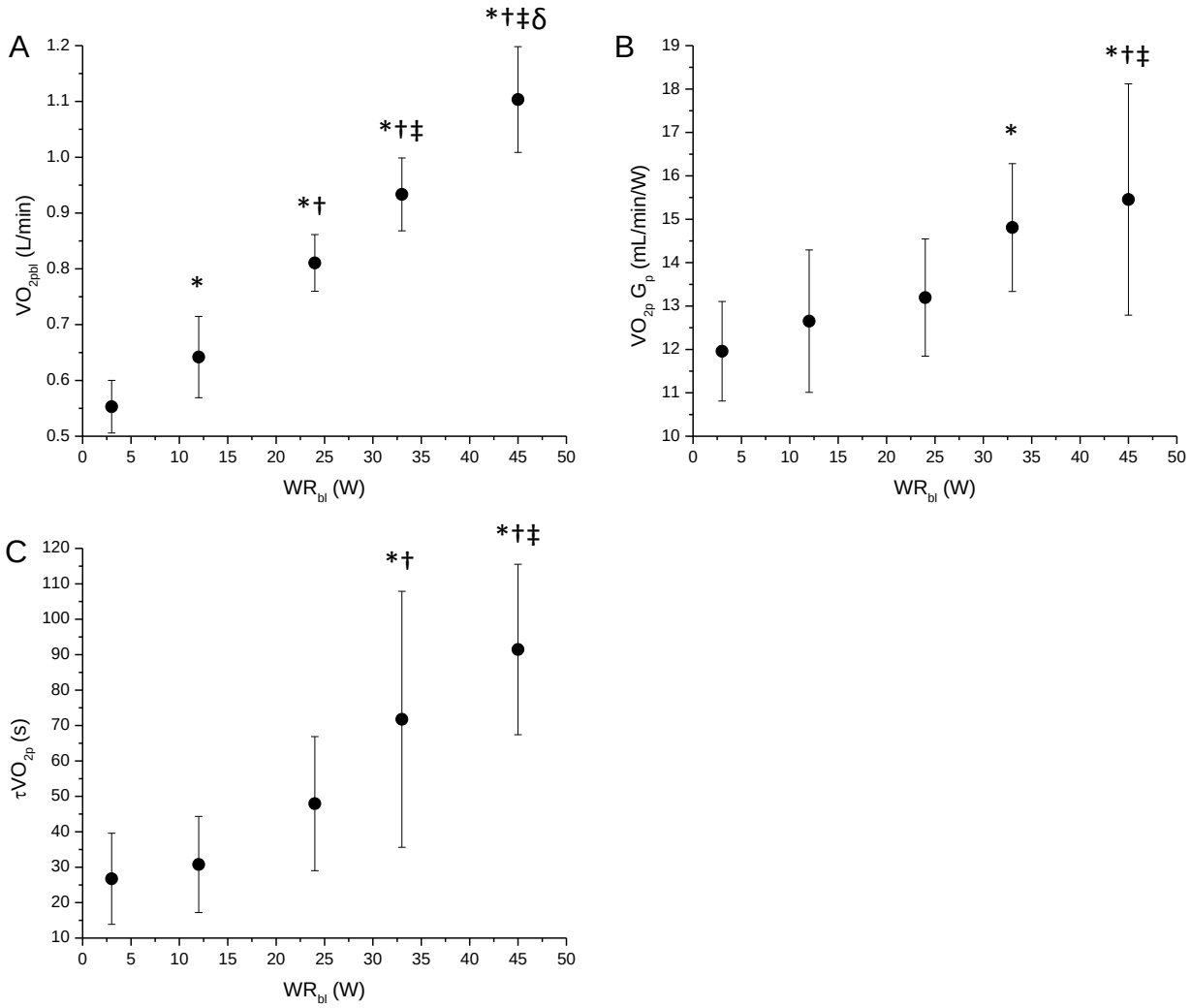

Figure 4.3. Pulmonary O_2 uptake ($\dot{V}O_{2p}$) parameter estimates from increasing work rate baselines (WR_{bl}) with a common work rate amplitude (21 W) for each trial (bl_3-bl_{63}) for, A: baseline $\dot{V}O_{2p}$; B: $\dot{V}O_{2p}$ gain of the primary component (G_p); C: time constant of the primary component for $\dot{V}O_{2p}$. Symbols denote a significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}; ‡, bl_{24}; δ, bl_{33}. Δ, amplitude.

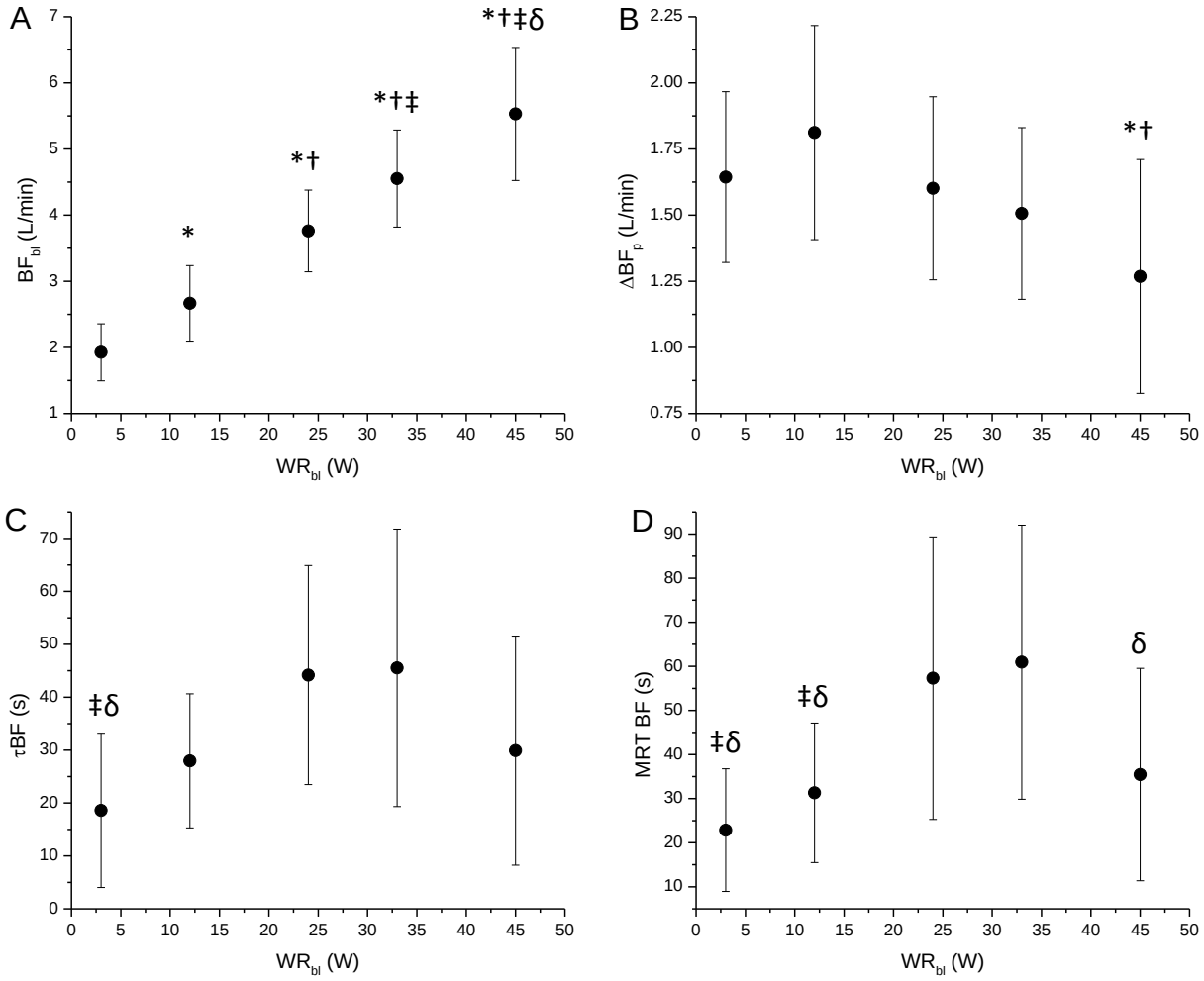

Figure 4.4. Blood flow (BF) parameter estimates from increasing work rate baselines (WR_{bl}) with a common work rate amplitude (21 W) for each trial (bl_3-bl_{63}) for, A: baseline BF; B: BF amplitude for the primary component (determined from the pulmonary O_2 uptake ($\dot{V}O_{2p}$) primary component); C: time constant of BF for the primary component (determined by $\dot{V}O_{2p}$); D: time constant of BF for the entire transition. Symbols denote a significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}; ‡, bl_{24}; δ, bl_{33}. MRT, mean response time of the entire BF response; τBF, time constant for the BF response fit in the same time frame as the $\dot{V}O_{2p}$ primary component; Δ, amplitude.

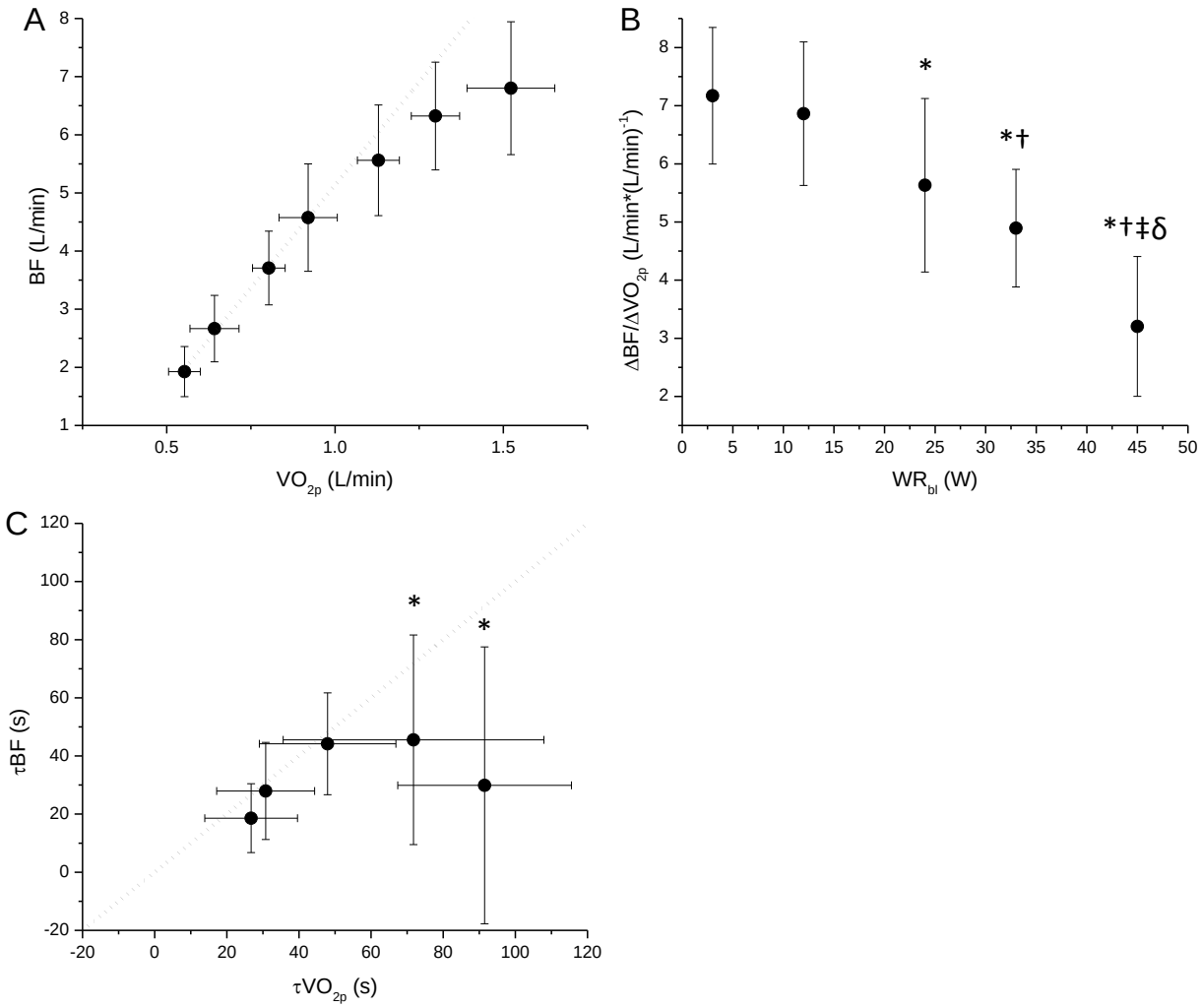

Figure 4.5. Blood flow (BF) vs. pulmonary O_2 uptake ($\dot{V}O_{2p}$) for, A: end-exercise values of BF vs. $\dot{V}O_{2p}$ across the various work rates (i.e., 3, 12, 24, 33, 45, 54, 66 W); B: increase in BF amplitude relative to the increase in $\dot{V}O_{2p}$ amplitude across the various transitions (i.e., bl_3-bl_{63}); C: time constant of the primary component across the various transitions (i.e., bl_3-bl_{63}). Symbols denote a significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}; ‡, bl_{24}; δ, bl_{33}; NOTE, for panel A, * denotes a significant difference between τBF and $\tau\dot{V}O_{2p}$ for that transition. Dotted line in panel A denotes linear regression of the lower 4 transitions (y = 7.0849x – 1.9499); dotted line for panel C denotes the line of identity (y = x). τ, time constant.

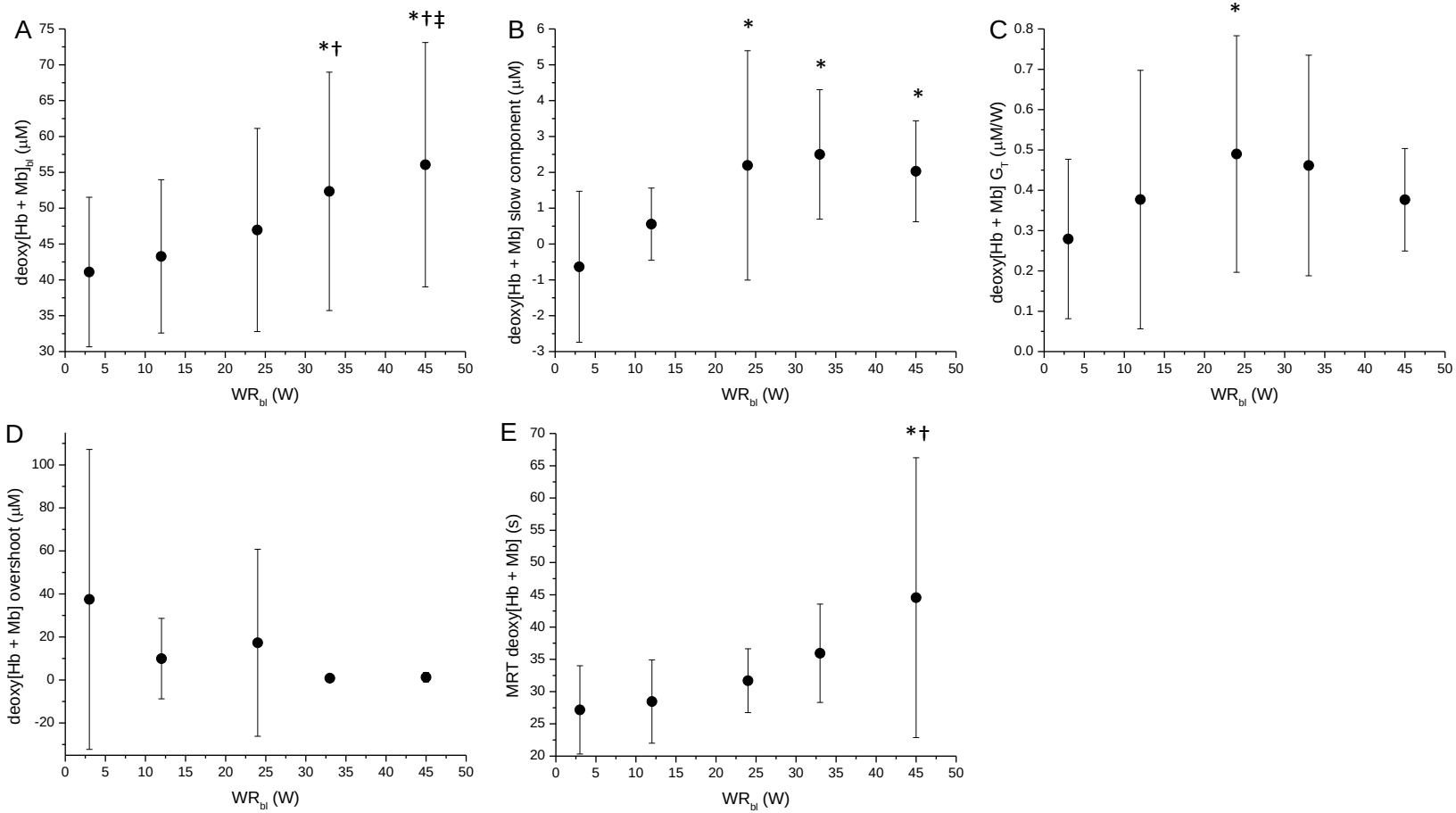

Figure 4.6. Muscle deoxygenation (deoxy[Hb + Mb]) parameter estimates from increasing work rate baselines (WR_{bl}) with a common work rate amplitude (21 W) for each trial (bl_3-bl_{63}) for, A: deoxy[Hb + Mb] baseline; B: deoxy[Hb + Mb] 'slow component'; C: deoxy[Hb + Mb] gain; D: deoxy[Hb + Mb] 'overshoot'; E: deoxy[Hb + Mb] mean response time (time constant + time delay). Symbols denote a significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}; ‡, bl_{24}; δ, bl_{33}. G_T, gain of full response; MRT, mean response time.

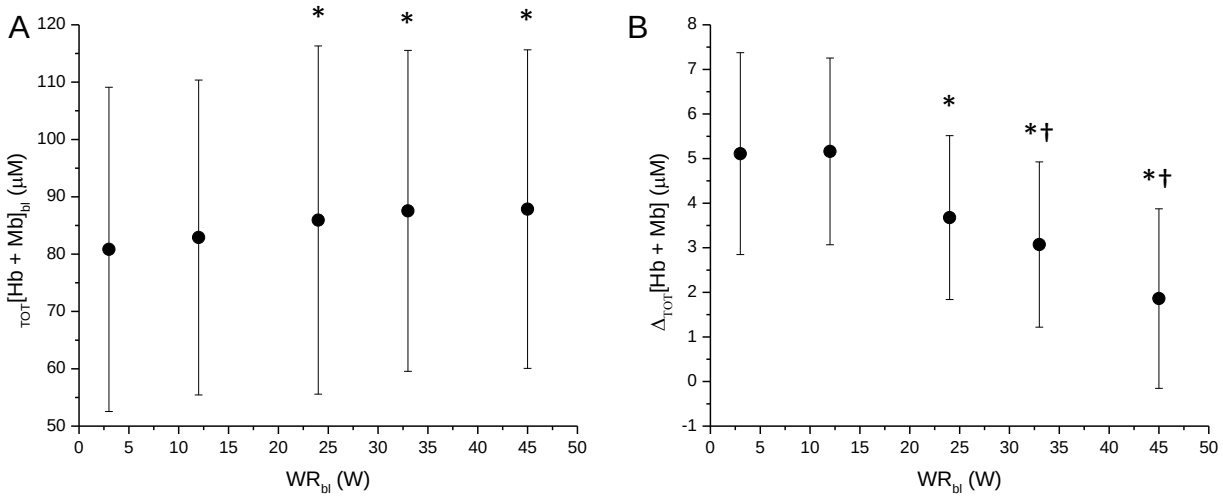

Figure 4.7. Total haemoglobin + myoglobin ($_{TOT}$[Hb + Mb]) parameter estimates from increasing work rate baselines (WR_{bl}) with a common work rate amplitude (21 W) for each trial (bl_3-bl_{63}) for, A: [Hb_{tot}] baseline; B: [Hb_{tot}] amplitude for the entire transition. Significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}. μM, micromolar.

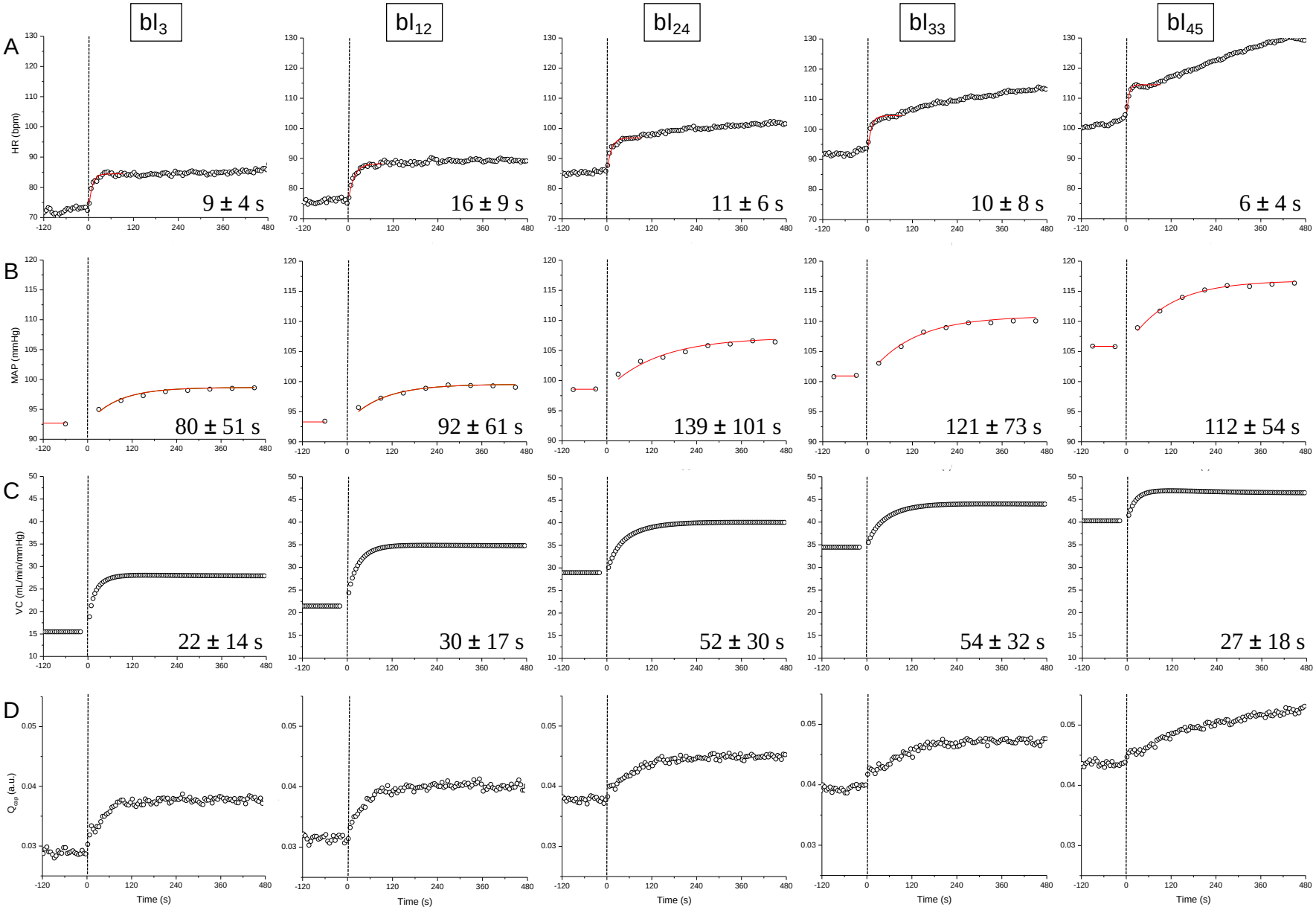
bl3
bl12
bl24
bl33
bl45
A
B
C
D
HR (bpm)
MAP (mmHg)
VC (mL/min/mmHg)
Qsip (a.u.)
9 ± 4 s
16 ± 9 s
11 ± 6 s
10 ± 8 s
6 ± 4 s
80 ± 51 s
92 ± 61 s
139 ± 101 s
121 ± 73 s
112 ± 54 s
22 ± 14 s
30 ± 17 s
52 ± 30 s
54 ± 32 s
27 ± 18 s
Time (s)

Figure 4.8. Group mean responses of: A, heart rate (HR); B, mean arterial pressure (MAP); C, vascular conductance (VC); and D, microvascular blood flow ($\dot{Q}_{cap}$) for trials bl_3-bl_{45}. Vertical dashed lines indicate the onset of the exercise transition (time = 0 s). The group mean primary component responses for each trial are superimposed on the data (red lines, fit by a monoexponential using group mean parameter estimates). Time constant (τ) values for HR and VC, and mean response time (MRT) values for MAP (mean ± SD) are displayed inset in each graph.

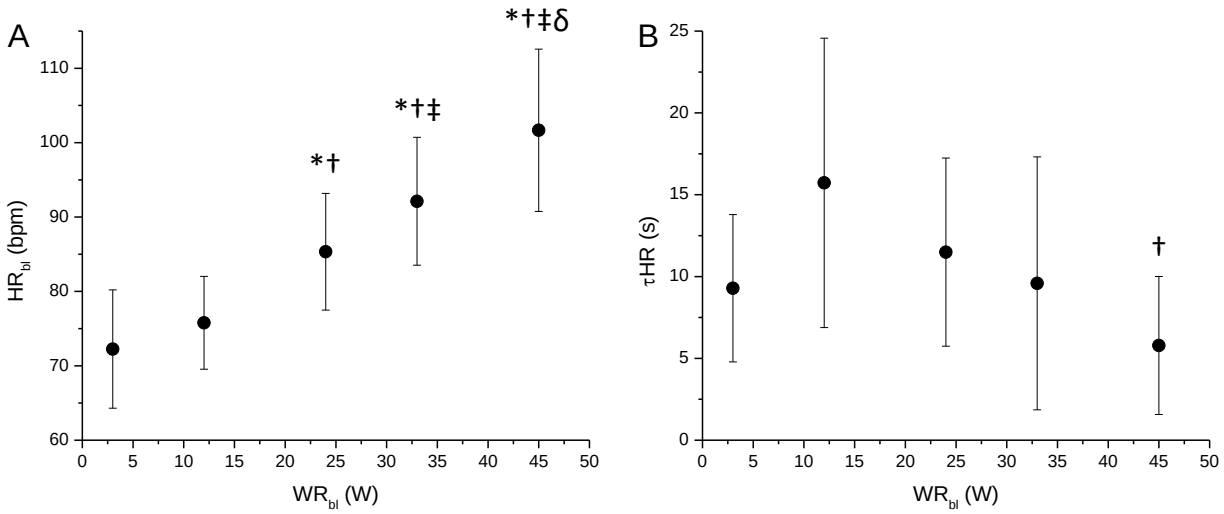

Figure 4.9. Heart rate (HR) parameter estimates from increasing work rate baselines (WR_{bl}) with a common work rate amplitude (21 W) for each trial (bl_3-bl_{63}) for, A: baseline HR; B: time constant (τ) for HR. Symbols denote a significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}; ‡, bl_{24}; δ, bl_{33}. bpm, beats per minute.

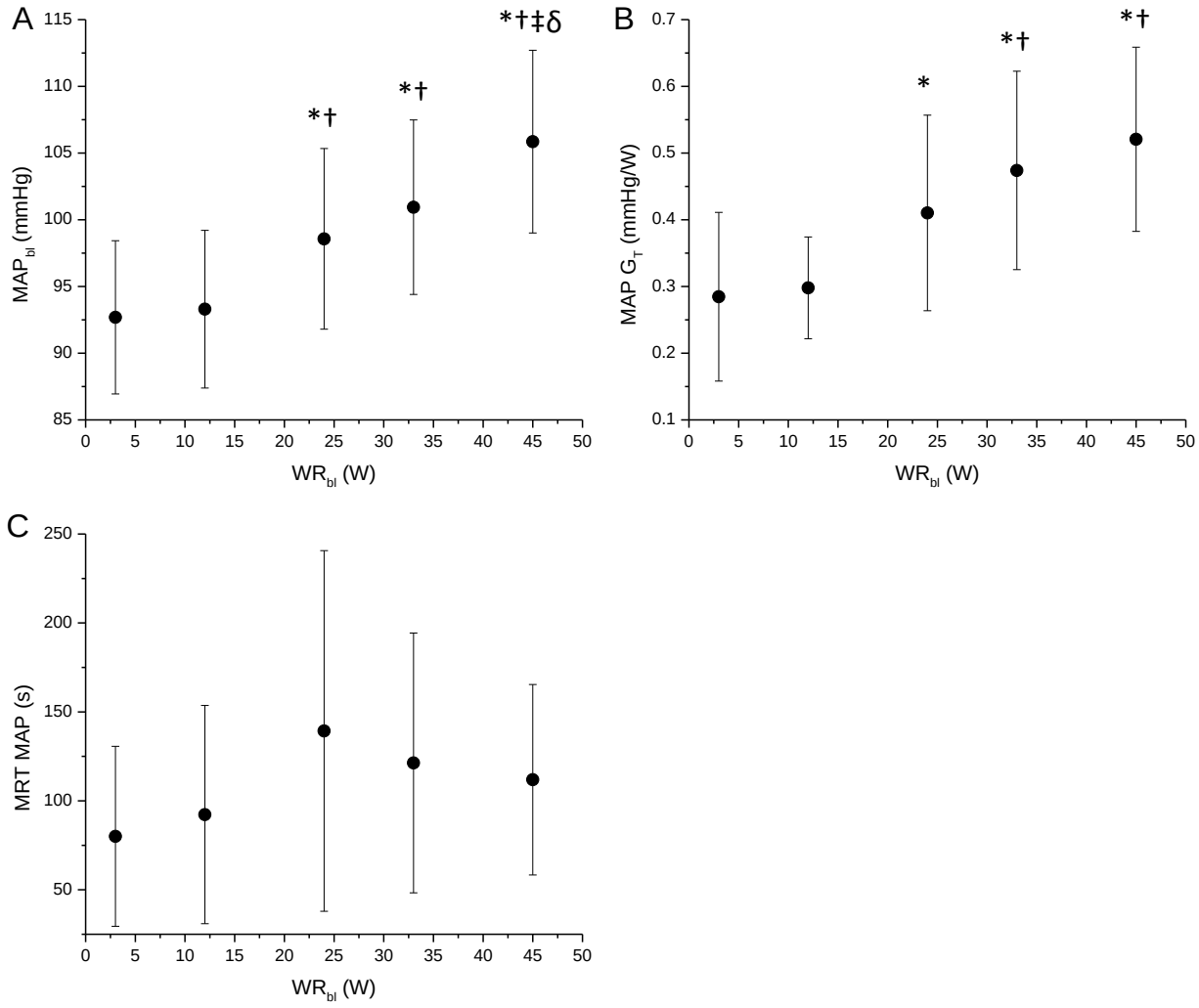

Figure 4.10. Mean arterial pressure (MAP) parameter estimates from increasing work rate baselines (WR_{bl}) with a common work rate amplitude (21 W) for each trial (bl_3-bl_{63}) for, A: baseline MAP; B: amplitude of MAP; C: time constant of MAP. Symbols denote a significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}; ‡, bl_{24}; δ, bl_{33}. Δ, amplitude; MRT, mean response time.

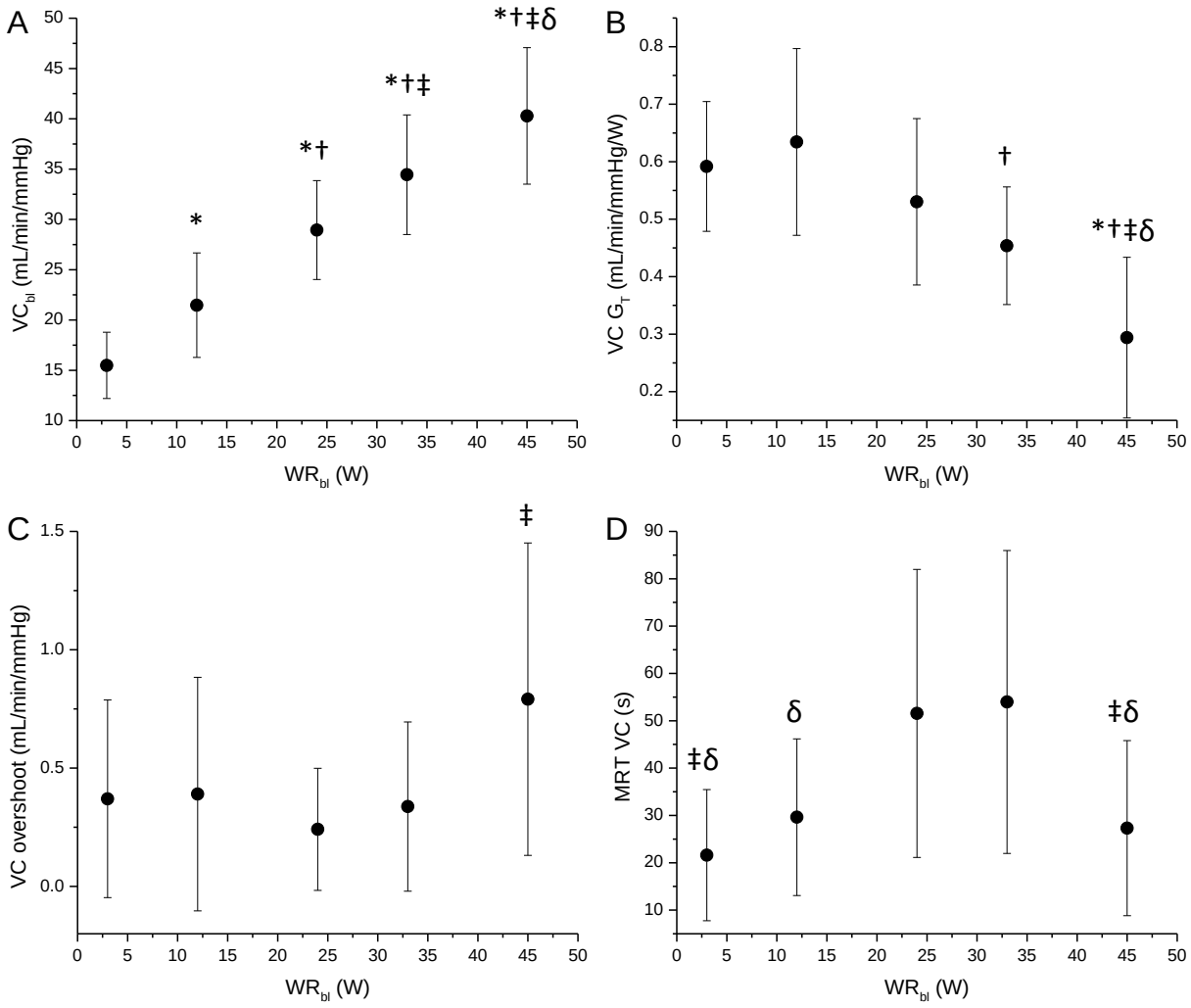

Figure 4.11. Vascular conductance (VC) parameter estimates from increasing work rate baselines (WR_{bl}) with a common work rate amplitude (21 W) for each trial (bl_3-bl_{63}) for, A: baseline VC; B: gain of the 'primary' component of VC; C: VC overshoot; D: Mean response time of VC (MRT VC). Symbols denote a significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}; ‡, bl_{24}; δ, bl_{33}. G_p, gain of the primary component; MRT, mean response time.

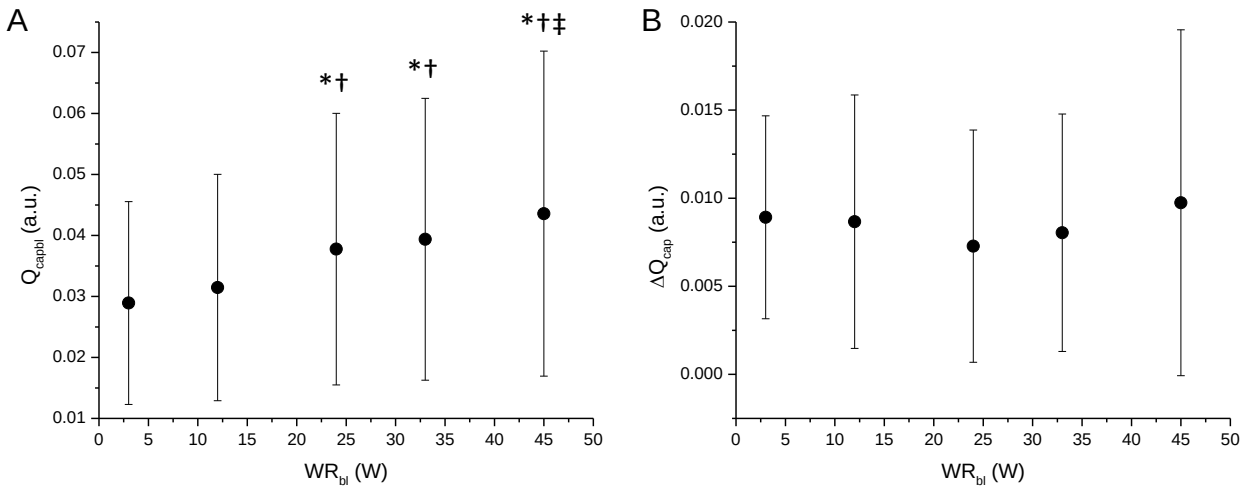

Figure 4.12. Calculated microvascular blood flow ($\dot{Q}_{cap}$) parameter estimates from increasing work rate baselines (WR_{bl}) with a common work rate amplitude (21 W) for each trial (bl_3-bl_{63}) for, A: baseline $\dot{Q}_{cap}$; B: $\dot{Q}_{cap}$ amplitude. Symbols denote a significant difference ($p < 0.05$) from: *, bl_3; †, bl_{12}; ‡, bl_{24}; δ, bl_{33}. Δ, amplitude; a.u., arbitrary units.

Discussion

In the present study exercise transitions in response to a constant WR change (ΔWR) were initiated from different baseline metabolic rates which ranged in intensity across the MOD to HVY to VH domains to examine the effect of metabolic rate on the kinetics and integration amongst bulk arterial blood flow (BF), pulmonary $\dot{V}O_2$ ($\dot{V}O_{2p}$), and quadriceps muscle deoxygenation (deoxy[Hb + Mb]), as well as heart rate (HR), mean arterial pressure (MAP), and vascular conductance (VC) kinetics. To the best of the authors' knowledge, only one study has previously investigated the effect of an elevated WR_{bl} on BF kinetics in humans, with their findings limited to only two transitions which were constrained within the MOD domain (MacPhee et al., 2005). The main findings of the present study were: i) $\tau\dot{V}O_{2p}$ and $\Delta\dot{V}O_{2p}$ generally increased with increasing WR_{bl} (despite the same absolute step change in ΔWR); ii) bulk BF kinetics (τBF, MRT BF) slowed with increasing WR_{bl} with the exception of the highest WR_{bl} where there was a significant speeding compared to transitions from lower WR_{bl}'s; iii) the increase in BF amplitude (ΔBF) relative to the increase in metabolic demand ($\Delta BF/\Delta\dot{V}O_{2p}$) became smaller as WR_{bl} increased; iv) the MRT deoxy[Hb + Mb] became progressively larger with increasing WR_{bl}; v) the gain in MAP increased with increasing WR_{bl}; and vi) the gain in VC became smaller with increasing WR_{bl}.

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

Recent studies from our laboratory (Keir et al., 2016a/b) using leg cycling exercise reported that a progressively increasing WR_{bl}, while keeping ΔWR constant, results in a curvilinear slowing of $\dot{V}O_{2p}$ kinetics as evidenced by an increasing phase II $\tau\dot{V}O_{2p}$. The results of the present study agree well with these previous findings, and those of others (Nederveen et al., 2017, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001), by also showing that with knee-extension exercise, $\tau\dot{V}O_{2p}$ becomes larger with increasing WR_{bl}. This slowing of $\dot{V}O_{2p}$ kinetics with exercise transitions initiated from progressively higher baseline metabolic rates has been suggested to be due to the progressive recruitment of less efficient muscle fibres in order to support the metabolic requirements associated with the new step-increase in WR (Nederveen et al., 2017, Keir et al., 2016a/b, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001) or to a greater muscle instability (Grassi et al., 2011) and

decrease in Gibbs free energy associated with ATP hydrolysis in the newly and/or already recruited muscle fibres (Wust et al., 2014, Bowen et al., 2011). A decrease in metabolic efficiency is evidenced in the present study by the increase in the $\dot{V}O_{2p}$ G_p (Figure 4.3B) and overall $\dot{V}O_{2p}$ G_T (data not shown), which agrees well with the increased $\dot{V}O_{2p}$ gain reported previously (Nederveen et al., 2017, Keir et al., 2016a/b, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001). These less efficient type I and type II (especially type IIx) fibres that are recruited, have a higher capacity for glycolytic metabolism and lower capacity for oxidative metabolism, and are associated with slower $\dot{V}O_{2p}$ kinetics (Crow & Kushmerick 1982, Pringle et al., 2003a/b). Additionally, in animal muscle, fast, glycolytic fibres are supported by a lower muscle BF, and lower muscle O_2 delivery-to-muscle O_2 utilization ratio, relative to more oxidative fibres (McDonough et al., 2004, Behnke et al., 2003), which leads to greater microvascular O_2 extraction and fall in PO_2, thus lowering the driving pressure (ΔPO_2) for O_2 diffusion from the red blood cell into the myocyte. Furthermore, McDonough et al. (2005) found that in response to muscle contraction in rat gastrocnemius (primarily fast twitch fibres) compared to soleus (primarily slow twitch fibres) muscles, microvascular PO_2 was lower at rest, and in response to muscle contraction, the PO_2 decreased at a faster rate and displayed a transient undershoot before reaching a significantly lower "steady-state" value. These findings suggest that O_2 delivery to the less efficient fast twitch fibres is attenuated relative to the O_2 requirement and that BF kinetics may be slower to these fibre types. However, it is not clear from these data whether the slowing of $\dot{V}O_{2p}$ kinetics from elevated baseline metabolic rates is the result of 'sluggishness' in metabolic activation of the newly recruited fibres or to a lower O_2 delivery (i.e., BF) to these fibres, or to some other presently unknown factor.

BF

Due to the lack of information on the BF response during exercise transitions in these previous studies, one of the main goals of the present study was to investigate the effect of increasing WR_{bl} on BF kinetics. There was an increase in τBF (which was determined for the same time period as the primary component for $\dot{V}O_{2p}$) with increasing WR_{bl}, but a constant ΔWR, for all but the highest WR_{bl}, which generally confirms our hypothesis that BF kinetics would be slowed with increasing WR_{bl}, and agree with the findings of MacPhee

et al. (2005) which were confined to the MOD domain. Peripherally, this slowing of BF kinetics may be due to the recruitment of less efficient fibres that have a lower (McDonough et al., 2004, Behnke et al., 2003), and possibly slower (McDonough et al., 2005), BF response. In turn, a slowed response of the microvasculature surrounding these fibres, might be expected to produce a slowed response to the resistance vessels upstream (via conducted vasodilation (through the endothelium and/or smooth muscle cells), and/or flow-mediated dilation (due to shear stress)), which will ultimately affect the conduit artery. Alternatively, a slowed demand for O_2 from less efficient fibres, or a decrease in metabolic efficiency of already recruited fibres, may also translate into a smaller and/or slowed demand for convective O_2 delivery. Centrally, a slowing of BF kinetics could be due to a slowed adjustment of cardiac output ($\dot{Q}_T$), as suggested by MacPhee et al. (2005), in which those authors noted HR kinetics were slower when exercise was initiated from an elevated WR_{bl} in the MOD domain. However, the present study did not observe a slowing of HR kinetics with increasing WR_{bl} (see Figure 4.9B), suggesting that HR kinetics were unlikely to be responsible for the slowing of BF kinetics.

In contrast to our hypothesis, the BF dynamics for the highest exercise transition were faster than the previous transition, although this value only reached significance when BF kinetics were estimated across the entire exercise transition (i.e., MRT BF). While the reason for this speeding of BF kinetics at the highest WR, which has never been observed before, is not readily elucidated, we do offer a possible explanation. At the various baseline metabolic rates and exercise transitions studied in the present study, subjects were working within and across different intensity domains such that the physiological responses associated with these ΔWR interventions might be expected to vary depending on the intensity domain into which the transition is being made (e.g., sustainable (MOD and HVY) vs. unsustainable (VH and severe) exercise). In the current study we did not make the WRs relative to each individual's intensity domains, deciding instead to use the same absolute WRs and a ΔWR that provided a good signal-to-noise ratio for the variables, and allowed a relatively wide range of end-WR intensities and a peak WR (i.e., 66 W) that could be completed by all participants. Based on the intensity boundaries (i.e., LT, RCP) and blood [$lactate^-$] measures determined from the step-incremental KE exercise test, we were able to estimate, for each subject, in which

intensity domain each of the WRs was being performed. Based on this, it was established that for the final exercise transition, most subjects (7 of 10) were transitioning from the HVY into the VH intensity domain. In the HVY domain there is an increased accumulation of metabolites, many of which have been shown to induce vasodilation (e.g., H^+, K^+, adenosine, prostaglandins, CO_2, and nitric oxide). An enhanced vasodilatory response in the microvasculature may result in a faster increase in microvascular blood volume and flow during an exercise transition, with the dilatory signal extending upstream to facilitate bulk BF. If metabolites are indeed involved in a quickened response, the question could be raised as to whether it is the presence of these metabolites in the HVY baseline prior to the transition or because of a rapid accumulation of these metabolites during the VH WR of the transition that cause this response. Unfortunately, it is not possible to differentiate the contribution of these two potential mechanisms in the present study. Therefore, further investigation into the effects of HVY and VH exercise on bulk BF kinetics is warranted.

$\dot{V}O_{2p}$ and BF

The finding that the relationship between increase in bulk BF relative to the increase in metabolic demand ($\Delta BF/\Delta\dot{V}O_{2p}$) becomes smaller when WR_{bl} is increased is similar to what has been previously observed in our laboratory (MacPhee et al., 2005, Chapter III) and others (Van Beekvelt et al., 2001, Koga et al., 2005). Furthermore, there was a progressive attenuation of the increase in $\Delta[Hb_{tot}]$ with increasing WR_{bl} (Figure 4.7B), implying that both bulk and microvascular BF were attenuated for transitions from an elevated baseline metabolic rate. In accordance with the Fick equation, a decrease in ΔBF relative to a rise in $\Delta\dot{V}O_{2p}$ would necessitate an increase O_2 extraction and result in a widening of the $a\text{-}vO_{2diff}$. In the present study, despite the decrease in both the $\Delta BF\text{-to-}\Delta\dot{V}O_{2p}$ ratio and $[Hb_{tot}]$ with increasing WR_{bl} (Figure 4.5B), the NIRS-derived deoxy[Hb + Mb] signal, which can be considered a surrogate measure for $a\text{-}vO_{2diff}$, generally did not show a greater increase in amplitude with increasing WR_{bl} (Figure 4.6C), which was unexpected according to the Fick relationship. While these findings are not easily reconcilable, the discrepancy between $\Delta BF\text{-to-}\Delta\dot{V}O_{2p}$ ratio and O_2 extraction may be partly explained by the fact that the Doppler ultrasound measures muscle conduit artery (bulk) BF while the NIRS-derived deoxy[Hb + Mb] measure reflects the local muscle microvasculature O_2 delivery-to-O_2 utilization

relationship. Therefore, a comparison of measures between these two sites should be made cautiously.

While a progressive decrease in $[Hb_{tot}]$ G_T with increasing WR_{bl} does agree with the findings of BF at the level of conduit artery, it also is possible that at such high WRs there is a limitation of the NIRS, with the signal becoming 'saturated.' In such a situation, the $[Hb_{tot}]$ (and other NIRS measures, deoxy[Hb + Mb] and oxy[Hb + Mb]) would be unable to increase further, making the signal less reliable. In addition to this, there is a plateauing of the $_{TOT}$[Hb + Mb] response profiles in the highest transition (bl_{45}; Figure 4.2D) that is not present in any of the previous transitions. While it is possible that this plateau, and decrease in $_{TOT}$[Hb + Mb] G_T with increasing WR_{bl}, is due to the large contractile forces at this WR which may occlude BF to a greater degree than the prior transitions and prevent a further increase in $_{TOT}$[Hb + Mb], it also may be a result of a 'saturated' signal for NIRS. Indeed, the lack of an increase in deoxy[Hb + Mb] G_T with increasing WR could agree with a 'saturation' of the NIRS signal. It also should be noted that $_{TOT}$[Hb + Mb] is already high at the baseline of these higher WR_{bl}s, suggesting that diffusive capacity into the muscle is high and may not have a substantial need for further increases during the transition.

deoxy[Hb + Mb]

The dynamics of muscle microvascular deoxy[Hb + Mb] were slowest (i.e., MRT deoxy[Hb + Mb] largest) at the highest WR_{bl}, which suggests an increased BF and O_2 delivery relative to local muscle O_2 utilization, and thus a lower fractional O_2 extraction. In addition, there was a small, transient overshoot in the deoxy[Hb + Mb] response at the lowest WR_{bl} (bl_3) which tended to be smaller at the highest WR_{bl}'s ($p < 0.10$; Figure 4.6D), indicative of an improved matching of local microvascular BF and muscle O_2 utilization during the exercise transient of higher WR_{bl}s. Therefore, despite ΔWR being the same, when initiating an exercise transition from a higher WR_{bl} there may be a decreased reliance on widening the a-vO_{2diff} in the transitory phase than when a transition is initiated from a lower WR_{bl}, where microvascular BF seemingly adjusts more slowly. While this finding seems to be at odds with the decrease in $[Hb_{tot}]$ G_T and $\Delta BF/\Delta VO_{2p}$ ratio discussed above, it should be noted that these findings pertain to the kinetics rather than the overall response. Nevertheless, further

research is needed to discern the limitations of BF and/or NIRS that are present at higher WR_{bl}s.

HR

The adjustment of HR, which contributes to the adjustment of central $\dot{Q}_T$ and thus peripheral conduit artery BF, revealed that τHR was smaller in the highest WR_{bl} (bl_{45}) than bl_{12} (Figure 4.9B). This observation would seem to align well with the finding that BF kinetics were faster at the highest WR_{bl} (Figures 4.4C & D), suggesting that $\dot{Q}_T$ could be partially contributing to the faster rate of adjustment of bulk BF at this transition. Despite that, overall, the kinetics of HR and BF were not related (data not shown), with τHR showing a general decrease while τBF showed a general increase during the lower WR_{bl}'s, suggesting that the adjustment of HR has little influence on the rate of adjustment of bulk BF. However, as $\dot{Q}_T$ also is determined by stroke volume (SV), it is possible that the rate of SV increase was slower at the lower WR_{bl}s, due to a slower return of venous blood and/or a slower/smaller activation of the sympathetic nervous system (SNS). However, there were no measures taken for SV – or its influencing variables (venous return and SNS activity) – in the present study, and therefore, the full contribution of $\dot{Q}_T$ to the bulk BF response is unknown.

The increase in HR during exercise is determined by parasympathetic nervous system (PSNS) withdrawal and SNS activation. In the present study the progressive increases in HR associated with exercise transitions from the lowest to the highest WR_{bl} ranged between ~70 & ~100 bpm. In this range, HR increases are determined more by withdrawal of the faster-responding PSNS rather than by increases in SNS activation which is a slower process and predominates at HRs > ~100 bpm (see Karemaker 2017 for review). Therefore, the finding of a speeding of HR kinetics with increasing WR_{bl} is somewhat surprising given the known dynamics of PSNS and SNS regulation of the heart and the expected reduction in PSNS control of HR from an increased WR_{bl}.

MAP

The MAP gain (MAP G_T) increased with increasing WR_{bl} (Figure 4.10B), which is expected to contribute to the increase in the BF response to exercise (BF = MAP · VC). However,

despite the progressive increase in MAP G_T there was a relative decrease in BF gain (and $\Delta BF/\Delta \dot{V}O_{2p}$) with increasing WR_{bl}, which implies some restraint in the BF response. An increase in resistance to BF with increasing WR_{bl} does seem plausible, as higher WRs result in an increase in SNS output which causes an increase in vasoconstriction throughout the body (see Delp & O'Leary 2004 for review), particularly the inactive musculature. Since the present study has a relatively small active muscle mass (quadriceps only) that will be vasodilating, this leaves a large proportion of muscle in the body undergoing vasoconstriction, and thereby, increasing resistance. By this same premise, a high vascular resistance from the large amount of inactive muscle mass in the lower limbs may also be contributing to the decrease in $\Delta BF/\Delta \dot{V}O_{2p}$ at higher WR_{bl}'s. In addition to an increase in sympathetic vasoconstriction, the increase in muscle force production required to overcome the external resistance at higher WRs also would be expected to contribute to muscle BF restriction due to occlusion of the vasculature within the active muscle by the contracting fibres. Indeed, previous studies have shown that upon the cessation of exercise there is an increase in BF to the muscle (Van Beekvelt et al., 2001, Shoemaker et al., 1994), suggesting that BF is inadequate during the exercise period. Furthermore, the degree of this postexercise hyperemia may be related to intensity, as Van Beekvelt et al. (2001) found that the postexercise increase in BF following forearm exercise at 25% WR_{peak} was brief (~10 s) and not significantly greater than BF during the exercise bout, while postexercise hyperemia following a 75% WR_{peak} was significantly greater and longer (~90 s). These findings would seem to imply that there is a greater occlusion of BF at higher WRs. Indeed, Lutjemeier et al. (2005) reported that muscle contraction had a net increase in BF at low intensities, but a negative effect on BF at the highest WRs studied as the force of contraction resulted in greater impedance to BF. Taken together, these findings could suggest that the increase in resistance may exceed the increase in MAP, which could provide rationale for the attenuated increase in both bulk BF (Figure 4.5B) and $[Hb_{tot}]$ (Figure 4.7B) at higher WRs that were observed in the present study.

VC

Coincident with this increase in MAP G_T, there was a decrease in VC G_T with increasing WR_{bl}. This attenuation in VC across increasing WR_{bl}'s may be responsible for the smaller

BF gains seen across increasing WR_{bl}'s, as well as a possible contributor to the slowing of BF kinetics during the initial WR_{bl}'s. Additionally, in some, but not all subjects, an 'overshoot' in VC was present, with trial bl_{45} having a significantly greater 'overshoot' than trial bl_{24} (Figure 4.11C). While the cause of this VC 'overshoot' is not evident, it may be associated with an initial rapid increase in BF (caused by a rapid vasodilation, and/or the 'muscle pump') coupled with a gradual increase in resistance (caused by vasoconstriction).

With the concomitant measures of BF and MAP (and the calculation of VC), this study was able to assess the contribution of both MAP and VC to the adjustment of BF across the multiple different WR_{bl} transitions used in this study. The finding that MRT MAP was not significantly different between transitions (Figure 4.10C), while MRT BF became larger with increasing WR_{bl} before becoming significantly smaller at the highest WR_{bl} (Figure 4.4D), would seem to suggest that MAP does not play a central role in determining the overall kinetic response of BF. However, MRT VC became progressively larger with increasing WR_{bl}, before becoming significantly smaller at the highest WR_{bl} (Figure 4.11D), a finding which perfectly reflected MRT BF. This would seem to suggest that the speeding of BF kinetics in the highest WR_{bl} (bl_{45}) was predominantly caused by a speeding of VC kinetics. This could then suggest that vasodilation in the active musculature, rather than an increase in BP, is a key control mechanism for BF kinetics.

Limitations

This study does present with several limitations. Due to the number of trial repetitions needed to improve the signal-to-noise ratio, and the high intensity of some of the WRs, there is a high potential of a training effect. As training has been shown to speed $\dot{V}O_{2p}$ kinetics (Murias et al., 2016, Phillips et al., 1995, Bell et al., 2001) and cause changes in both metabolic (e.g., increased $\dot{V}O_2$ efficiency) and cardiovascular variables (e.g., increased capillarization), this warrants careful consideration. While the number of repetitions needed for each transition (3-6) was not reduced (so as to maintain a good signal-to-noise ratio), repetitions of a particular WR transition were not performed until every other intensity had been completed. By this design, if a training effect were to occur, it would be "distributed" between all of the trials proportionally. Furthermore, no observable differences in $\dot{V}O_{2p}$, BF,

or HR were found between the individual trials within each transition, suggesting little evidence of a training effect throughout the study.

Blood pressure was taken manually at one minute intervals, and although the profiles generated in our study closely resembled a monoexponential response, we recognize that caution should be used when quantifying this response due to the prolonged time between measurements (especially early in the transition). Similarly, as VC was calculated using the iterated response of MAP, caution also should be used in quantifying the VC response due to the large amount of generated data.

Lastly, the use of NIRS has several limitations which are well addressed by Schlup et al. (2015). Firstly, the transmitted wavelength of light detects both haemoglobin and myoglobin without knowing the proportion that each contributes to the overall signal. The oxy-haemoglobin dissociation curve displays a sigmoidal relationship which allows greater off-loading of O_2 for a given range of microvascular PO_2s within the range of approximately 60-10 mmHg ($P_{50} \approx 27$ mmHg). In contrast, the intramuscular oxy-myoglobin curve displays a hyperbolic relationship whereby significant O_2 off-loading does not occur until very low intracellular PO_2s ($P_{50} \approx 2.5 – 3$ mmHg; Schenkman et al., 1997, Richardson et al., 1995). Therefore, microvascular and intracellular PO_2 levels will effect the degree of desaturation of haemoglobin and myoglobin to different extents. However, at present, we do not have reliable values of intracellular PO_2 levels during exercise, making it difficult to determine the contribution of deoxy-Mb to the NIRS signal. While it has been shown that myoglobin contributes ~50-70% of the NIRS signal at rest, and ~70% of the change in the NIRS signal from rest to maximal exercise is from myoglobin (Davis & Barstow 2013), the diffusion pathway as a whole would be unaffected, and therefore, this should not compromise the use of NIRS in looking at the O_2 delivery-to-O_2 utilization ratio. Secondly, the NIRS signal investigates a small volume of muscle, and with a limited depth of penetration of the NIR light (~3.5 cm) and the distribution of muscle fibre types (type II fibres predominate in superficial muscle and type I fibres predominated in deeper muscle regions (Lexell et al., 1983, Johnson et al., 1973)), this signal should not be considered as representative of the entire muscle. Thirdly, the contribution of each of the microvascular vessels (arterioles, capillaries, venules) to the NIRS signal is not known. However, Poole et al. (1995) observed

that the vast majority (~84%) of the microvascular volume in the rat diaphragm is comprised of capillaries, suggesting that the deoxy[Hb + Mb] signal represents mainly capillary O_2 extraction. However, to date, no studies have reproduced this, with several studies theorizing a different composition of the microvasculature (arterioles vs. capillaries vs. venules) without providing a source for these compositions, leaving the true division of the microvasculature unknown at present (Barstow 2019). Nevertheless, care was taken to ensure the same placement of the probes on each subject throughout the study, and therefore, the differences observed between trials should represent true differences, albeit limited perhaps to the muscle fibres investigated. Fourthly, the NIRS signal must pass through a layer of skin and adipose tissue, which may have their own microvascular volumes and tissue region-specific BF. However, the microvascular volume of these tissues is considerably less than in skeletal muscle (Maehara et al., 1997), and little O_2 extraction would be expected in these tissues, making their contribution to the absolute deoxy[Hb + Mb] signal insignificant (see Barstow 2019 for review).

Conclusions

In summary, this study examined the effect of exercise transitions from increasing baseline metabolic rate, with a common ΔWR, on the responses of $\dot{V}O_{2p}$, BF, deoxy[Hb + Mb], HR, MAP, $\dot{Q}_{cap}$, and VC. It was found that $\tau\dot{V}O_{2p}$ became larger with increasing WR_{bl}, a finding which corroborates the results of Keir et al. (2016a/b) that $\dot{V}O_{2p}$ kinetics become progressively slower as baseline metabolic rate is elevated. However, although increasing baseline metabolic rate resulted in a progressive slowing of conduit artery BF kinetics over the lower four transitions investigated in this study, which was in line with our hypothesis, there was a significant speeding of BF kinetics at the highest WR_{bl}, which was contrary to our hypothesis. The findings that BF kinetics follow a similar response to that of $\dot{V}O_{2p}$ over the lower exercise intensities (i.e., similar time constants and becoming slower with increasing WR_{bl}), suggests that conduit artery BF could be limiting to $\dot{V}O_{2p}$ kinetics in this situation. However, the finding of a disconnect between $\dot{V}O_{2p}$ and BF kinetics at the higher WR_{bl}s (i.e., a speeding BF kinetics with slowing $\dot{V}O_{2p}$ kinetics), implies that conduit artery BF kinetics cannot be limiting $\dot{V}O_{2p}$ kinetics at these intensities. Furthermore, the slowing of deoxy[Hb + Mb] kinetics with increasing WR_{bl} implies that O_2 delivery to the muscle is

in excess of what is being utilized, and therefore, microvascular O_2 delivery/diffusion is also unlikely to be limiting $\dot{V}O_{2p}$ kinetics. This would then suggest that in this situation (elevated WR_{bl}) the limitation on $\dot{V}O_{2p}$ kinetics lies within the muscle (i.e., a metabolic 'sluggishness'). Future studies should individualize the WRs to be within a given intensity domain for each subject so as to gain a more accurate understanding on whether this 'disconnect' between BF and $\dot{V}O_{2p}$ kinetics is a result of the physiological changes occurring in these different intensity domains. Lastly, we observed a significant decrease in the $\Delta BF/\Delta\dot{V}O_{2p}$ ratio with increasing WR_{bl}, showing that bulk BF does not increase in proportion to metabolic demand. This may indicate a more 'efficient' distribution of BF amongst the active muscle fibres in the leg when WR_{bl} is elevated, but further investigation is needed.

Acknowledgements

We gratefully thank each of our participants for their time and effort in this study. We thank Brad Hansen for his technical support of the lab equipment. We also thank Maureen MacDonald and her lab for their guidance on measuring blood flow. We also extend our gratitude to Professor P.A. Robbins, University of Oxford, for providing the "End-tidal Forcing" software for breath-by-breath pulmonary oxygen uptake measurement.

Grants: This study was supported by the National Science and Engineering Research Council of Canada (NSERC) research and equipment grants.

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CHAPTER V: Conclusions

Summary of Studies

This thesis investigated the role of $\dot{V}O_{2p}$, BF, deoxy[Hb + Mb], HR, MAP, $\dot{Q}_{cap}$, and VC, and the integration amongst these variables, in response to step-transitions of multiple different baseline metabolic rates (WR_{bl}) and metabolic amplitudes (ΔWR) across the MOD, HVY, and VH domains. While many studies have investigated the effect of different ΔWRs (Nyberg et al., 2017, Keir et al., 2016b, Spencer et al., 2013, Jones et al., 2012, Koga et al., 2005, MacPhee et al., 2005, Ozyener et al., 2001, Scheuermann et al., 2001, Koga et al., 1997, Koga et al., 2001, Koga et al., 1999, Engelen et al., 1996, Paterson & Whipp 1991) and WR_{bl}s (Nederveen et al., 2017, Keir et al., 2016a/b, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001) on $\dot{V}O_{2p}$ kinetics, relatively few have looked at the response of BF kinetics, or other physiological variables, to these same transitions. Those studies that have investigated BF kinetics have often looked at only two ΔWRs (McNarry et al., 2014, Jones et al., 2012, Koga et al., 2005, MacPhee et al., 2005, Hughson et al., 1996), with the only study looking at BF kinetics from an elevated WR_{bl} doing so being constrained within the MOD domain (MacPhee et al., 2005). To expand upon these previous studies, the present thesis investigated the contribution and interaction of many physiological variables across multiple different WR_{bl} and ΔWR transitions.

It was first necessary to discern how to properly (or best) quantify the BF signal, therefore, Chapter II investigated the effect of some of the most prevalent analysis techniques on BF kinetics by averaging the BF signal over 1, 2, or 5 cycles ('binned' and 'rolling') using either the R-spike interval of the cardiac cycle (HR) or the contraction-relaxation (CR) profiles to demarcate a 'cycle' of interest. The use of HR or CR to quantify the BF signal is necessary because of the physiological fluctuations in the BF profile caused by the systolic and diastolic phases of the cardiac cycle, as well as periodic BF occlusion caused by the muscle contraction-relaxation cycle. To date, those studies that have examined the effect of different analysis techniques on quantifying BF have used only steady-state conditions (Osada & Radegran 2006a/b, Osada 2004, Shoemaker et al., 1996), without

consideration given to the non-steady-state conditions associated with changes (transitions) in exercise intensity in which a clean signal with good temporal resolution is fundamental.

Although the lack of a 'gold standard' for analyzing BF constrains the ability to define a 'true' BF for each exercise transition, comparisons of the various BF analysis techniques used in the present study across several kinetic parameters (e.g., baseline, amplitude, time constant, and confidence interval) yielded several noteworthy findings. The findings of Chapter II corroborated the findings of previous studies showing that analysis technique does not influence "steady-state" BF, but when focusing on "non-steady-state" conditions associated with changes in exercise intensity, it was found that: i) estimating MRT from a single CR cycle (CR1) within the MOD domain resulted in values that were smaller than observed for most other analysis techniques; ii) analysis using CR1 yielded BF amplitude (ΔBF) values that were higher than (or similar to) the other analysis techniques in HVY, but not in MOD; iii) analyses using a single cardiac cycle (HR1) had the highest confidence interval (CI_{95}; i.e. largest variability), while those using CR1 had the lowest CI_{95} for both MOD and HVY; and iv) when comparing the difference between 'model determined' and 'data determined' steady-state BF values for MOD and HVY, those analysis techniques that incorporated a 'rolling' average (HR2r, HR5r, CR2r, CR5r) had a significantly larger difference in these values for HVY than MOD. In considering the outcomes for the kinetic parameters estimated using the various analysis techniques, the CR1 method was chosen as the preferred method of BF analysis, and was used throughout the thesis, because of its low variability, reliability in modeling end-exercise values, and shorter interval duration which minimizes 'overlap' of the steady-state and non-steady-state data.

Several studies have reported that the kinetics for the primary component of the $\dot{V}O_{2p}$ response are similar for step-transitions in WR (ΔWRs) initiated from a common, light-intensity, baseline metabolic rate into the MOD or HVY domains (Nyberg et al., 2017, Keir et al., 2016b, Spencer et al., 2013, Jones et al., 2012, Koga et al., 2005, MacPhee et al., 2005, Ozyener et al., 2001, Scheuermann et al., 2001, Koga et al., 1997). Furthermore, studies that have investigated the relationship amongst BF or O_2 delivery and $\dot{V}O_{2p}$ kinetics, in general, report no differences in BF kinetics between transitions into the MOD or HVY domain if initiated from a common, light-intensity, baseline metabolic rate

(Nyberg et al., 2017, McNarry et al., 2014, Jones et al., 2012, Koga et al., 2005, MacPhee et al., 2005, Hughson et al., 1996). However, previous studies only investigated and report the response of BF kinetics to two different ΔWR transitions. Although recent studies from our laboratory using leg-cycling ergometry (Keir et al., 2016b, Spencer et al., 2013) reported no differences in $\dot{V}O_{2p}$ kinetics with step-transitions using multiple, different ΔWRs, BF dynamics and the relationships of BF and $\dot{V}O_{2p}$ responses were not investigated. Therefore, in Chapter III alternate-leg knee-extension exercise was used which allowed the simultaneously measurement of $\dot{V}O_{2p}$, BF, deoxy[Hb + Mb], HR, and MAP (and the calculation of $\dot{Q}_{cap}$, and VC) across multiple transitions of different absolute ΔWRs from a common, low baseline metabolic rate. The purpose of this study was to investigate the effect of these different ΔWRs (which spanned the MOD, HVY, and VH domains) on the dynamic adjustment of $\dot{V}O_{2p}$, BF, deoxy[Hb + Mb], HR, MAP, $\dot{Q}_{cap}$, and VC, and the interactions of these variables throughout these transitions. It was hypothesized that $\dot{V}O_{2p}$, BF, deoxy[Hb + Mb], HR, and VC kinetics would be similar across the different ΔWR transitions and that these variables would increase proportionally with the increasing metabolic demand of larger ΔWRs.

The main findings of Chapter III were that: i) phase II $\dot{V}O_{2p}$ kinetics became progressively slower (i.e., $\tau\dot{V}O_{2p}$ larger) in a linear manner with increasing ΔWR; ii) phase II BF kinetics (τBF) were not different amongst the different ΔWRs (although there was a trend for a progressive slowing of BF kinetics with increasing ΔWR, with the largest ΔWRs being significantly slower when fitting the entire transition (MRT BF)); iii) the increase in BF per increase in $\dot{V}O_{2p}$ ($\Delta BF/\Delta\dot{V}O_{2p}$) became smaller as ΔWR became larger; iv) muscle deoxygenation kinetics became progressively faster (i.e., MRT deoxy[Hb + Mb] smaller) in a linear manner with increasing ΔWR; v) HR kinetics were similar across all ΔWRs; and vi) VC kinetics had a tendency to become slower with increasing ΔWR. Collectively, these findings suggest that to compensate for a relative decrease in bulk BF (O_2 delivery) with increasing ΔWR, there is an increase in O_2 extraction to help augment the O_2 requirements of the muscle. This decrease in the $\Delta BF/\Delta\dot{V}O_{2p}$ ratio may be attributable to the increased force production of the muscle – to meet the larger ΔWR – which increases vascular occlusion causing an attenuated increase in BF. Alternatively, it is possible that an increase

in sympathetic nerve activity at the higher WRs leads to an increase in vasoconstriction in the large amount of non-active muscles of the leg, and therefore, a relative decrease in bulk BF to the leg. In turn, this could cause a redistribution of BF to the relatively small amount of active musculature (quadriceps) in the leg where the metabolic demand is highest. Although these higher ΔWRs will lead to a greater recruitment of less efficient fibres (in particular, type II fibres) to meet force production requirements, the BF may be higher to type I fibres (McDonough et al., 2004, Behnke et al., 2003). This will lead to type II fibres relying more on O_2 extraction than type I fibres, which will create a smaller pressure head for 'driving' O_2 into the type II muscle fibres. Therefore, the slowed $\dot{V}O_{2p}$ kinetics with increasing ΔWR may be due to a smaller BF and O_2 delivery to these newly recruited type II fibres, or a metabolic sluggishness of the oxidative pathways in these less efficient type II fibres.

Recent studies have investigated the effect of performing exercise transitions from an elevated baseline metabolic rate (i.e., higher WR_{bl} and $\dot{V}O_{2p}$) with the consistent findings that $\dot{V}O_{2p}$ kinetics become slower when transitions are initiated from elevated WR_{bl}s and the gain (G_p; $\Delta\dot{V}O_{2p}/\Delta WR$) becomes larger (Nederveen et al., 2017, Keir et al., 2016a/b, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001). Although the mechanism has not been identified, it was suggested that the slower $\dot{V}O_{2p}$ kinetics and greater $\dot{V}O_{2p}$ G_p associated with transitions from the higher compared to the lower baseline metabolic rate, perhaps, was related to recruitment of a less efficient muscle fibre pool to meet the increased muscle force/power requirements of the imposed, higher WRs (Nederveen et al., 2017, Keir et al., 2016a/b, Bowen et al., 2011, MacPhee et al., 2005, Brittain et al., 2001). The delayed adjustment of muscle O_2 utilization in response to an instantaneous increase in WR or ATP requirement has been attributed to a sluggish activation of rate limiting enzymes and metabolic pathways responsible for providing oxidizable substrate (other than O_2) to the mitochondria, to a slow increase in muscle BF and O_2 delivery, or a combination of these. To date, only one study has investigated the effect of a raised WR_{bl} on the response of BF, with the finding that, like $\dot{V}O_{2p}$, BF kinetics also are slowed with a transition from an elevated WR_{bl} (MacPhee et al., 2005). However, this study used only two WR_{bl}'s and the entire response profile was constrained within the MOD domain. Therefore, the purpose of Chapter IV of this thesis was to extend these findings by expanding the range

of baseline metabolic rates to include exercise across the MOD, HVY, and VH intensity domains and examining the response and integration of $\dot{V}O_{2p}$, BF, deoxy[Hb + Mb], HR, MAP, $\dot{Q}_{cap}$, and VC between these different transitions.

The main findings for Chapter IV were: i) $\dot{V}O_{2p}$ kinetics were slower at the highest WR_{bl}s compared to the lowest WR_{bl}s; ii) conduit artery BF kinetics were slower (i.e., τBF & MRT larger) when WR_{bl} was increased, with the exception of the highest exercise transition where BF kinetics were faster than what was seen in the previous, penultimate, transition; iii) the increase in BF relative to the increase in metabolic demand ($\Delta BF/\Delta\dot{V}O_{2p}$) became progressively smaller as WR_{bl} increased; iv) the MAP gain ($\Delta MAP/\Delta WR$) was larger at the higher WR_{bl}s; v) HR kinetics were fastest at the highest WR_{bl}; and vi) deoxy[Hb + Mb] MRT became progressively larger with increasing WR_{bl}. While the slowing of BF kinetics with increasing WR_{bl} at the lower transitions is in line with initial hypotheses and previous results (MacPhee et al., 2005), the significant speeding of BF kinetics at the highest WR_{bl} may be related to the intensity domain in which the subjects are exercising and the vasodilation-inducing metabolites that have accumulated during the elevated baseline metabolic rate. At the same time, there was a decrease in the absolute bulk BF relative to the metabolic demand of the muscle as WR_{bl} increased. This relative decrease in bulk O_2 delivery appeared to be offset by a redistribution of microvascular BF to the active muscle fibres as the observed slowing of deoxy[Hb + Mb] kinetics suggests an enhanced O_2 delivery to the active muscle relative to O_2 utilization within the microvasculature.

One of the most unanticipated observations of this thesis was a speeding of BF kinetics when exercise was initiated from the highest compared to the second highest WR_{bl} (see Chapter IV). This speeding may be the result of vasodilation-inducing metabolites released by the muscle as a result of the high intensity of exercise being performed as most subjects were transitioning from the HVY (WR_{bl}, 45 W) into the VH domain (WR_{bl}, 66 W). Based on the data from Chapter IV, it was not possible to discern whether this speeding of BF kinetics in the highest transition was attributable to the metabolic conditions present at baseline exercise prior to the transition (45 W; HVY domain for 7 of 10 subjects; see APPENDIX III), or the metabolic instability that arose from the increased metabolic demand of the transition (66 W; VH for 7 of 10 subjects). However, from the data collected

in Chapter III, in which these same subjects performed an exercise transition to the same absolute WR of 66 W, but from a baseline metabolic rate of light-intensity (3 W) exercise, there was no speeding of BF kinetics compared to those transitions to a lower WR (in fact, kinetics generally became slower with increasing ΔWR from a common light-intensity baseline). With this comparison, it is tempting to speculate that since there was no significant speeding of BF kinetics at the 66 W WR when the transition was initiated from a low metabolic baseline (3 W), but there was a speeding when the transition was initiated from a high metabolic baseline (45 W), that it was the pre-transition metabolic rate that resulted in a speeding of BF kinetics, and not necessarily the metabolic rate of the transition. However, it should also be noted that for both of these transitions to 66 W, the time constant values were similar (MRT BF: 35 ± 16 s, 35 ± 24 s; τBF: 25 ± 12 s, 30 ± 22 s, for a low WR_{bl} (3 W) and a high WR_{bl} (45 W), respectively). Thus, the physiological conditions of the 66 W transition may predispose BF towards a given rate of adjustment, with this response possibly associated with the metabolic instability of a VH exercise domain. However, with the study design of this thesis, any attempted explanation for the speeding of BF kinetics at the highest WR_{bl} is largely speculation, and therefore, there is a need for further research to more accurately investigate this response.

Limitations

A large portion of this thesis was the investigation of BF kinetics in response to step-transitions in WR. Essential to this is a proper quantification of the mean blood velocity (MBV) (and BF) response signal. However, at present, there is no 'gold standard' for quantifying the BF signal. Therefore, in Chapter II the effect of different techniques for quantifying BF was investigated. Based on the findings, none of the techniques investigated stood out as being superior although the CR1 analysis was chosen as being better and used throughout the thesis to estimate the BF response to exercise.

The same absolute WRs were used for all subjects in Chapters III and IV which resulted in each absolute WR having a different number of subjects within each intensity domain (MOD, HVY, and VH). While this setup was necessary for enhancing the signal-to-noise ratio, creating a range of WRs to compare (i.e., 5 total), and ensuring that subjects could

complete the 8 min transition for all WRs (i.e., 66 W max), it does restrict the ability to definitively distinguish the physiological responses associated with the different, individual, exercise-intensity domains. This is particularly important for interpreting the $\dot{V}O_{2p}$ and BF response profiles as profiles will differ depending on which (or into which) intensity domain the exercise transitions are taking place (see Poole & Jones 2012 for review). Additionally, due to the high knee-extension-specific WRs used in these studies, participants were generally more fit, making it difficult to extrapolate responses to the general population.

In Chapters III and IV, NIRS was used to measure muscle deoxygenation (i.e., deoxy[Hb + Mb]) and to infer the fractional O_2 extraction. In humans, it is known that there is a heterogeneity of fibre types within the muscle (e.g., ~50% type I fibres), dispersion of fibre types within the muscle (with type II fibres being more superficial than the deeper type I fibres), and heterogeneity of O_2 utilization and BF amongst muscle fibre types and within muscle regions. Due to the relatively shallow depth to which NIRS is able to penetrate, and the very small area of interrogation, the deoxy[Hb + Mb] signal cannot be considered to be representative of the whole muscle. Nevertheless, care was taken to ensure that the NIRS probe was positioned on the same location over the muscle for each trial, which improves the likelihood that differences in the NIRS signal can be attributed to changes in the metabolic activity and O_2 delivery within the same muscle region and muscle fibre pool.

Ideally measures of blood pressure (BP) and calculation of mean arterial pressure (MAP = 1/3 SBP + 2/3 DBP) should have been made on a more continuous basis that simply once per minute. However, when data collection for the different studies began we did not have access to a beat-by-beat blood pressure (BP) unit and so BP was measured by one of the investigators experienced in sphygmomanometry. Because the min-by-min MAP profile appeared to change with a monoexponential time course during the exercise transition it was decided to model the MAP profile and estimate MAP kinetics. This also allowed calculation of vascular conductance (VC = BF/MAP) and estimation of VC kinetics. In doing so, it was recognized that the fidelity of the entire non-steady-state and steady-state response profile was compromised because of such a low density of actual, measured data. Also, although the increase in HR during exercise is accomplished by a decrease in the

duration of both systole and diastole in the cardiac cycle, there is a larger reduction in the diastolic phase, which offset the proportion of the cardiac cycle that was spent in systole and diastole. In this study no attempt was made to compensate for this when calculating MAP. However, even at the highest WR, HR was still relatively low (~130 bpm), which quite possibly precludes a large influence of cardiac cycle phase durations on MAP calculations.

While $\dot{Q}_{cap}$ has been quantified previously as a measure of microvascular BF (Ferreira et al., 2005, Harper et al., 2006, Schlup et al., 2015), its calculation does require the use of two measures that are surrogate values within the Fick equation, namely the replacing of $\dot{V}O_{2m}$ with $\dot{V}O_{2p}$ and a-vO_{2diff} with deoxy[Hb + Mb]. In particular, Murias et al. (2012) described a "proportionality issue" in which $\dot{V}O_{2p}$ increases by a relatively small range (e.g., 80-100%), whereas deoxy[Hb + Mb] can show a vastly larger range of increase (e.g., 10-160%), with the authors finding that when Δdeoxy[Hb + Mb] values were greater than ~50% of deoxy[Hb + Mb]$_{bl}$ values the $\dot{Q}_{cap}$ response showed a nonexponential or negative adjustment during exercise, which is deemed unphysiological. However, in their study, Murias et al. (2012) used a bout of 'priming' exercise (which would create an excess of BF), which was not used in the present studies. Nevertheless, caution should be exercised when using $\dot{Q}_{cap}$ as a representation of microvascular BF.

Future Directions

One of the most intriguing (and unexpected) findings of this thesis was the speeding of bulk BF kinetics when an exercise transition was initiated from the highest WR_{bl}. While it is postulated that this was a result of the intensity domain from which the transition was initiated (HVY), or perhaps the intensity domain that the transition finished in (VH), this cannot be stated decisively. This is largely limited by the decision to not individualize each subject to their own relative intensity domains. Therefore, future studies should look to adjust WRs to each subject's relative intensity domain to help elucidate the source of this speeding in bulk BF kinetics. Furthermore, a more direct comparison for determining whether this speeding of BF kinetics is due to the WR_{bl} or the WR that the subject is transitioning to would provide better focus on the cause of this speeding.

Additionally, in Chapter III there was a trend ($p < 0.10$) for BF kinetics to be slowed with increasing ΔWR. Further study on bulk BF kinetics in response to increasing WRs may want to include a larger sample size and use of actual "intensity domains," rather than simply "absolute WRs," may also help to better define this potential relationship.

The decrease in the $\Delta BF/\Delta \dot{V}O_{2p}$ ratio with transitions of both an increasing WR_{bl} as well as an increasing ΔWR was also an intriguing finding in these studies. A greater focus on whether this relative decrease in bulk BF for the metabolic rate is compensated for by a more efficient redistribution of microvascular BF within the active musculature could help reveal the source of this response.

Lastly, future studies should consider incorporating a more direct measure of microvascular BF, such as contrast enhanced ultrasound, instead of calculated $\dot{Q}_{cap}$. This would allow for a more accurate quantification of the role of microvascular BF on the bulk BF response, especially at higher WRs in which the microvascular network may be fully perfused within the exercising muscle.

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APPENDIX I: Ethics approval

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. John Kowalchuk
Department & Institution: Health Sciences\Kinesiology, Western University

Review Type: Full Board
HSREB File Number: 107156
Study Title: The effect of elevated baseline work rates and increasing work rate amplitudes on blood flow kinetics.
Sponsor: Natural Sciences and Engineering Research Council

HSREB Initial Approval Date: October 20, 2015
HSREB Expiry Date: October 20, 2016

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Advertisement	In-Class Announcement Script	2015/09/02
Other	PAR-Q form	2015/09/02
Western University Protocol		2015/10/05
Letter of Information & Consent		2015/09/23

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

[redacted]

Ethics Officer, on behalf of [redacted], HSREB Chair

Ethics Officer to Contact for Further Information [redacted]

This is an official document. Please retain the original in your files

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APPENDIX II: Letter of information and consent

LETTER OF INFORMATION & CONSENT

Western

The effect of baseline metabolic rate and work rate amplitude on the dynamic adjustment of pulmonary O_2 uptake, muscle blood flow and deoxygenation during transitions to exercise

Principle Investigator: John M. Kowalchuk, PhD

Purpose of the Study:

You are being invited to participate in a research study examining how different levels of light-to-heavy intensity exercise affect the way the human body adjusts muscle blood flow and uses oxygen (O_2) when going from lower to higher exercise intensities. Each exercise protocol will begin with a bout of very light/easy alternate-leg knee-extension ('kicking') exercise after which you will perform either a single-step or a series of step-increases in work rate (for example: from "light-" to "moderate-intensity exercise" and from "moderate-" to "heavy-intensity exercise"), with each "step" lasting six to eight minutes. The effect of step increases in work rate either i) from a common, light-intensity baseline but by increasing work rate change, or ii) from progressively higher baseline intensities but by a constant work rate change, on the rate of adjustment of blood flow and muscle O_2 utilization is unclear. Therefore, in this study, blood flow, lung O_2 uptake, and leg muscle deoxygenation will be examined during single-step or multiple step-incrementing exercise tests spanning from "light-" to "heavy-" intensity exercise.

Participation in this study involves visits to the research laboratory at the Canadian Centre for Activity and Aging (Arthur and Sonia Labatt Health Science Centre, Room 313) on approximately 34-50 different occasions, with each visit taking approximately 30-40 minutes (for a total time commitment of ~24-33 hrs).

A total of 16 adult males will be invited to participate in this study. In order to participate you must be between 18-39 years of age and healthy. You will not be able to participate in the study if you have been diagnosed previously with any respiratory, cardiovascular, metabolic or musculoskeletal disease; or you are currently on medication; or you are a smoker; or you respond to the exercise protocol in an irregular manner or cannot tolerate the exercise protocol; or if you respond "YES" to any of the questions on the Physical Activity Readiness Questionnaire (PAR-Q). If you are participating in another study at this time, please inform the investigator to determine if it is appropriate for you to participate in this study.

Research Testing Protocol

Should you decide to participate in the study, testing will include one ramp-incremental cycle ergometer test, and two step-incremental exercise tests on an alternate-leg knee-extension ergometer to volitional fatigue (see **Figure 1**). The tests will begin with the exercise intensity being very light and easy (very little resistance). After several minutes, the exercise intensity will increase constantly (cycle ergometer) or every minute (knee-extension ergometer) until you are unable to continue and have to stop because you are either unable to pedal or 'kick' against the resistance and maintain the required rate or cadence, because the intensity is too high, or you perceive the exercise as being too uncomfortable. These visits should last no more than 40 minutes.

In addition to the ramp-incremental cycle exercise test and step-incremental knee-extension exercise tests to volitional fatigue, you will be asked to complete up to six repetitions of six different single-step exercise protocols with each protocol beginning with 6 minutes of 3 W 'kicking' exercise followed by 8 minutes exercise at intensities of either: 12, 33, 45, 54, 66, or 75 W (total = 14 minutes; see **Figure 1**).

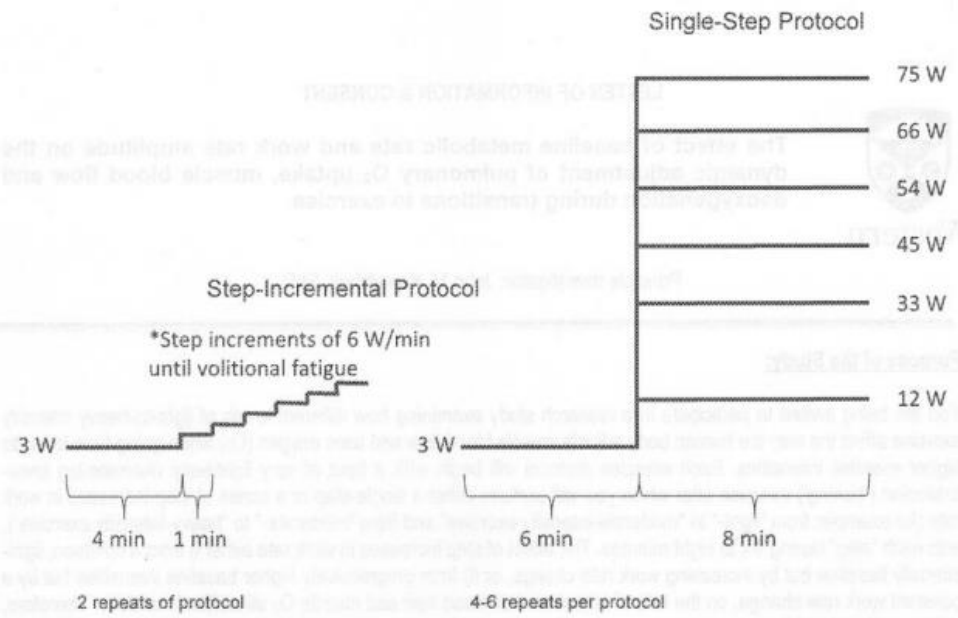

Figure 1: Step-Incremental and Single-Step Protocols

You will also be asked to perform 4-6 repetitions of two different step-incremental exercise protocols. Each exercise protocol will begin with a 6 minute period of either 3 W or 12 W 'kicking' followed by an instantaneous increase in resistance by 21 W (to either 24 W [from 3 W baseline – Protocol A] or 33 W [from 15 W baseline – Protocol B]). You will exercise at this intensity for an additional 6 minutes, and then the work rate will instantaneously increase (again) by 21 W and this intensity will be sustained for 8 minutes. This exercise intensity will be followed by one additional instantaneous 21 W increase in resistance lasting another 8 minutes (total = 28 minutes; see **Figure 2**). Half of the exercise (approximately 12 minutes) will be of "light"- to "moderate-intensity" (exercise that can be performed for a prolonged period of time, e.g., fast walking, easy jogging or bicycling) while the other half (approximately 16 minutes) will be of "heavy-intensity" (exercise where it will be difficult to carry a conversation due to increased breathing rate, e.g., running or bicycling uphill).

Repeat testing in each condition is required in order to get cleaner data associated with such testing and to increase the underlying signal-to-noise ratio which means the amount of good/accurate data points to the amount of bad data points. Bad, unusable data may result from loss of signal of the Doppler ultrasound for blood flow or breaths associated with coughs or swallowing for O_2 uptake.

Research Procedures:

During each of the exercise tests you will be required to wear a nose-clip (to prevent you from breathing through your nose) and a rubber mouthpiece (similar to breathing through a snorkel or diving mask); nose-clips and mouthpieces are disinfected before each test. This will enable us to measure the volume of air that you breathe in and out, and measure the gas concentration in that air.

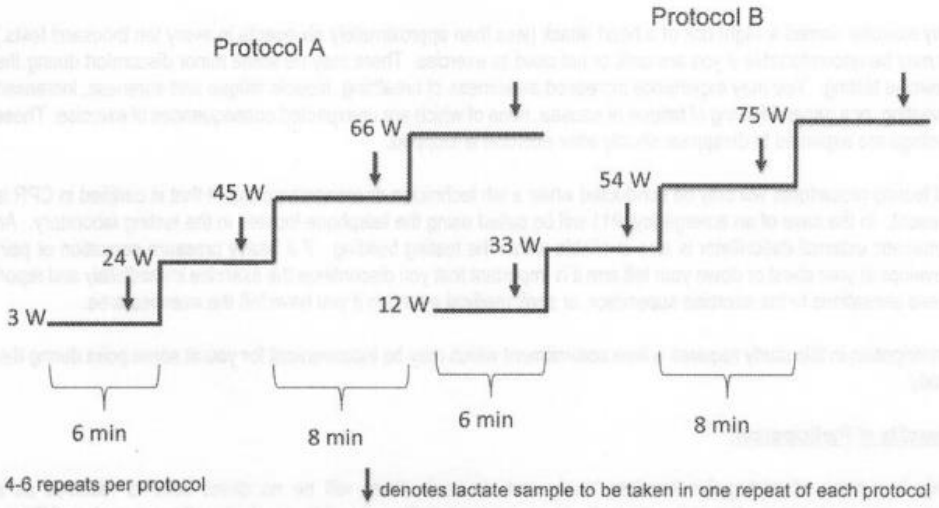

Figure 2: Exercise Protocols

During each of the exercise tests, an investigator will hold a Doppler ultrasound probe over the common femoral artery (a major artery for blood flow on the inside of the upper thigh and hip) to determine blood flow to the exercising muscles in your leg. You may feel a very slight pressure over the area from the probe, but this is a non-invasive measure with no known risks.

During each of the exercise tests, the amount of oxygen in your leg muscle will be measured using near-infrared spectroscopy which projects light into a specific location of your leg muscles and measures the amount of light coming out at another location. Two near-infrared probes will be placed on your leg approximately midway between your hip and your knee. They will be secured with tape, covered to prevent light from entering or leaving the area, and bound with elastic bandage to minimize movement. You might experience a bit of discomfort by having this equipment secured to your leg during the exercise period; however, this is a non-invasive procedure and any discomfort usually becomes less noticeable with time during the exercise.

Heart rate and rhythm will be monitored continuously by electrocardiogram. One electrode will be placed on each of the following areas: left chest, right chest, and left side under your ribs and connected to an electrocardiograph. The electrodes use adhesive tape to secure to the skin. There are no known risks or discomforts associated with this procedure.

Blood lactate (or muscle acid) levels will be measured during two of the exercise tests (a total of eight times) at select time points (see **Figure 2**). A small pin prick will be made to your earlobe in order to draw a droplet of blood. You may experience some pain or discomfort related to the earlobe prick.

Possible Risks and Discomforts:

You may experience some minor discomfort from wearing the nose-clip and rubber mouthpiece, or by having the ultrasound probe and NIRS probes secured to your leg during the exercise period. These sensations often become less noticeable with time during the exercise.

Any exercise carries a slight risk of a heart attack (less than approximately six events in every ten thousand tests.) or may be uncomfortable if you are unfit or not used to exercise. There may be some minor discomfort during the exercise testing. You may experience increased awareness of breathing, muscle fatigue and soreness, increased sweating, or a general feeling of fatigue or nausea, none of which are unexpected consequences of exercise. These feelings are expected to disappear shortly after exercise is stopped.

All testing procedures will only be conducted when a lab technician or research assistant that is certified in CPR is present. In the case of an emergency, 911 will be called using the telephone located in the testing laboratory. An automatic external defibrillator is also available within the testing building. If a heavy pressure sensation or pain develops in your chest or down your left arm it is important that you discontinue the exercise immediately and report these sensations to the exercise supervisor, or seek medical attention if you have left the exercise area.

Participation in this study requires a time commitment which may be inconvenient for you at some point during the study.

Benefits of Participation:

This is a basic physiology/biochemistry study and, as such, there will be no direct benefits received as a consequence of participating in the study. If you are interested, the rational for conducting the research and theory and significance of each of the tests will be explained, as will your individual results from each of the tests. You will also have the opportunity to learn about and better understand your physiological responses to an exercise situation.

Confidentiality:

Records from this study are confidential and will be stored securely at the Canadian Centre for Activity and Aging, Sonia Arthur Labatt Health Sciences Building. Your records will be identified by a number rather than your name. Your de-identified data will be kept indefinitely so that it is available for analysis within the research group. Published reports resulting from this study will not identify you by name. If you wish to withdraw your data at any time you can do so by contacting the Principal Investigator, Dr. John Kowalchuk at [redacted]. Representatives of The University of Western Ontario Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research.

Voluntary Participation:

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your academic or employment status.

You will be given a copy of this letter of information and signed consent forms. You do not waive any legal rights by signing the consent form. If you have any questions regarding this study please contact Dr. John M. Kowalchuk [redacted], or Lorenzo Love [redacted] at the Canadian Centre for Activity and Aging, Sonia and Arthur Labatt Health Sciences Building, The University of Western Ontario, London. If you have any question about the conduct of this study or your rights as a research subject you may contact the Director of the Office of Research Ethics, The University of Western Ontario [redacted].

LETTER OF INFORMATION & CONSENT

Western

The effect of baseline metabolic rate and work rate amplitude on the dynamic adjustment of pulmonary O_2 uptake, muscle blood flow and deoxygenation during transitions to exercise

Principal investigator: John M. Kowalchuk, PhD

Records from this study are confidential and will be stored securely at the Canadian Centre for Activity and Aging, Sonia Arthur Labatt Health Sciences Building. Your records will be identified by a number rather than your name. The data will be available for analysis within the research group. Published reports resulting from this study will not identify you by name.

We would like to keep your de-identified study related data and use it in potential future analyses. Please check and initial the box below to indicate whether you consent to have your data kept and used in future analyses.

☐ I agree _____ (initials)

☐ I do not agree _____

I have read the Letter of Information, have had the nature of this study explained to me and I agree to participate. All questions have been answered to my satisfaction and I will receive a copy of the Letter of Information and signed consent form.

Participant:

_____ _____

Name (please print) Signature

_____

Date

Investigator (i.e., Person Responsible for Obtaining Informed Consent):

_____ _____

Name (please print) Signature

_____

Date

APPENDIX III: Figures

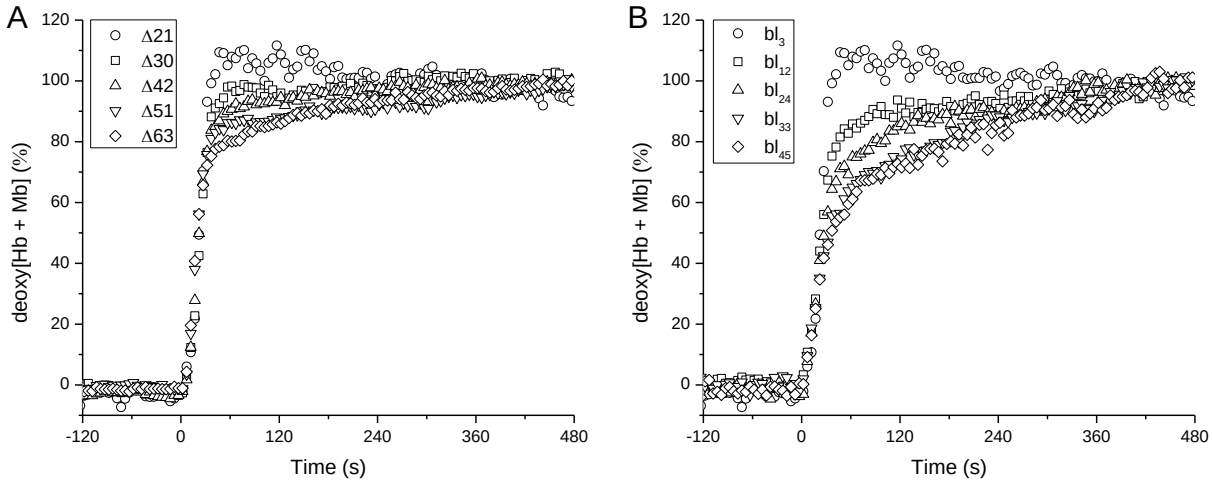

Figure A. Normalized muscle deoxygenation (deoxy[Hb + Mb]) response profiles for: A, transitions from a common baseline WR (3 W) to different ΔWRs; B, transitions of a common ΔWR (21 W) from elevated baseline WRs.

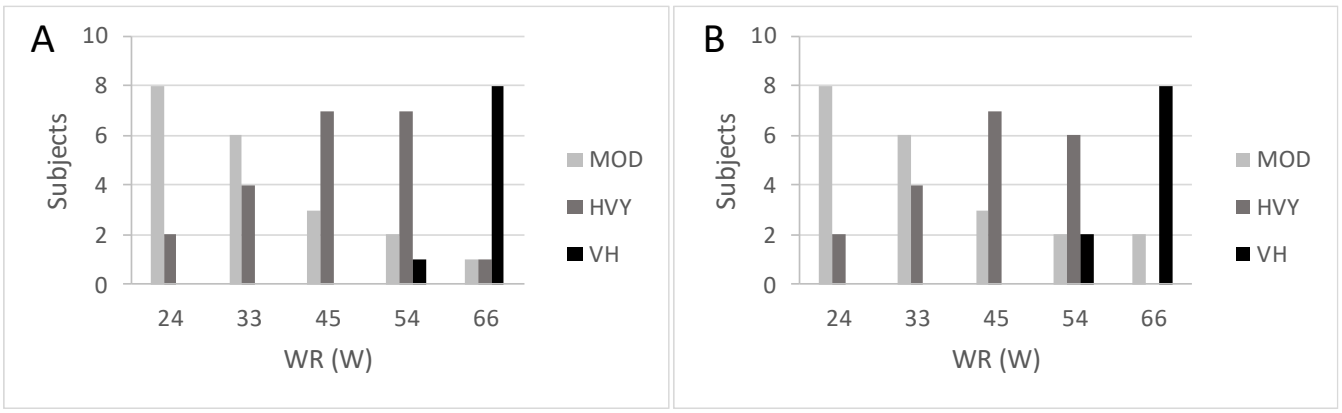

Figure B. Number of subjects in each intensity domain across the various WRs for: A, transitions from a common baseline WR (3 W); B, transitions from elevated baseline WRs. MOD, moderate-intensity; HVY, heavy-intensity; VH, very heavy-intensity; WR, work rate; W, watts.

CURRICULUM VITAE

LORENZO K. LOVE

School of Kinesiology, Faculty of Health Sciences
Canadian Centre for Activity and Aging
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EDUCATION:

Doctorate of Philosophy in Kinesiology – 2020
Western University, London, ON

Master of Science in Kinesiology – 2009
Brock University; St. Catharines, ON

Bachelor of Kinesiology – 2006
Brock University; St. Catharines, ON

TEACHING:

Sessional Assistant Professor
Redeemer University College, Ancaster, Ontario, July 2020 - present
Kinesiology Department
Courses taught:

- KPE-318: Exercise Physiology (laboratory)
- KPE-313: Motor Learning
- KPE-312: Care & Prevention of Injuries
- KPE-218: Human Physiology
- KPE-159: Fundamentals of Strength Training
- KPE-142: Fundamentals of Fitness
- KPE-118: Introduction to Anatomy I

Instructor
Redeemer University College, Ancaster, Ontario, Sept. 2016 - Apr. 2020
Kinesiology Department
Courses taught:

- KPE-318: Exercise Physiology
- KPE-312: Care & Prevention of Injuries
- KPE-218: Human Physiology
- KPE-159: Fundamentals of Strength Training
- KPE-142: Fundamentals of Fitness

Teaching Assistant
Western University, London, Ontario, Sept. 2012 - Dec. 2013
Kinesiology Department
Course taught:

- KIN 3330: Laboratory in Exercise Physiology

Brock University, St. Catharines, Ontario, Sept. 2006 - April 2009
Physical Education & Kinesiology Department
Courses taught:

- PEKN 2P90: Physiological Responses to Physical Activity
- PEKN 2P97: Muscle Physiology & Exercise Metabolism
- PEKN 3P02: Functional Fitness Assessment & Exercise Prescription
- PEKN 4P97: Regulation of Human Metabolism

REFEREED CONFERENCE PRESENTATIONS:

Canadian Society for Exercise Physiology, Kelowna, BC, Nov. 2019
The response of microvascular blood flow (Q_{cap}) and muscle deoxygenation ([HHb]) between elevated baseline work rates and increasing work rate amplitudes (poster presentation)

Canadian Society for Exercise Physiology, Niagara Falls, ON, Nov. 2018
Data analysis technique influences blood flow kinetics parameter estimates for moderate- and heavy-intensity exercise transitions (poster presentation)

Canadian Society for Exercise Physiology, Winnipeg, MB, Oct. 2017
Influence of elevated baseline work rates and increasing work rate amplitudes on blood flow kinetics (poster presentation)

Canadian Society for Exercise Physiology, Victoria, BC, Oct. 2016
Influence of elevated baseline work rates and increasing work rate amplitudes on blood flow kinetics (poster presentation)

Canadian Society for Exercise Physiology, Hamilton, ON, Oct. 2015
Effect of carbohydrate availability on the metabolic response to exercise following high-fat and high-carbohydrate diets (poster presentation)

Canadian Society for Exercise Physiology, Banff, AB, Nov. 2008
Human skeletal muscle pyruvate dehydrogenase phosphatase activity and expression: the effect of aerobic capacity (poster presentation)

PUBLICATIONS:

Nederveen, J.P., Keir, D.A., **Love, L.K.**, Rossiter, H.B. and Kowalchuk, J.M. Effect of heavy-intensity 'priming' exercise on oxygen uptake and muscle deoxygenation kinetics during moderate-intensity step-transitions initiated from an elevated work rate. *Resp Physiol Neurobiology* 235: 62-70, 2017

Keir, D.A., Benson, A.P., **Love, L.K.**, Robertson, T.C., Rossiter, H.B. and Kowalchuk, J.M. Influence of muscle metabolic heterogeneity in determining the VO_{2p} kinetic response to ramp-incremental exercise. *J Appl Physiol* 120: 503-513, 2016.

Raper, J.A., **Love, L.K.**, Peters, S.J., Heigenhauser, G.J.F., Paterson, D.H. and Kowalchuk, J.M. The effect of high fat and high carbohydrate diets on pulmonary O_2 uptake kinetics during the transition to moderate-intensity exercise. *J Appl Physiol* 117: 1371–1379, 2014

Love, L.K., LeBlanc, P.J., Inglis, J.G., Bradley, N.S., Choptiany, J., Heigenhauser, G.J.F. and Peters, S.J. The relationship between human skeletal muscle pyruvate dehydrogenase phosphatase activity and muscle aerobic capacity. *J Appl Physiol* 111: 427–434, 2011

MANUSCRIPTS IN PREPARATION:

Love, L.K., Hodgson, M.D., Keir, D.A. and Kowalchuk, J.M. The effect of increasing work rate baselines on the kinetic response of VO_{2p}, blood flow, and muscle deoxygenation

Love, L.K., Hodgson, M.D., Keir, D.A. and Kowalchuk, J.M. The effect of increasing work rate amplitudes from a common baseline on the kinetic response of VO_{2p}, blood flow, and muscle deoxygenation

Love, L.K., Hodgson, M.D., Keir, D.A. and Kowalchuk, J.M. Data analysis technique influences blood flow kinetics parameter estimates for moderate- and heavy-intensity exercise transitions.

Love, L.K., Lee, D., Bright-McCurdy, T.S., Doherty, T.J., Kowalchuk, J.M. The effect of carbohydrate availability during a high fat diet on pulmonary O_2 uptake kinetics during transitions to moderate- and heavy-intensity exercise.

Leckie, J.R., Nederveen, J.P., Whitfield, J., **Love, L.K.**, Doherty, T.J., Paterson, D.H., Spriet, L.L. and Kowalchuk, J.M. The effect of low and high carbohydrate diets on pulmonary oxygen uptake, muscle deoxygenation kinetics, and pyruvate dehydrogenase activation during exercise transitions in the heavy-intensity domain.

CERTIFICATIONS:

- Clinical Exercise Physiologist - Canadian Society for Exercise Physiology
- Certificate in University Teaching and Learning, Western University, London, ON
- Graduate Teaching Assistant Instructional Skills Workshop, Brock University, St. Catharines, ON
- Emergency first aid/CPR/AED

AWARDS:

- Ontario Graduate Scholarship, Sept. 2015-Aug. 2016
- Ontario Graduate Scholarship, Sept. 2014-Aug. 2015
- Dean's Honour List, 2004-2006